EVALUATION OF ETIOLOGIC CHEMICAL FACTORS AND OXIDATIVE STRESS STATUS ASSOCIATED WITH PREVALENCE OF UROLITHIASIS IN THE URBAN AREAS OF MIZORAM

A Thesis submitted
in partial fulfilment of the requirements for the Degree of

Doctor of Philosophy

in

Chemistry

By

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This is to certify that the thesis entitled "E*valuation of etiologic chemical factors and oxidative stress status associated with prevalence of urolithiasis in the urban areas of Mizoram*" submitted by ***Ms. R Lawmzuali,*** **(Regd. No.:MZU/PhD/548 of 20.05.2013)** for the degree of ***Doctor of Philosophy*** **in Chemistry** in the Mizoram University, Aizawl, Mizoram, embodies the record of original investigations carried out by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any degree in any other university.

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I, R Lawmzuali, hereby declare that the subject matter of this thesis "*Evaluation of etiologic chemical factors and oxidative stress status associated with prevalence of urolithiasis in the urban areas of Mizoram*" is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

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ACKNOWLEDGEMENT

I would like to take this privilege to thank scores of people from the core of my heart who with their constant support, affection, inspiration and encouragement gave me strength to successfully complete this venture.

I wish to express my sincere thanks with a deep sense of gratitude, to my respected supervisors Dr. N. Mohondas Singh, Assistant Professor, Department of Chemistry and Dr. K. Birla Singh, Assistant Professor, Department of Zoology, PUC for their immense guidance, help, dedicated support, intellectual supervision, uncompromised commitment and professional expertise for the timely completion of my research.

My sincere gratitude to Dr. Muthukumaran R, Head, Department of Chemistry for the timely support and guidance throughout my course and providing research facilities to complete my research.

I am also thankful to Dr. Diwakar Tiwari, Professor and former Head, Department of Chemistry, for his constant support, valuable suggestions, encouragement to keep my moral high in time of difficulties and setting an example of professional dedication.

I deeply owe my honest regards to Prof. R.C. Tiwari, Dean, School of Physical Sciences, for his constant support and valuable suggestions.

I would like to convey my heartfelt thanks to *UGC-DAE CSR Kolkata Centre*, for their financial support and help. I also thank Prof. M Sudharshan and his associates for their valuable co-operation.

My heartfelt thanks to all the teaching staff, *Dr. Muthukumuran R., Dr. Zodinpuia Pachuau, Prof. Diwakar Tiwari, Dr. Raj Kumar Mishra, Dr. Ved Prakash singh and Dr. A. Bimolini Devi,* Department of Chemistry, Mizoram University, for valuable support during my research work.

My sincere thanks to my colleagues and friends, Mr. Lalzawnpuia, Mr. Premjit Singh, Mr. Lalrosanga, Ms. Olivia Lalmuanzuali, Mr. Lalhmingliana Hnamte, Mr. Joseph Lalhruaitluanga, Mr. C. Lalhriatpuia, Mr. Thanhmingliana, Mr. Lalsaimawia Sailo, Mr. Lalnuntluanga, Mr. Zirlianngura, Ms. Rebecca Lalmuanpuii, Ms. Lalchhingpuii, Ms. Jacqueline Lalthlengliani, Ms. Lalneihpuii, Ms. Lalrintluangi for their valuable co-operation and being a great source of help.

My sincere thanks to all the non-teaching staff, Department of Chemistry, who have always extended their full hearted support during my research work.

Above all Thank You to the Almighty God, who has given me life and opportunity to express my gratitude to all those people who have helped and guided me throughout my life.

R. Lawmzuali

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1. INTRODUCTION AND REVIEW OF LITERATURE

1.1. UROLITHIASIS

Urolithiasis, the process of formation of stones in the kidney, bladder and/or urethra is a complex phenomenon not yet thoroughly understood. It is one of the most common urological disorders and has afflicted humans since time immemorial. It is a longstanding medical illness and still a common public health problem. A large portion of the world population suffers from urinary tract and kidney stones, formed due to deposition of calcium, phosphates and oxalates. In this process, the chemicals start accumulating over a nucleus, which ultimately takes the shape of a stone. These stones may persist for an indefinite period of time, leading to secondary complications causing serious consequences to patient's life. It is very painful and a proper cure is very much needed to get rid of the problem (Kshetrimayum BS *et al.*, 2013). Many parts of the world including India are now suffering from the stone diseases. It affects 20 % of the general population worldwide. In the United States, up to 12% of men and 6 % of women will develop urolithiasis at some point in their life. In Thailand, the highest prevalence (16.9 %) was reported in the Northeast provinces, while in Middle Eastern countries, the lifetime prevalence of kidney stone is even higher. Urolithiasis usually recurs and it poses difficulty in the management and burdensome medical costs. Recurrence rates as high as 50 % in 10 years have been documented (Ramello *et al.*, 2000; Stamatelou *et al.*, 2003). Epidemiological studies indicate many factors like age, sex, industrialization, socioeconomic status, diet and the environment influence urolithiasis. In urolithiasis, calcareous stone is the most common type of kidney stone disease. It accounts for more than 80% of all stones. The primary chemical complexes are calcium oxalate (CaOx) and calcium phosphate (CaP). Urinary stones contain both crystalloid and colloid components. The crystalloid components are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium-ammonium phosphate, uric acid and creatinin. Uric acid (UA)

stone represents about 4.5–23% and the other less frequent types of kidney stones are magnesium ammonium phosphate (MAP) or struvite stones, ammonium urate stones, cystine stones, xanthine and other miscellaneous stones(Pak *et al.*, 1997).

In urolithiasis, formation of urinary calculi (urinary stones) occurs anywhere in the urinary system (Pearle *et al*., 2007). It comprises nephrolithiasis (the formation of kidney stones), ureterolithiasis (the formation of stones in the ureters), and cystolithiasis (the formation of bladder stones) (Mc Nutt., 1893). One of the common types of urolithiaisis is renal calculus also known as kidney stone which is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Kidney stones typically leave the body by passage in the urine stream. However, many stones are formed and passed without causing symptoms. If stones grow to a sufficient size (usually at least 3 millimeters, they can cause obstruction of the ureter and causes post renal azotemia and hydronephrosis (distension and dilation of the renal pelvis and calyces), as well as spasm of the ureter. This leads to pain, most commonly felt in the flank (the area between the ribs and hip), lower abdomen, and groin (a condition called renal colic). Renal colic is commonly associated with nausea, vomiting, fever, blood in the urine, pus in the urine, and painful urination. Renal colic typically comes in waves lasting 20 to 60 minutes, beginning in the flank or lower back and often radiating to the groin or genitals.

It has been reported that kidney or ureter stones occur in 1 in 20 people at some period in their life time (Figure-1). The development of the stones is usually related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate. The stones formed in the urine collecting area (the pelvis) of the kidney and may range in size from tiny to staghorn stones the size of the renal pelvis itself. The diagnosis of kidney stones is made on the basis of information obtained from the history, physical examination, urinalysis, and radiographic studies besides ultrasound examination.

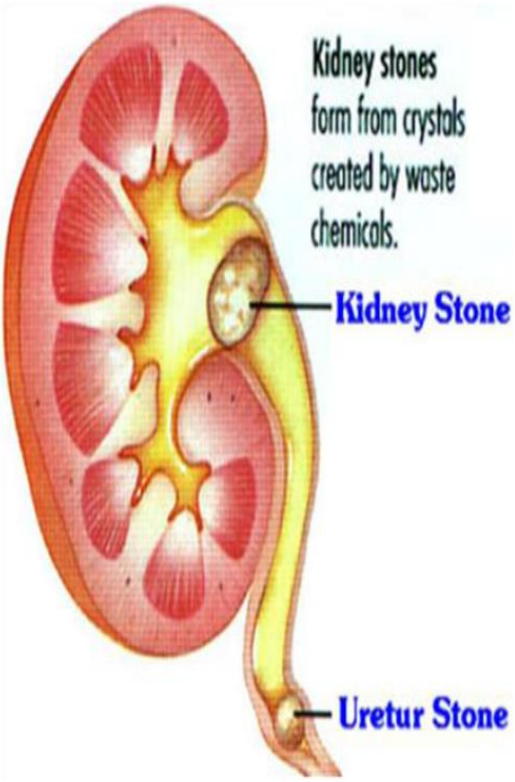

Figure 1. Stones in the kidney and ureter (Kshetrimayum BS and Saitluangpuii S, 2013**)**

Since the last two decades, there has been huge effort and different studies have been undertaken from the scientific communities towards clarifying the etiology of the disease in different population and ethnic communities of the world in order to contain the disease. Safarinejad in 2007, determined the prevalence, incidence, and risk factors of adult urolithiasis in Iran in a cross sectional study. In this study, data on risk factors for urolithiasis including age, race, education, body mass index, hypertension, and current use of medication were also obtained by a self-administered questionnaire and reported that urinary stones were more in number among men and women who lived in south central and southwest counties, with odds increasing from west to east and from north to south. It was also reported that a positive association was found between urolithiasis and unemployment, consumption of tea,

consumption of cola and meat consumption. This study provides a quantitative estimate of the prevalence, incidence, and main risk factors for adult urolithiasis in the Iranian population. He further warranted for further studies in order to determine the incidence and prevalence of urolithiasis in different ethnic groups. In another study, a nationwide survey was conducted to investigate the prevalence of upper urinary calculi in Taiwan with the help of postal questionnaire and revealed that alcohol consumption and family history of kidney stone were significant risk factors for stone occurrence with reported overall prevalence being 9.6% (14.5% in males and 4.3% in females). The subtropical temperature and gradually higher socioeconomic standards of living may also contribute to the high prevalence in this region. Further, it has been reported that urinary stones from endemic patients had higher fluoride, oxalate and Ca levels than those from non-endemic patients. *In vitro* studies also suggested that fluoride did not influence the heterogonous mineralization of calcium oxalate and fluoride *in vivo* may behave as a mild promoter of urinary stone formation by (a) excretion of insoluble calcium fluoride, (b) increasing oxalate excretion and (c) mildly increasing the oxidative burden. In an interesting study, Bastian and Vahlensieck in 1975, determined the excretion of Mg, Ca, Zn and citric acid in the urine of 11 patients with urinary calculi and in 5 healthy subjects on a standard diet for a period of 5 days and found that there was a more or less distinct dependence of electrolytes and citric acid on the standard diet and the excretion of Mg and citric acid in the urine was reduced in patients with calcium oxalate stone, while that of calcium and zinc were increased. Moreover, Roswitha *et al.*, in 2005, designed a study to evaluate the effect of dietary intervention on urinary risk factors for recurrence in calcium oxalate stone formers and suggested that nutrition is the major environmental risk factor in idiopathic calcium oxalate stone disease. In this study, the evaluation of urinary risk profiles of the patients on their usual dietary habits revealed a high risk for calcium oxalate stone formation. They also reported that a low fluid intake and an

increased intake of protein and alcohol were identified as the most important dietary risk factors and a shift to a nutritionally balanced diet according to the recommendations for calcium oxalate stone formers significantly reduced the stone forming potential. In addition to this, Fardellon *et al.,* in 2001, administered a frequential type self-questionnaire enabling evaluation of the calcium content of the diet of an individual as well as of a given population on the basis of 20 different types of food (items) rich in calcium and/or frequently eaten in metropolitan France. The self-questionnaire with its 20 items thus offers a simple and rapid method for estimation of the daily calcium intake of a given individual to within an accuracy of 20% and provided a dietetic evaluation technique suitable for both clinical and epidemiological use. In order to determine whether kidney stone disease prevalence increased in the United States over a 20-year period and the influence of region, race/ethnicity, and gender on stone disease risk, Stamatelou *et al*., in 2003 measured the prevalence of kidney stone disease history from the United States National Health and Nutrition Examination Survey (II and III), population-based, cross-sectional studies, involving 15,364 adult United States residents in 1976 to 1980 and 16,115 adult United States residents in 1988 to 1994. The study reported that the prevalence of kidney stone disease history in the United States population increased between 1980 and 1994 and history of stone disease was strongly associated with race/ethnicity and region of residence.

Mithani S *et al*., in 2005, studied to identify difference in urinary excretion between stone formers and healthy volunteers as a metabolic factor and reported that urinary citrate excretion level among the stone patients is similar to normal volunteers and is not a predisposing factor for the lithogenesis. Boonla C *et al* in 2006 also investigated the composition of the urinary tract stones and prospectively identify the risk of urinary stone in Udon Thani province of Thailand and reported that high carbohydrate and low fat diet consumptions combined with low citrus fruit intake are chief dietary risks of stone

development in the population. Moreover, in an systematic study to explore the etiopathogenesis of disease in Kathmandu region of Nepal, Sanjiv R *et al.,* in 2006, studied the qualitative composition of 47 renal stones collected from surgical patients admitted to NMCTH over a period of 13 months and suggests that calcium oxalate stones are predominant and the prevalence was very high among 20 yrs age group. Monthira *et al.,* in 2005 also studied the epidemiology of urolithiasis in southern Thailand and revealed that oxalate and uric acid was found in all the renal calculi.

In another study, Naghii and Hedayati in 2010 studied the influence of sex hormones on the stone formation and suggested the association between serum gonadal steroids and urolithiasis in males received only limited attention and the recommendation for steroid investigation as a basic evaluation to rule out treatable systemic causes in urolithiasis patients is warranted. In addition to this, Iguchi M *et al.,* in 1999, studied the effect of the female sex hormone on urinary stone formation by using ethylene glycol and vitamin –D induced rat urolithiasis model and suggested that the female sex hormones can inhibit renal crystal deposition in ethylene glycol treated rats by suppressing urinary oxalate excretion. Moreover, Paryani and Ather in 2002, assessed mean serum creatinine in order to confirmed whether definitive treatment of urolithiasis following relief of obstruction in patients with renal insufficiency reported in further improvement in renal function as determined by serum creatinine and reported that renal calculi and concurrent mild to moderate renal insufficiency warrants aggressive treatments and patients demonstrate significant improvement in renal function independent of relief of obstruction.

In India, urolithaisis constituted one of the commonest afflictions requiring surgical intervention and there are about 5-7 million patients suffering from urinary calculus disease. It is for these reasons that the Indian Council of Medical Research (ICMR) has classified this disease as one of the refractory diseases and stressed that efficient effort should be made to

find out the causes of the disease and to search for suitable drugs for its cure (Satyawati GV, 1982). Pushpa D *et al.,* in 2010, analyze qualitatively the uroliths obtained by surgical intervention at Krishna hospital Karad, a South West region in Maharashtra (India), to evaluate the predominant constituent present in them reported that urolithiasis was more suffered by individuals between the age group of 30 to 60 years with more predominance in males than females. In this study, the chemical analysis of uroliths showed that all the assessed stones were of mixed heterogeneous type with magnesium ammonium phosphate (71.2%) was predominant constituent followed by calcium oxalate (68.8%), calcium carbonate (64.0%), urate (44.8%), cystine (12.8%), xanthine (2.4%) and fibrin (1.6%). The study provides simple qualitative laboratory based method for assessing chemical composition of various uroliths and a reliable diagnosis of stone contents whose data may be useful in advising the people of this region for taking preventive measures for reducing the risk of prevalence and recurrence of urolithiasis in them. Rao TVRK *et al.,* in 2006, carried out epidemiology of the urolithiasis in the Purnia division of Bihar and analyzed the chemical composition of the stone and suggested that there is gradual increase in the urolithiasis during the past ten years and most of the stones are found to be mixed crystalloid composition containing calcium oxalate, phosphates, magnesium ammonium phosphate and ammonium ureate etc. Moreover, Girija EK *et al.,* in 2007 also analyzed the urinary calculi of population of southern Indian States and found that majority of the stones are the pure or calcium phosphate mixed calcium oxalate stones.

Jawalekar SL *et al.,* 2010 in order to find out risk factors for urolithiasis analyzed the urinary constituents and serum parameters in the patients of urolithiasis in Maharastra and revealed the positive association between parathyroid hormone and urolithiasis. Rathee N *et al*., in 2004 measured fluoride content in 100 urinary stones retrieved by open surgery of stone formers admitted at PGIMS Rohtak and their respective urine and serum and compared

with those of healthy individuals. The concentration of fluoride was also measured in the sources of drinking water of these stone formers and reported that the concentration of fluoride was probably significantly higher in drinking water of these stone formers than the normal ones. This study further reported that there is a positive correlation between the content of fluoride of urinary stones and urine of stone patients, stone and serum, drinking water and stone and their urine and serum, urine and drinking water and serum and water.

On the contrary, Rajkiran *et al.*, in 1996, studied the nutrient intake of 69 stone formers (SFs) from three subsets of the local population (urban 22, rural tribal 22 and rural nontribal 25) and 69 age, sex, weight and socioeconomically matched control subjects (NSs) (urban 20, rural tribal 22 and rural nontribal 27). The influence of dietary intake of protein, carbohydrate, fat, fiber, calcium and oxalic acid on urinary excretion of Ca, oxalic acid, uric acid, inorganic phosphorus, Mg and citric acid was examined using the chi-square test. The study reported that low nutrient intake did not influence the lithogenic process since there was no association between them. Pendse and Singh in1996, studied fifty-two(52) cases of urinary tract calculus disease for dietary habits, routine chemical and microscopic urinalysis, bacterial culture, quantitative analysis of 24h urine sample and qualitative analysis of the stones. The results of the study strongly suggested the multifactorial etiology of stone disease in this region and reported that imbalanced nutrition and urinary tract infection were the principal risk factors for urolithiasis in this study.

1.2. TYPES AND NATURE OF STONES IN UROLITHIASIS

Urinary stones can be classified according to size, location, X-ray characteristics, aetiology of formation, composition, and risk of recurrence (Leusmann DB, 1990; Leusmann DB, 2000; Kim SC *et al*., 2007; Hesse A *et al*., 2003). Accordingly, the following types were recorded:

(i) Stone size: Stone size is usually given in one or two dimensions, and stratified into those measuring up to 5 mm, 5-10 mm, 10-20 mm and > 20 mm in largest diameter.

(ii) Stone location: Stones can be classified according to anatomical position: upper, middle or lower calyx; renal pelvis; upper, middle or distal ureter; and urinary bladder.

(iii) X-ray characteristics: Stones can be classified according to plain X-ray appearance which varies according to mineral composition (Kim SC *et al.,* 2007). Non-Contrast-Enhanced Computer Tomography (NCCT) can be used to classify stones according to density, inner structure and composition. Accordingly, stones were classified as Radiopaque (Calcium oxalate dehydrate, Calcium oxalate monohydrate, Calcium phosphates), Poor radiopacity (Magnesium ammonium phosphate, Apatite, Cystine) and Radiolucent(Uric acid, Ammonium urate, Xanthine and 2,8-dihydroxyadenine (Leusmann DB, 2000; Kim SC *et al.,* 2007).

(iv) Aetiology of stone formation: Stones can also be classified into those caused by: infection, or non-infectious causes (infection and non-infection stones); genetic defects or adverse drug effects (drug stones). Non-infection stones are calcium oxalate, Calcium phosphate (including brushite and carbonate apatite), and Uric acid while infection stones are Magnesium ammonium phosphate, Carbonate apatite and Ammonium urate. Genetic causes/ Drug stones comprises Cystine, Xanthine and 2,8-dihydroxyadenine (Yasui T *et al., 2013*).

(v) Stone composition - Metabolic aspects are important in stone formation, and metabolic evaluation is required to rule out any disorders and thus Analysis in relation to metabolic disorders is the basis for further diagnostic and management decisions. Stones are often formed from a mixture of substances. The clinically most relevant substances and their mineral components are calcium oxalate monohydrate ($CaC_2O_4.H_2O$), Calcium oxalate dehydrate($CaC_2O_4.2H_2O$), Basic calcium phosphate($Ca_{10}(PO_4)_6.(OH)_{2)}$, b-tricalcium phosphate($Ca_3(PO_4)_{2)}$, Carbonate apatite phosphate($Ca_5(PO_4)_3OH$), Calcium hydrogen

phosphate ($CaHPO_4.2H_2O$), Calcium carbonate($CaCO_{3)}$, Octacalcium phosphate($Ca_8H_2(PO_4)_6.5H_2O$),Uric acid dehydrate($C_5H_4N_4O_{3)}$, Ammonium urate($NH_4C_5H_3N_4O_{3)}$, Sodium acid urate monohydrate($NaC_5H_3N_4O_3.\ H_2O$), Magnesium ammonium phosphate ($MgNH_4PO_4.6H_2O$), Magnesium acid phosphate trihydrate ($MgHPO_4.\ 3H_2O$), Magnesium ammonium phosphate, Monohydrate ($MgNH_4\ (PO_4).1H_2O$), Cystine ($[SCH_2CH\ (NH_2)\ COOH]_{2)}$, Gypsum, 2,8-dihydroxyadenine, Proteins, Cholesterol, Calcite, Potassium urate, Trimagnesium phosphate, Melamine, Matrix, Drug stones.

(vi). Stone classification based on chemical composition: Urinary stones are composed of a combination of crystals (both inorganic and organic) and proteins. Calcium-based stones, which include calcium oxalate monohydrate, calcium oxalate dihydrate, and calcium phosphate stones, account for 70%–80% of upper urinary tract stones. Struvite stones account for 5%–15% of stones and are composed of magnesium ammonium phosphate. In contrast, uric acid stones are unique in that they can often be dissolved with urinary alkalinization; they account for 5%–10% of stones and occur in acidic urine (pH <5.8). Other stones, including cystine, xanthine, and protein matrix stones, as well as drug (eg, triamterene, indinavir)–induced calculi, account for less than 5% of stones (Moe OW, 2006*;* Sandhu C et al., 2003). Major types of stones according to the chemical components are calcium stones (70-80%), Uric Acid stone (5-10%), Cysteine stone (1%), Struvite (1%), xanthine stone (1%), Mixed stones (50-60%) while major subtypes are calcium oxalate monohydrate (40-60%), calcium oxalate dehydrate (40-60%), calcium hydrogen phosphate(brushite) (2-4%), calcium orthophosphate (<1%), mixed calcium oxalate-phosphate (35-40%) and mixed uric acid- calcium oxalate (5%).(Barnela *et al.*, 2012).

1.3. CAUSATIVE FACTORS OF UROLITHIASIS

Urolithiasis often has no definite, single cause, although several factors may increase the risk. It may be linked to environmental, genetical and dietary factors etc. Kidney stones form when urine contains more crystal-forming substances — such as calcium, oxalate and uric acid — than the fluid in urine can dilute. At the same time, urine may lack substances that prevent crystals from sticking together, creating an ideal environment for kidney stones to form. Factors that increase risk of developing kidney stones include:

(i). **Genetic factors and candidate genes for Urolithiais:** Genetic factors are known to play a major role in urolithiasis. Studies have tried to identify genes related to ureter calculi in an effort to clarify the cause of urolithiasis and to advance the diagnosis and treatment of urolithiasis (Danpure CJ., 2000; McGeown MG., 1968). It has been reported that the use of single-nucleotide polymorphisms (SNPs) associated with genetic diseases has been fruitful in identifying candidate disease genes. Recent genetic advances in urolithiasis indicate the potential of a new approach towards the gene polymorphism (Resnick M *et al.*, 1968; Goodman HO *et al*., 1995; Chen WC *et al.*, 2001a; Chen WC *et al*., 2001b) Moreover, polymorphism in manganese superoxide dismutase gene (Mn-SOD) is a new approach to identify its probable association with urolithiasis through oxidative stress. MnSOD is one of the primary enzymes that directly scavenge poten-tial harmful oxidizing species. It has been reported that A valine (Val) to alanine (Ala) substitution at amino acid 16, occurring in the mito-chondrial targeting sequence of the MnSOD gene, has been associated with an increase in urolithiasis risk (Tugcu *et al.*, 2007). Moreover, number of studies has been carried out by many scientists in many parts of the world to identify the probable candidate genes responsible for urolithiasis. Some of the results as reported from the studies done by scientists are shown below:

Candidate Genes	Type of patients	References
TaqI and ApaI gene polymorphism	Calcium stone patients	Nishijima *et al*.2002
BsmI endonuclease polymorphism	Calcium oxalate stone patients	Wen-Chi Chen *et al*.2001
VEGF gene BstUI polymorphism	Calcium oxalate stone patients	Chen *et al*.2001a
FokI and TaqI VDR genes polymorphism	Calcium oxalate stone patients	Mittal *et al*.2010

Numerous studies have been dedicated to interpreting the possible association between the polymorphisms of genes and urolithiasis susceptibility. However, the results were remained in-conclusive. The controversial results across many of these studies could possibly be related to the small sample size from an individual study, ethnic difference or the biological genetic model applied for the analysis. Therefore, it was necessary to quantify the potential between-study heterogeneity and summarize results from all eligible studies with rigorous methods. In view of this, very recently, Yiwei Lin *et al*.2011 carried out a meta-analysis with the most updated data in order to revisit the association between VDR (vitamin-D receptor) variants (i.e., *Apa*I, *Bsm*I, *Fok*I and *Taq*I) and urolithiasis risk and reported that some VDR gene polymorphisms are associated with an increase in the probability of urolithiasis with certain populations under an indicated genetic model. Considering the

predictive value, this meta-analysis study also warrants further investigation in this field to better clarify these SNP-urolithiasis associations and to rein-force their findings.

(ii). Family or personal history: There is a higher risk of developing kidney stones if a member of the family already has it.

(iii) Dehydration: Not drinking enough water each day can increase risk of kidney stones. And it has been reported that people who live in warm climates and those who sweat a lot may be at higher risk than others.

(iv) Certain diets: Eating a diet that's high in protein, sodium and sugar content may increase risk of some types of kidney stones. This is especially true with a high-sodium diet. Too much sodium in the diet increases the amount of calcium in the kidneys significantly increases risk of kidney stones.

(v) Being obese: It has been reported that High body mass index (BMI), large waist size and weight gain have been linked to an increased risk of kidney stones.

(vi) Digestive diseases and surgery: Gastric bypass surgery, inflammatory bowel disease or chronic diarrhea can cause changes in the digestive process that affect absorption of calcium and water and thus increasing the levels of stone-forming substances in urine.

(**vii) Other medical conditions.** Diseases and conditions that may increase risk of kidney stones include renal tubular acidosis, cystinuria, hyperparathyroidism, certain medications and some urinary tract infections.

1.4. INFLUENCE/ROLE OF DIET IN UROLITHIASIS

Urolithiasis as a multifactorial recurrent disease has various etiological and risk factors. It includes both intrinsic factors such as demographic, anatomic and genetic disposition, and extrinsic factors such as geographic predilection, climatic conditon, lifestyle predilection as well as dietary habits. Understanding how the stone forms *in vivo* will herald means to prevent its development. Dietary factors play an important role in kidney stone

formation, and dietary modification can reduce the risk of stone recurrence. Super saturation of urinary lithogenic promoters such as calcium, oxalate, phosphate and uric acid are considered as the risk factors of renal stone formation. On the other hand, a marked decrease in the urinary concentration of stone inhibitors such as citrate, potassium and magnesium is also a critical risk. And the urinary levels of these stone modulators are greatly influenced by diet (Siener *et al.,* 2003; Siener *et al.,* 2005).

Though only 10% to 20% of urinary oxalates come from dietary sources (Morton, Iliescu & Wilson., 2002), dietary reduction is commonly advised for calcium oxalate stone formers. It has been suggested that because there is much less oxalate in the urine than calcium in the urine, urinary oxalate concentration is much more critical to the formation of calcium oxalate crystals than is the urinary calcium concentration; reducing urine oxalates may have a more powerful effect on stone formation than can reduction of urine calcium (Morton *et al.,* 2002). Patients with calcium oxalate stones, particularly those with documented hyperoxaluria, should avoid foods high in oxalates. Vitamin C is a precursor to endogenous production of oxalates, so some clinicians recommend avoiding mega-doses of vitamin C. The rare genetic condition of primary hyperoxaluria is only slightly impacted by dietary reduction, and causes serious medical problems besides kidney stones. The effect of excess animal protein (purine) is also most obvious for the uric acid stone former. Uric acid, a byproduct of purine metabolism, is excreted in large quantities in the urine. Excess protein consumption creates urine leading potentially super saturation of uric acid, and a low pH, a necessary condition for formation of uric acid stones. There is no inhibitor of uric acid crystal formation (Menon & Resnick., 2002), so dietary measures focus on reducing uric acid and increasing urine volume. In this regard, reduction of animal protein to 350 gram per day for adults is recommended. This is enough to meet the dietary needs of most population in India, many of whom typically consume several more grams of animal protein daily than the

recommended level. Protein from plant sources (beans, legumes, etc.) can be substituted as a dietary alternative without negative consequences however calcium oxalate stone formers reducing their animal protein should also take into consideration the oxalate content of substitute proteins. The role of excess protein in promoting calcium stone formation is equally important as high dietary protein is associated with increased urinary calcium. Thus, there is a link between meat consumption and both uric acid and calcium stone formation. In fact, vegetarians form stones at one-third the rate of those eating a mixed diet (Lemann J., 2002). Clearly, the benefits of protein restriction for stone formers are many. The eating habits of people can make them prone to suffer from higher risks of having cases of Urolithiasis. Studies have been done on many places where it is found that in places where the rate of Urolithiasis is high, the diet of the people are usually to be blamed.

Epidemiological studies and metabolic investigations of the chemical composition of urine have suggested that a number of nutrients may influence the formation of stones in the upper urinary tract. These stones, which are predominantly calcium oxalate in composition, are more common in affluent countries where there is relatively high consumption of protein and fat and low consumption of carbohydrate (Anderson DA., 1972). The availability of calcium for stone formation depends ultimately on the dietary intake, intestinal absorption, excretion in the faeces, transport across cell membranes from the extracellular to the intracellular components of the body fluids and the renal tubular reabsorption of calcium.

With regard to Urolithiasis, the lithogenic potential of dietary protein is believed to be through different mechanisms. It results from a combination of a higher renal load of lithogenic substances, and a tendency towards their increased precipitation in the kidneys. Animal proteins are rich in sulfur-containing amino acids such as cystine and methionine. Oxidation of sulfur to sulfate generates acid load that aggravates calcium mobilization from bones (Arnett TR., 2008). Further, calcium forms soluble complex with sulfate generated

from the oxidation of sulfur in proteins. The acid load increases calcium mobilization from the bones, and causes hypercalciuria and low urine citrate levels, which is considered the strongest complexing agent for calcium in urine (Sharma AP *et al.,* 2007). The acid load also implemented decreases renal tubular reabsorption and imposes an additional risk for negative calcium balance and osteopenia, since urinary calcium excretion raises further (*Robertson* WG *et al.,* 1982; Goldfarb S, 1988). In addition, chronic over consumption of animal protein may increase renal mass and thereby up-regulate calcitriol production (Hess B *et al.,* 1995). This contributes to hypercalciuria by down-regulating parathyroid hormone secretion with subsequent reduction in renal calcium reabsorption (Hess B *et al.,* 1995).

It has been also reported dietary sodium increases the risk of urolithiasis. Salt intake expands intravascular volume, which can increase urinary calcium level, likely by decreasing renal tubular calcium reabsorption. Increased in salt intake can also induced mild systemic metabolic acidosis, this can lower urinary citrate levels, and increases the risk of calcium precipitation in kidneys (Sakhaee K *et al*., 1993). Further, sodium intake is another significant dietary risk factor for kidney stone disease and hypercalciuria because urinary sodium excretion is directly correlated with urinary calcium excretion, such that increasing the excretion of one leads to an increase in excretion of the other. High sodium intake contributes to stone formation in several ways; first it increases the urinary calcium level by reducing renal tubular reabsorption of calcium. Second, high sodium intake can cause a mild reduction in urinary citrate level by provoking mild bicarbonaturia and metabolic acidosis. Third it can increase urinary saturation of monosodium urate, causing urate induced calcium oxalate crystallization (Breslau NA *et al.,* 1982).

On the other hand, potassium-rich foods decreases stone formation through a decrease in urinary calcium excretion & high urinary potassium is believed to increase renal tubular phosphate absorption and consequently inhibit 1, 25- dihydroxyvitamin synthesis which

slows intestinal calcium absorption(Osorio AV *et al.,* 1997). Potassium rich foods offer the additional advantage of high citrate content thus decreasing the precipitation of urinary calcium (Srivastava T *et al.,* 2005). There are studies which reported that urine specific gravity, which measures urine density function, may be a better indicator of urinary dilution and a stronger predictor of stone formation and linked to diet one's consumed (Chen Y *et al.,* 2001). It was also suggested that maintaining urine specific gravity below a certain level might reduces the occurrence of urinary stones, along with appropriate fluid intake. A diet high in animal proteins results in higher acid excretion and lower urinary pH compared with a vegetarian diet and the formation of calcium phosphate crystals is highly dependent on the urinary pH (Robertson WG *et al*., 1975), where as the formation of calcium oxalate in a solution seems to be independent of variations in pH within the physiological range.

Correlation of the dietary pattern with the incidence of kidney stone disease in the Indian subcontinent had revealed that kidney stone occurred more frequently in the areas where the staple diet has been wheat than among the rice eaters (Teotia M *et al.,* 1976).Whole-wheat flour when consumed as a staple food leads to the production of urine supersaturated with uric acid, which appears to be an essential pre-requisite for the formation of primary stone. In addition to this increased animal-protein intake increases the excretion of uric acid and calcium and lowers urinary citrate excretion, all of which predispose a person to the formation of calcium stones (Gary C Curhan *et al.,* 1993). Increased uric acid saturation may favour the nucleation and growth of calcium oxalate and / or ammonium acid urate by blocking the action of acid mucopolysaccharide inhibitors. Another possibility could be that whole wheat flour when eaten as a staple food for a long period may lead to the production of some unknown chemical factor that inhibits the reabsorption of uric acid in the proximal renal tubule and thus causes increased urinary concentration of uric acid. Whether or not such a mechanism is genetically determined requires further studies (Teotia M *et al.,* 1976).

Dietary protein intake also increases net fixed acid production and acid excretion, thus inhibiting renal tubular reabsorption of calcium and imposes an additional risk for negative calcium balance and osteopenia, since urinary calcium excretion rises further (Lemann J *et al.,* 1986).

It has also been reported that vegetarian diets have been associated with increase excretion of calcium, oxalate and uric acid and a significant increase in oxalate excretion. Dietary intake of oxalate also varies among individuals based on food choices and it is especially high in vegetarians because green leafy vegetables contain large amounts of oxalate. On the other hand Ca restriction increases the absorption of oxalate in the gastrointestinal tract leading to an increase of urinary oxalate excretion suggesting that the inverse relation between dietary calcium and kidney stones may be due to increased binding of oxalate by calcium in the gastrointestinal tract. In this case, urinary oxalate may be more important than urinary calcium for stone formation, because calcium oxalate saturation of urine increases rapidly with small increases in the oxalate concentration. In this aspect, calcium restriction could actually be harmful in that it may lead to increased urinary oxalate excretion (Borsatti A, 1991).

Increase intake of Mg is favorable as it decreases calcium absorption and increases magnesium absorption which as an inhibitor reduces risk factors of the disease. Mg is a divalent cation is a complexing agent for oxalate. Magnesium inhibits oxalate absorption and excretion thus prevents its supersaturation. Normally magnesium is complexed with calcium as well as oxalate and decreases its excretion. Thus decreased magnesium in nephrolithiasis results in increased urinary oxalate level, as sufficient magnesium is not available to form the magnesium oxalate complex (Schwille PO *et al.,* 1999).Intake of increased phosphorus has an effect it decreases calcitriol production and enhance urinary excretion of a natural inhibitor of oxalate precipitation, pyrophosphate. Phosphorus has also been shown to partially protect

the kidney against calcium-induced damage. Phosphorus acts as a urinary acidifier and helps prevent stones from forming in the kidney (Tieder M *et al.,* 1985).

Majority of urinary stones contain oxalate and it seems logical to restrict dietary oxalate to lessen the stone risk. Dietary oxalate contributes to 10- 20 % (more recent studies suggest up to 80 %) of urinary oxalate and the rest comes from body metabolism. Reduction in dietary oxalate is a standard recommendation to individuals with calcium oxalate stone disease. Diets rich in oxalate are spinach, rhubarb, beetroot, beet, cocoa, chocolates, coffee and tea, nuts (pea nut, almond, and cashew), asparagus, cumin seed, cranberry, raspberry, dried beans, bran flakes, wheat bran, and strawberries. Other high-oxalate foods are grits, bran cereals, berries, figs, citrus peels, kiwis, tangerines, green leafy vegetables, okra, olives, beans, parsley, zucchini, potatoes and sweet potatoes, peppers, eggplant, black pepper, marmalade and soy sauce. Beverages with oxalate include coffee, chocolate milk and hot chocolate, dark beers, black tea, soy drinks and juices made out of high-oxalate fruits.

There was also report of some dissolved chemicals present in the drinking water could be an etiological factor for this disease. Siener in 2006 reported that for a majority of male kidney stone patients (88.46 per cent), the source of water was hand pump (groundwater). Only 2.56 per cent and 8.98 per cent of males were using water from tap (surface water) and *babdi* (stagnant water), respectively. In case of females kidney stone patients, 61.54 per cent were using water from hand pump while 28.85 per cent were using tap water. 9.61 per cent were still dependent on *babdi* for drinking water. Different rocks contain different minerals and groundwater is always in contact with these rocks, for example, sandstone, limestone and basalt and minerals. As a result, groundwater often contains more dissolved minerals than surface water. Consumption of groundwater may be one of the reasons of stone formation in patients (Siener R., 2006).

1.5. OXIDATIVE STRESS AND UROLITHIASIS

Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them". In other words, Oxidative stress is the general phenomenon of oxidant exposure and antioxidant depletion or oxidant-antioxidant balance. It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. During, normal oxidation process in the body, oxygen combines with reduced molecules, such as carbohydrates or fats, and provides energy and when there is decreased oxidation or decreased energy production, the cells can no longer function efficiently and disease results. However, this normal process propagates short- lived intermediates known as free radicals, and some free radicals escape and initiate further oxidation setting up a chain reaction. So, potentially harmful reactive oxygen species are produced as a consequence of biological metabolism, and by exposure to environmental factors. Free radicals are then usually removed or inactivated by a team of natural antioxidants which prevent these reactive species from causing excessive cellular damage.

Although reactive oxygen/nitrogen species (ROS/RNS) play an important role in immunemediated defense against invading microorganisms and serve as cell-signalling molecules, a high concentrations of ROS/RNS are capable of damaging host tissues and can modify or damage DNA, lipids, and proteins. In human body, ROS/RNS levels are controlled through an intricate network of endogenous and exogenous antioxidant molecules that are responsible for scavenging and consumption of specific reactive species. In this regard, intake of dietary antioxidants has received much attention, with the concept being that these molecules can affect disease by modulating the biological reactivity of free radicals.

In humans, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms (Peter H Proctor., 1989; Peter Proctor *et al*.,

1984). These include cancer (Halliwell B., 2007), Parkinson's disease, Alzheimer's disease (Valko M *et al*., 2007), atherosclerosis, heart failure (Singh N *et al*., 1995), myocardial infarction (Ramond A *et al*., 2011), Schizophrenia (Proctor P, 1972; Boskovic M *et al*., 2011) Bipolar disorder (Rodrigo MV *et al*., 2008; Dean OM *et al*., 2011), fragile X syndrome (Diego OY *et al*., 2009), Sickle Cell Disease (Amer J *et al*., 2006), lichen planus (Aly DG *et al*.,2010), vitiligo (Arican O *et al*., 2008), autism (James SJ *et al*., 2004), and chronic fatigue syndrome (Gwen Kennedy *et al*., 2005).

From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs. In addition to this, oxidative stress has not only a cytotoxic effect, but also plays an important role in the modulation of messengers that regulate essential cell membrane functions, which are vital for survival. It affects the intracellular redox status, leading to the activation of protein kinases, including a series of receptor and non-receptor tyrosine kinases, protein kinase C, and the MAP kinase cascade, and hence induces various cellular responses. These protein kinases play an important role in cellular responses such as activation, proliferation, and differentiation, as well as various other functions. Accordingly, the protein kinases have attracted the most attention in the investigation of the association between oxidative stress and disease. In addition to this, oxidative stress may also be involved in the development of stone formation in the renal system. Manganese superoxide dismutase (MnSOD) is one of the primary enzymes that directly scavenge potentially harmful oxidizing reactive oxygen species. It has been reported that a valine (Val) to alanine (Ala) substitution at the site of amino acid 16, in the mitochondrial targeting sequence of the MnSOD gene, has been associated with an increase in Urolithiasis risk (Tugcu *et al.*, 2007). For most human diseases, increased formation of reactive oxygen species is a secondary to primary disease process

(Halliwell B., 1991). Similarly association of Urolithiasis and free radicals has been reported (Selvam and Kalaiselvi, 2001). Experiments performed on animals (Muthukumar and Selvam., 1997), cultures (Thamilselvan *et al.*, 2003) and human sera (Singh and Barjatia, 2002) have revealed that there is an enhanced oxidative stress in stone forming conditions. Oxalate is known to induce lipid peroxidation by unknown mecha- nism which causes disruption of the structural integrity of the membranes (Thamilselvan *et al.*, 1997). The levels of serum malondialdehyde, nitrite, α-tocopherol, plasma ascorbate and erythrocyte superoxide dismutase are biomarkers for the pathogenesis of urolithiasis (Pillai and Pillai, 2002).

For most human diseases, oxidative stress characterized by increased formation of reactive oxygen species and a state of damage caused by reactive oxygen species (ROS) is considered secondary to primary disease process (Halliwell B., 1991). Similarly, an association of enhance oxidative stress and stone forming conditions have been reported both in animals (Selvam and kalaisel., 2001) and human studies(Singh PP and Barjatia., 2002). One of the consequences of this is manifested in the formation of lipid peroxides (LPO) in cell membranes, resulting in dysfunction of the same. There are studies in experimental animals which reported induction of lipid peroxidation and acute phase proinflammatory cytokines (IL-1, IL-6, and TNF-α) by oxalate and oxalate load which causes disruption of the structural integrity of the membranes (Thamilselvan S and Khan SR., 1997) and of which free radical plays a prominent role. Given the higher reactivity of the ROS, living things have been developed several efficient mechanisms that enable stabilization and disposal of them to mitigate their harmful effects. Among them are inbuilt antioxidant scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and small molecular antioxidants such as reduced glutathione (GSH).

1.6. FREE RADICALS

The role of free radical is gaining worldwide attention since so many physiological and pathophysiological phenomenons are related to redox status of the cell. A free radical is any atom or molecule that contains one or more unpaired electrons. The unpaired electron alters the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding nonradical (Halliwell and Gutteridge, 1989). Despite being essential for most form of life, the high content of O_2 in atmosphere means that oxidation reactions are common place in our environment. Although, our body uses O_2 and oxidation reactions to good effect for generating energy and killing invaders, unwanted side reaction are unavoidable. Therefore to support the aerobic metabolism, mechanisms had to evolve for the biological control of O_2. One such mechanism involves its complete reduction to water which produces free radical O^{2-} by 1 electron reduction of molecular oxygen as the first intermediate in this pathway (Darley-Usmar *et al.*, 1995). Free radicals are also molecules that contained an unpaired electron in its outer orbit and that can exist independently. Molecular oxygen is a diradical, containing 2 unpaired electrons with parallel spin configurations. Because electrons must have opposite spin to occupy the same orbit, electron added to molecular oxygen must be transferred one at a time during its reduction (Sen, 1995; Yu, 1994) resulting several highly reactive intermediates (Yu, 1994). The complete reduction of oxygen to H_2O requires 4 steps and the generation of several free radicals and H_2O_2. H_2O_2 is not free radical in itself because it contains no unpaired electrons. H_2O_2 is however considered as reactive oxygen species because of its ability to generate highly reactive hydroxyl free radicals through interactions with reactive

transition metals. The complete reduction of oxygen is summarized in the following equations:

$O_2 + e^- \rightarrow O_2^{-\cdot}$ Superoxide radical…….(i)

$O_2^{-\cdot} + H_2O \rightarrow HO_2^{\cdot} + OH^-$ Hydroperoxyl radical…….. (ii)

$HO_2^{\cdot} + e^- + H \rightarrow H_2O_2$ hydrogen peroxide……. (iii)

$H_2O_2 + e^- \rightarrow {\cdot}OH + OH^-$ hydroxyl radical…… (iv)

Each of these oxygen derived intermediates is considered highly reactive because its unstable electron configurations allow for the attraction of electrons from other molecules resulting another free radicals that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to the lipid peroxidation (Hochstein and Ernsster, 1963), DNA damage (Kasai *et al.*, 1986) and protein degradation (Griffith *et al.,* 1988) during oxidatively stressful events. Although all the intermediates are potentially reactive, the intermediates vary in their biological importance. The superoxide radical ($O_2^{-\cdot}$) is the most well known oxygen derive free radicals and unlike other free oxygen radicals can lead to the formation of other additional species (Harris, 1992). Hydrogen peroxide although not a free radicals by definitions is biologically important oxidant because of its ability to generate the hydroxyl radicals, which are extremely potent radicals (Aruoma and Halliwell, 1987). Further because of its nonionized and low charge state, H_20_2 is able to diffuse through hydrophobic membranes as seen with leakage of H_20_2 from mitochondria (Yu, 1994). The hydroxyl radicals are formed not only by the reduction of hydrogen peroxide but also through the interaction of superoxide with hydrogen peroxide and the reduced form of the metal ions i.e copper and zinc (Ross and Moldeus, 1991).The ability of hydroxyl radical to

remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems.

Free radicals are very unstable due to their high reactivity (Paolisso *et al.*, 1996; Halliwell and Gutteridge, 1989).Because of their nature, they have short life time and are difficult to measure and accurately determined in *vivo* as well as in biological materials such as plasma or other fluids (Granic, 2001; Sano *et al.*, 1998; Borcea *et al.*, 1999). In clinical states, their existence is determined by their influence on other molecules or antioxidant mechanisms which they cause (Borcea *et al.*, 1999). It is more reliable to measure the consequence of their action, mostly decreased level of antioxidant enzyme activity (Bambolkar and Sainani, 1995).

Free radicals formation occurs continuously in cells as a consequence of the both enzymatic and non enzymatic reactions. Enzymatic reactions which serve as sources of free radicals include those involved in the respiratory chain, phagocytosis, prostaglandin synthesis and in cytochrome P-450 system. Free radicals also arise in non enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. Some internally generated sources of free radicals are mitochondria, phagocytes, xanthene oxidase reaction involving iron and other transition metals, arachidonate pathways, exercise, inflammation. Some externally generated sources of free radicals are cigarette smoke, environmental pollutants, radiation, ultraviolet light, certain drugs, pesticides, aneaesthetic and industrial solvents and ozone (Langseth, 1996).

1.7. LIPID PEROXIDATION

Lipid peroxidation (LPO) is a complex process whereby polyunsaturated fatty acids (PUFAs) in the phospholipids of cellular membranes undergo reaction with oxygen to yield lipid hydroperoxides (LOOH). The reaction occurs through a free radical chain mechanism

initiated by the abstraction of a hydrogen atom from a PUFA by a reactive free radical, followed by a complex sequence of propagative reactions. The LOOH and conjugated dienes that are formed can decompose to form numerous other products including alkanals, alkenals, hydroxyalkenals, malondialdehyde (MDA) and volatile hydrocarbons (Halliwell B *et al*., 1989). LPO is often the first parameter to confirm the involvement of free radicals in cell damage as LPO is an extremely likely consequence if a reactive free radical is formed in a biological tissue where PUFAs are generally abundant and extremely demaging to cells. Moreover, a vast array of analytical techniques has been developed to measure lipid peroxidation, though not all of them are applicable to the situation in vivo (Cheeseman K, 1989).

For all assays it's important that artifactual changes in lipid peroxidation products are minimized both during and after sampling. Radicalscavenging antioxidants and metal-chelating agents are added to prevent the further formation of lipid hydroperoxides and the breakdown of existing lipid hydroperoxides. Enzymic reactions that may affect levels of products are inhibited by mixing the sample with acid or organic solvents. It is generally advisable to assay samples as quickly as possible after taking them, since a tendency to increased lipid peroxidation on storage has been reported (Young IS *et al*., 1991; Duthie GG *et al*., 1992). Conversely, lipid hydroperoxides can deteriorate on storage (Holley A *et al*., 1991).

The lipid peroxidation's reaction in biological membranes causes impairment of membrane functioning (Slater TF *et al.,* 1971; Comporti M, 1987), decreases fluibility, inactivation of membrane-bound receptors and enzymes and increases non-specific permeability to ions such as Ca^{2+}. Additionally, lipid hydroperoxides decompose upon exposure to iron or copper ions, simple chelates of these metal ions (e.g. with phosphate esters), haem, and some iron proteins, including haemoglobin and myoglobin. Products of

these complex decomposition reactions include hydrocarbon gases (such as ethane and pentane), radicals that can abstract further hydrogen atoms from fatty acid side chains and cytotoxic carbonyl molecules, of which the most harmful are the unsaturated aldehydes such as 4- hydroxy-2-trans-nonenal. Indeed, a major contributor to extracellular antioxidant defence in mammals is the existence in body fluids of proteins that bind copper ions (caeruloplasmin and albumin), iron ions (transferrin), haem (haemopexin) or haem proteins (haptoglobins) and stop them from accelerating lipid peroxidation and other free radical reactions (Kappus H, 1987; Gutteridge JMC *et al.,* 1988).

The measurement of putative "elevated end products of lipid peroxidation" in human samples is probably the evidence most frequently quoted in support of the involvement of free radical reactions in tissue damage by disease or toxins. Studies beginning in the 1950s provided good evidence that several halogenated hydrocarbons exert some, or all, of their toxic effects by stimulating lipid peroxidation *in vivo*. This early choice of halogenated hydrocarbons for study was both casual (in that it gave early emphasis to the important biological role of free radical reactions) but also unfortunate, since later studies have shown that most toxins stimulating oxidative damage to cells do not appear to act by accelerating the bulk peroxidation of cell membrane lipids (Gutteridge JMC *et al.,* 1988):

toxin → lipid peroxidation → cell damage

In the process of LPO, oxidation of lipids can be measured at different stages, including:

a. Losses of unsaturated fatty acids;

b. Measurement of primary peroxidation products and

c. Measurement of secondary carbonyls and hydrocarbon gases.

Between phases a, b and c it is possible detect carbon-and oxygen-centered radicals and identify these radicals by their ESR spectra (Halliwell B, 1989).

LPO, however, is often a late event, accompanying rather than causing final cell death. Indeed, cell and tissue destruction whether mediated by radicals or otherwise can often lead to more lipid peroxidation because antioxidants are diluted out and transition metal ions that can stimulate the peroxidation process are released from disrupted cells (Halliwell B, 1987). This stimulation of lipid peroxidation as a consequence of tissue injury can sometimes make a relevant contribution to worsening the injury. For example, in atherosclerosis there is good evidence that lipid peroxidation occurs within the atherosclerotic lesion and leads to foam cell generation and hence lesion growth (Halliwell B *et al*., 1984). In traumatic injury to the brain and spinal cord, good evidence again exists that iron ion release into the surrounding area, and consequent iron-stimulated free radical reactions, worsen the injury (Steinberg D *et al*., 1989).It is equally likely that in some other diseases, the increased rates of free radical reactions induced as a result of tissue injury make no significant contribution to the disease pathology. Each proposal that free radicals in general or lipid peroxidation in particular, are important contributors to the pathology of a given disease must be carefully evaluated on its merits. This obviously requires accurate methodology for measuring these processes in cells, tissues and whole organisms. It should be noted that the chemical composition of the end products of peroxidation will depend on the fatty acid composition of the lipid substrate used and upon what metal ions (if any) are present.

1.8. ANTIOXIDANT ENZYMES (SUPEROXIDE DISMUTASE AND CATALASE)

During the evolution, aerobic organisms have developed protection and defense mechanisms against oxidants and free radicals. The antioxidant systems include numerous enzymes and non enzyme type antioxidant groups that are located in the cell and extracellular fluid. It has been speculated that the susceptibility of an organisms to oxidative damage is influenced by the antioxidant defense system's

ability to cope with stress, which in turn can be influenced by the nutritional intervention with antioxidants (Chow, 1988). Inherent antioxidant defense system consisting of enzymes such as catalases and superoxide dismutase etc. and nutrients may participate in coping oxidative stress (Wills, 1985; Machlin and Bendich, 1987; Leibovitz *et al.*, 1990). As antioxidant enzymes have important role in protection against free radical damages, decrease in the activities or expression of these enzymes may predispose tissues to free radical damage (Hwang and Fwu, 1993; L'Abbe *et al.*, 1991).

Antioxidant enzymes dependent defences play an important role in scavenging free radicals produced under oxidative stress (Harris, 1992). In mammalian cells, there are several mechanisms in which organisms defend themselves against oxidative stress. Among them, there are small molecular antioxidants such as reduced glutathione(GSH) and antioxidant scavenging enzymes such as cellular Cu, Zn-superoxide dismutase (SOD), catalase (CAT), cellular glutathione peroixdase (GPx) and Glutathione-s-transferase (Kasapoglu and Ozen, 2001; Touyz, 2000). Sato *et al.*, (2002) postulated that SOD is the key enzyme in protecting the vessel wall against oxidative stress. Organisms, depending on the oxidative metabolism, have evolved number of enzymes to reduce O_2^- which is formed as an intermediate. SOD is one such enzyme which catalyzes the reaction of 0_2^- with an electron and 2 protons to form H_2O_2. Three mammalians SODs have so far been identified: copper- zinc SOD (SOD-1), MnSOD (SOD-2) and extracellular SOD (SOD-3).The three SODs gene have been characterized and cloned (Groner *et al.*, 1985; Wan *et al.*, 1994; Folz and Crapo, 1994). SOD is an enzyme that repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body. SOD catalyzes the destruction of the O^{2-} free radical.

$$2O^{2-} + 2H^{+} \rightarrow O_2 + H_2O_2$$

It protects oxygen-metabolizing cells against harmful effects of superoxide free-radicals (Petkau *et al*. 1975; Fridovich 1972, 1973; Lavelle *et al*. 1973; Paschen and Weser 1973). McCord in the year 1974 reported that SOD protects hyaluronate against depolymerization by free-radicals and indicated that exogenous SOD might have an anti-inflammatory effect (Salin and McCord 1975). The O^{2-} ion, which has been considered important in aging, lipid peroxidation and the peroxidative hemolysis of red blood cells is formed by the univalent reduction of O_2 during various enzymatic reactions or by ionizing radiation (Fee and Teitelbaum 1972, Fee *et al*. 1975). In addition to this, there is also superoxide radical formation during leukocyte phagocytosis indicating that SOD deficiency might lead to Heinz body hemolytic anemia (Allen *et al*., 1974; DeChatelet *et al*., 1974; Dionisi *et al*., 1975; Winterbourn *et al*., 1975).

SOD is also reported to be found in both the dermis and the epidermis and is the key to the production of healthy fibroblasts. Studies have shown that SOD acts as both an antioxidant and anti-inflammatory in the body, neutralizing the free radicals that can lead to wrinkles and precancerous cell changes. Researchers are currently studying the potential of SOD as an anti-aging treatment, since it is now known that SOD levels drop while free radical levels increase as we aged. There are two types of SOD: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD. Each type of SOD plays a different role in keeping cells healthy. Cu/Zn SOD protects the cytoplasm of the cell, and Mn SOD protects their mitochondria from free radical damage. It has been reported that abnormalities in the Cu-Zn SOD gene may contribute to the development of Amyotrophic Lateral Sclerosis (ALS), or Lou Gehrig's disease, in some people. ALS is a fatal disease that causes deterioration of motor nerve cells in the brain and spinal cord. It has been theorized that low levels of SOD in those with ALS leaves nerve cells unprotected from the free radicals that can kill them, so researchers have

been studying the effect of vitamin E and other antioxidant supplements on the progression of this disease. It was hoped that regular doses of antioxidants could make up for the lack of SOD and help neutralize free radicals. Initial studies were promising, and indicated that vitamin- E supplementation could potentially slow the progression of ALS, with some researchers claiming that the risk of death from ALS was as much as 62 percent lower in regular vitamin E users compared to nonusers.

CAT on other hand is important in antioxidant defense against hydrogen peroxide (Robertson *et al.,* 2003; Hansen *et al.,* 1999). The tretameric peroximal catalase converts H_2O_2 to water and molecular oxygen and in the presence of H^+ donors it facilitates the reduction of organic hydroperoxides. CAT gene expression is regulated by H_2O_2. In mammals H_2O_2 is detoxified by CAT and GPx. CAT protects the cells from H_2O_2 generated within them and plays an important role in inactivation of ROS and in adaptation to oxidative stress. The mechanism responsible for the down regulation of CAT in disease animals is uncertain. However, it may be the consequence of decreased Cu -Zn SOD and the reduction of H_2O_2 production which regulates CAT expression (Mates *et al.,* 1999).

Catalase (CAT) is considered a common enzyme found in nearly all living organisms exposed to oxygen and catalyzed the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen (Chelikani P *et al.,* 2004).

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

While the complete mechanism of CAT is not currently known, the reaction is believed to occur in two stages:

$$H_2O_2 + \text{Fe (III)-E} \rightarrow H_2O + \text{O=Fe (IV)-E (. +)}$$

$$H_2O_2 + \text{O=Fe (IV)-E (. +)} \rightarrow H_2O + \text{Fe (III)-E} + O_2$$ (Boon EM *et al.,* 2007)

Here Fe ()-E represents the iron center of the heme group attached to the enzyme. Fe (IV)-E (.+) is a mesomeric form of Fe(V)-E, meaning the iron is not completely oxidized to +V, but receives some "supporting electrons" from the heme ligand. This heme has to be drawn then as a radical cation (.+).

As hydrogen peroxide enters the active site, it interacts with the amino acids Asn147 (asparagine at position 147) and His74, causing a proton (hydrogen ion) to transfer between the oxygen atoms. The free oxygen atom coordinates, freeing the newly formed water molecule and Fe(IV)=O. Fe(IV)=O reacts with a second hydrogen peroxide molecule to reform Fe(III)-E and produce water and oxygen. The reactivity of the iron center may be improved by the presence of the phenolate ligand of Tyr357 in the fifth iron ligand, which can assist in the oxidation of the Fe (III) to Fe (IV). The efficiency of the reaction may also be improved by the interactions of His74 and Asn147 with reaction intermediates and the rate of the reaction can be determined by the Michaelis-Menten equation (Maass E, 1995).

CAT is also a very important enzyme in reproductive reactions as it has highest turnover numbers of all enzymes and can convert millions of molecules of H_2O_2 to water and oxygen each second (Goodsell DS, 2004). CAT is a tetramer of four polypeptide chains, each over 500 amino acids long (Boon EM *et al.,* 2007). It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for human CAT is approximately 7 and has a fairly broad maximum pH in the ranged between 6.8 and 7.5. (Maehly A *et al*., 1954; Aebi H, 1984) however, the pH optimum for other CAT varies between 4 and 11 depending on the species (Toner K *et al*., 2000). CAT can also catalyze the oxidation, by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It does so according to the following reaction:

$$H_2O_2 + H_2R \rightarrow 2H_2O + R$$

Any heavy metal ion (such as copper cations in copper (II) sulfate) can act as a non-competitive inhibitor of catalase. Also, the poison cyanide is a competitive inhibitor of CAT, strongly binding to the heme of catalase and stopping the enzyme's action. The true biological significance of CAT is not always straightforward to assess: Mice genetically engineered to lack catalase are phenotypically normal, indicating this enzyme is dispensable in animals under some conditions (Ho YS *et al.*, 2004). A catalase deficiency may increase the likelihood of developing type 2 diabetes (Laszlo Goth *et al.*, 2001; Laszlo Goth, 2008). Some humans have very low levels of catalase (acatalasia), yet show few ill effects.

1.9. ANTIOXIDANTS VITAMINS (VITAMIN-C AND VITAMIN-E)

Antioxidant is a substance that protects the biological tissue from damage by the free radicals and can be recycled or regenerated by biological reducers (Rosen *et al.*,2001).Vitamin C or L-ascorbic acid/or ascorbate is an essential antioxidant for humans and certain other animal species. Vitamin C refers to a number of vitamers that have vitamin C activity in animals, including ascorbic acid and its salts, and some oxidized forms of the molecule like dehydroascorbic acid. Ascorbate and ascorbic acid are both naturally present in the body when either of these is introduced into cells, since the forms interconvert according to pH. Vitamin C is a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy (FSA, 2007). In animals, these reactions are especially important in wound-healing and in preventing bleeding from capillaries. Ascorbate may also act as an antioxidant against oxidative stress (Padayatty SJ *et al.,* 2003). However, the fact that the enantiomer D-ascorbate has identical antioxidant activity to L-ascorbate, yet far less vitamin activity underscores the fact that most of the function of L-ascorbate as a vitamin relies not on its antioxidant properties, but upon enzymic reactions that are stereospecific (Aboul-Enein HY *et al.*, 2003).

The biological role of ascorbate is to act as a reducing agent, donating electrons to various enzymatic and a few non-enzymatic reactions. The one- and two-electron oxidized forms of vitamin C, semidehydroascorbic acid and dehydroascorbic acid, respectively, can be reduced in the body by glutathione and NADPH-dependent enzymatic mechanisms (Meister A, 1994; Michels A *et al*., 2012). The presence of glutathione in cells and extracellular fluids helps maintain ascorbate in a reduced state (Gropper SS *et al.,* 2005). The vast majority of animals and plants are able to synthesize vitamin C, through a sequence of enzyme-driven steps, which convert monosaccharides to vitamin C. In case of plants, this is accomplished through the conversion of mannose orgalactose to ascorbic acid while in case of some animals, glucose needed to produce ascorbate in the liver (in mammals andperching birds) is extracted from glycogen; ascorbate synthesis is a glycogenolysis-dependent process (Wheeler GL *et al*., 1998 Banhegyi G *et al*., 2001).

Vitamin-C is absorbed in the body by both active transport and simple diffusion. Na-dependent active transport—Na -Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs)—are the two transporters required for absorption. SVCT1 and SVCT2 import the reduced form of ascorbate across plasma membrane (Savini I *et al.,* 2008). GLUT1 and GLUT3 are the two glucose transporters, and transfer only dehydroascorbic acid form of Vitamin C (Rumsey SC *et al.,* 1997). Although dehydroascorbic acid is absorbed in higher rate than ascorbate, the amount of dehydroascorbic acid found in plasma and tissues under normal conditions is low, as cells rapidly reduce dehydroascorbic acid to ascorbate (May JM *et al.,* 2003; Packer L, 1997). Thus, SVCTs appear to be the predominant system for vitamin C transport in the body. SVCT2 is involved in vitamin C transport in almost every tissue, the notable exception being red blood cells, which lose SVCT proteins during maturation (May JM *et al.,* 2007). "SVCT2 knockout" animals genetically engineered to lack this functional gene, die

shortly after birth suggesting that SVCT2-mediated vitamin C transport is necessary for life (Sotiriou S *et al.*, 2002).

Although the body's maximal store of vitamin C is largely determined by the renal threshold for blood, there are many tissues that maintain vitamin C concentrations far higher than in blood. Biological tissues that accumulate over 100 times the level in blood plasma of vitamin C are the adrenal glands, pituitary, thymus, corpus luteum, and retina (Hedige MA, 2002). Those with 10 to 50 times the concentration present in blood plasma include the brain, spleen, lung, testicle, lymph nodes, liver, thyroid, small intestinal mucosa, leukocytes, pancreas, kidney and salivary glands. Ascorbic acid can be oxidized (broken down) in the human body by the enzyme L-ascorbate oxidase. Ascorbate that is not directly excreted in the urine as a result of body saturation or destroyed in other body metabolism is oxidized by this enzyme and removed.

On the other hand vitamin- E consists of two families of compounds namely the tocopherols and tocotrienols, characterised by a 6-chromanol ring and an isoprenoid side chain. The members of each family are designated alpha (α)-, beta (β)-, gamma (g)-, or delta (d)- according to the position of methyl groups attached to the chroman nucleus. Therefore, 8 stereoisomers of the large vitamin E family are possible but only the RRR-form occurs naturally. Tocopherols and tocotrienols are differentiated by their phenyl "tails" as these are saturated in the tocopherols but unsaturated in the tocotrienols (Combs, 1992). Unlike other antioxidant vitamins, a specific role for vitamin E in a required metabolic function has not been found. Major functions of vitamin-E appear to be as a non-specific chain-breaking antioxidant that prevents the propagation of free-radical reactions and especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and in plasma lipoproteins. It has been indicated that vitamin E functions primarily as an antioxidant in biological systems by trapping peroxyl free radicals (Combs, 1992; IOM, 2000). In this

regard, vitamin E is found in cellular membranes associated with PUFA in phospholipids. In the case of vitamin-E deficiency, the oxidation of PUFA is more readily propagated along the membrane, leading to cell damage and eventually symptoms, mainly neurological.

Vitamin-E is known to transport in the blood by the plasma lipoproteins and erythrocytes. It is absorbed with the fat component of food, "piggy-rides" on chylomicrons (formed in intestinal mucosal cells) through the lymphatic system and are finally released into the blood stream, though the efficiency of vitamin -E absorption is low in humans (IOM, 2000). In human, the primary human vitamin-E deficiency symptom is a peripheral neuropathy characterized by the degeneration of the large-caliber axons in the sensory neurons. Other symptoms observed in humans include spinocerebellar ataxia, skeletal myopathy, and pigmented retinopathy. Vitamin- E requirements have thus been reported to increase when intakes of polyunsaturated fatty acids (PUFAs) are increased. It has been suggested that a ratio of at least 0.4 mg (1 μmol) a-tocopherol per gram of PUFA should be consumed by adults. However, the method of determining the vitamin E requirement generated by PUFA intakes is not universally accepted. There are also data to suggest that low-density lipoprotein (LDL) oxidation susceptibility *in vitro* is dependent upon its PUFA content. Although it is clear that the relationship between dietary PUFA and vitamin E needs is not simple, high PUFA intakes should certainly be accompanied by increased vitamin E intakes.

Along inbuilt antioxidant enzymes, the synergestic effect of α–tocopherol and ascorbic acid has proved to be an efficient protector to the membrane integrity in response to the damaging peroxidative effect (Pillai CK and Pillai KS, 2002). A combined study relating peroxidative stress and antioxidant capacity in stone forming conditions in humans has not been cited yet. Therefore in light of the above concepts the present study was planned to quantitate the levels of serum malondialdehyde, nitrite, α-tocopherol, plasma ascorbate and

erythrocyte superoxide dismutase and also to investigate their possible bearings in pathogenesis of urolithiasis is very important. While oxidative stress is a well-known mechanism of action in the genesis of cell injury in different pathologies in the context of urolithiasis, most of the studies supporting this hypothesis are experimental, with few clinical data available (Broche F et al 1997).

1.10. STATUS OF UROLITHIASIS IN NORTHEAST INDIA:

The northeastern states of India, which border Burma (Myanmar) on one side and can be said to fall in the broad belt area of stone disease covering south-east, middle-east, north-east Asia and facing an acute problem of stone diseases. Due to lack of research facilities, the remote-ness, difficult geographical situations, the prevalence of urolithiasis is virtually unknown outside of these states. A preliminary survey from the laboratory highlighted the fact that urolithiasis is a major problem in these regions and required urgent attention. The incidence of urolithiasis is very high among the natives of these regions who are different in food habits, and also socially, culturally and ethnically from the rest part of India. (Singh PP, 1978). Most of the living population in these states has different food habits like rice as staple diet, high consumption of fermented fishes, soybeans, bam-boos and other types of indigenous food stuffs. Non-vegetarian foods are one of the major recipes in the daily menu of the most of the people living in these regions. But study of literatures revealed that there have been non-existent of data on the studies of etiologic chemical factors of urolithiasis found in the in the different vegetables and meat foodstuffs commonly available and consumed by the natives of these regions. Moreover, no publish literature have been reported on clinical studies from the patients of urolithiasis from these regions of India. In fact, the following key questions are still needed to consider as far as the prevalence of urolithiasis in the northeastern states of India is concern:

(a) Why the prevalence of urolithiasis is very high in the living population of north eastern states of India as compared to other parts of India and World? And is the epidemiology of urolithiasis endemic to these regions only?

(b) Is the different dietary habit of the natives of these regions playing any role for the high prevalence of urolithiasis?

(c) Is genetic factor playing a role in the pathogenesis of urolithiasis in the natives of these regions?

(d) Is different climatic condition from other parts of the country play a major role for the epidemiology of urolithiasis in these regions?

(e) What are the remedies and preventive measures which can be look out by the scientist and health professionals to contain this disease?

Among the North Eastern States of India, Mizoram State is facing an acute problem of stone diseases. It is commonly believed that every family has a member afflicted with this disease. But literature reports revealed that there have been non-existent of data or limited studies on the role elements in the genesis of urolithiasis and their determination in the various vegetables and meat foodstuffs commonly available and consumed by the natives of this region using sophisticated techniques. Moreover, no published data has been reported on the role of oxidative stress in the pathogenesis of urolithiasis in the living population of this region of India. The study of literature reveals that etiology of urolithiasis is multilateral and multifactorial resulting primarily from diet and imbalances free radicals and antioxidants or antioxidant enzymes activities. If such factors was identified properly and underlying physiological mechanism for the causes of urolithiasis in the living population is established, the increasing prevalence of urolithiasis can be checked. Therefore, the scope of the present study is evaluation of the etiologic factors and oxidative stress status related to high prevalence of urolithiasis in the urban natives of Mizoram taking into account both the

physical and biological parameters and their correlation with this ailment. In view of the conflicting data reported from other parts of the world on the role of diet in the pathogenesis of urolithiasis and no clinical data to date from this region prompted us to investigate further in-depth studies in this field. Accordingly the present research proposal entitled "Evaluation of etiologic chemical factors and oxidative stress status associated with prevalence of urolithiasis in the urban areas of Mizoram" has been selected as a research thesis for the Ph.D. degree of the Mizoram University, Aizawl. The results of the study are given in the following pages.

2. MATERIALS AND METHODS

2.1. WORKING PRINCIPLES OF ANALYTICAL INSTRUMENTS

The analytical instruments employed in the study and their working principles are given below.

2.1.1. Evolution 220 UV-Visible Spectrometer

In UV-Visible spectroscopy, a sample is probed with photons of wavelength in the range of ultraviolet-visible light and absorbance is measured relative to an appropriate control. UV-Vis has immense analytical applications because analytes in mixtures that exclusively absorb photon radiation at particular wavelengths can be quantified independently of other components and an unknown material can be characterized by comparing with known materials. Solutions of transition metal ions, organic compounds with a high degree of conjugation, or those with alternating single and multiple bonds, and charge transfer complexes can be analyzed by UV-Vis spectroscopy.

In its simplest form, a sample is placed between a light source and a photodetector, and the intensity of a beam of light is measured before and after passing through the sample. These measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a "blank" – a cuvette filled with only the dispersing medium – to guarantee that spectral features from the solvent are not included in the sample extinction spectrum. Evolution 220 is an excellent choice to perform quantitative and qualitative analyses of a wide range of sample types by fixed wavelength, scan, quant and kinetic measurements with the help of comprehensive quantitative analysis solutions.

2.1.2. Energy Dispersive X-ray Fluorescence (ED-XRF) Spectrometer:

Energy Dispersive X-ray Fluorescence technology (EDXRF) provides one of the simplest, most accurate and most economic analytical methods for the determination of the chemical composition of many types of materials.

It can be used for a wide range of elements, from sodium (11) to uranium (92), and provides detection limits at the sub-ppm level; it can also measure concentrations of up to 100% easily and simultaneously. The atoms in the sample material are excited by x-rays emitted from a x-ray tube or radioisotope. For increasing sensitivity the primary excitation radiation can be polarised by using specific targets between the x-ray tube and the sample (ED-P (Polarisation)-XRF).

All element specific x-ray fluorescence signals emitted by the atoms after the photoelectric ionisation are measured simultaneously in a fixed mounted semi-conductor detector or sealed gas-proportional counter. The radiation intensity of each element signal, which is proportional to the concentration of the element in the sample, is recalculated internally from a stored set of calibration curves and can be shown directly in concentration units.

Analysis by EDXRF involves use of ionizing radiation to excite the sample. This excitation ejects electrons from the atomic shells of the elements in the sample. When a given atom replaces the ejected electron, by taking another electron from an outer atomic shell, x-ray energies are emitted.

Since each element generates a specific energy level in this replacement process, these energies are known as characteristic x-rays. For example, if an electron in the K shell of a manganese atom is ejected, an energy of 5.894 kilo-electron volts (keV) is generated when an electron from that atom's L shell is moved to the K shell.

EDXRF spectrometers use a semiconductor material (an x-ray detector) to convert characteristic x-rays into electrical signals. The spectrometer's electronics digitize the signals produced by the detector, and send this information to a PC or internal electronics for display and analysis. The Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer makes use of the fact that the pulse height of the detector signal is proportional to the x-ray photon energy, which is correlated with the wavelength.

These x-rays can be detected and displayed as a spectrum of intensity against energy: the positions of the peaks identify which elements are present in the sample (qualitative analysis) and in counting and comparing the number of energies at the same energy level reaching the detector (peak heights), we can determine percentages and so identify how much of each element is present in the sample (quantitative analysis).

One limitation of the technique is that only a thin layer, less than 0.1mm, is actually analysed. This can sometimes give misleading results on corroded or plated metals unless the surface is cleaned.

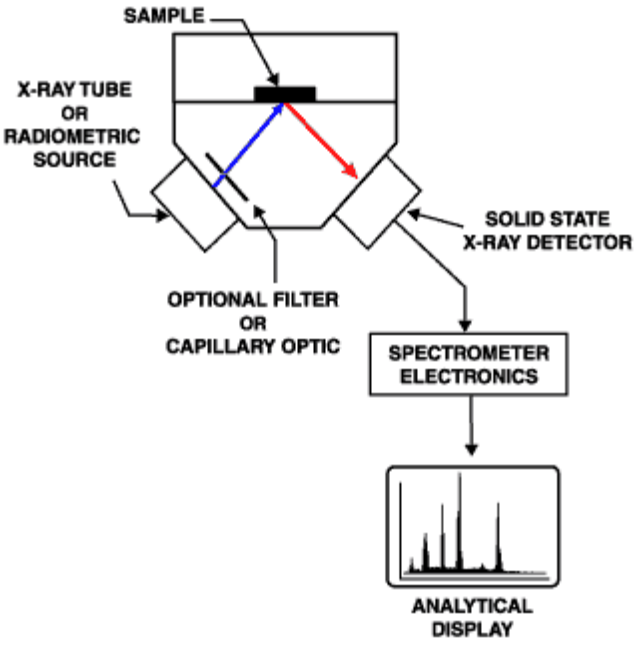

Figure: Working principle of ED-XRF

The ED-XRF analyzer also uses an x-ray source to excite the sample but it may be configured in one of two ways.

The first way is direct excitation, where the x-ray beam is pointed directly at the sample. Filter made of various elements may be placed between the source and sample to increase the excitation of the element of interest or reduce the background in the region of interest.

The second way uses a secondary target, where the source points at the target, the target element is excited and fluoresces, and then the target fluorescence is used to excite the sample. A detector is positioned to measure the fluorescent and scattered x-rays from the sample and a multichannel analyzer and software assigns each detector pulse an energy value thus producing a spectrum.

2.2. CHARACTERIZATION OF THE CHEMICAL COMPONENTS OF VEGETABLE FOODSTUFFS:

2.2.1. Selection and collection of vegetables foodstuffs

For the present investigations, the vegetable foodstuffs were selected on the basis of frequency of consumption by the native of urban population of Mizoram and availability in the markets after primary felid works and survey. After selection, twenty three (23) vegetables food samples were purchased or collected from the commercial settings such as Markets or grocery stores of urban areas of Mizoram. The sample collection was carried out in two phases to cover season based food items as some vegetables foodstuffs are seasonal. The first collections of vegetables food stuffs were carried out in the month of February and March while the second collection was conducted during September and October in a year.

2.2.2. List of vegetable foodstuffs samples collected

The scientific name, common and local name of the 23 vegetable foodstuffs were given below

Sl. No.	Local Name	Common Name	Scientific Name	Part of the Plant Used
1.	Phuihnam	East India Glory Blower	*Clerodendron colebrookianum*	Leaves
2.	Antam	Mustard Leaves	*Brassica juncea*	Whole plant
3.	Chingit	Indian pricky ash	*Zanthoxylum rhetsa L.*	Leaves
4.	Behlawi	Cowpea Leaves	*Vigna unguiculata L*	Leaves
5.	Iskut Hnah	Summer Squash Leaves	*Cucurbita pepo L*	Leaves
6.	Pelh	Padi Oats	*Gnetum gnemon L*	Leaves
7.	Maian	Pumpkin Leaves	*Cucurbita maxima*	Leaves
8.	Dawl	Elephant Leaves	*Colocasis esculenta L*	Leaves
9.	Tumbu	Plaintain	*Musa paradisiaca*	Fruit
10.	Mautuai	Bamboo Shoot	*Bambusa vulgaris*	Shoot
11.	Khanghu	Sha-byu	*Acacia gagaena*	Leaves
12.	Thingthupui	Rose Mahogany	*Dysoxylum gobara merr*	Leaves

13.	Tawkte	Thorn Apple/ African eggplant	*Solanum anguivi*	Fruits
14.	Hruizik	Rattan	*Calamus sp*	Stem
15.	Bekang	Soyabean	*Glycine max merr*	Seed
16.	Zawngtah	Tree Bean	*Parkia timoriana*	Fruit
17.	Zikhlum	Cabbage	*Brassica oleracea*	Leaves
18.	Anhling	Black Nightshade	*Solanum nigrum linn*	Whole plant
19.	Parbawr	Cauliflower	*Brassica oleracea*	Flower
20.	Bawkbawn	Brinjal/Eggplant	*Solanum melongena*	Fruit
21.	Samtawk		*Solanum incanum*	Fruit
22.	Ankasa	Toothache plant	*Acmella oleracea/ Spilanthes acmella*	Leaves and Flower
23.	Saptheihnah	Passion fruit leaves	*Passiflora edulis L*	Leaves

2.2.3. Preparation of vegetable foodstuffs samples for analysis

First, the plant parts of interests were destalked/ or peeled, cut and thoroughly washed with triple distilled water to eliminate contamination due to dust and environmental pollution, air-dried and then oven dried at 60^0C and stored in plastic bags until needed. After drying, they were ground into fine powder using mortar and pestle, and stored in a well labelled air tight container for analysis. The powdered samples were then formed into pellets (150mg each). The die used was 13mm and pressure of 300kg/cm^2 was used.

2.2.4. Analysis using ED-XRF Spectrometer

The elemental analysis of vegetables samples was carried out using a Xenematrix Ex-3600 Energy dispersive X-ray fluorescence (ED-XRF) spectrometer installed at UGC-DAE Consortium for Scientific Research, Kolkata Center, Kolkata, India, which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV). The measurements were carried out in vacuum using different filters (between the source and sample) for optimum detection of elements. For example, for P, S, Cl, K and Ca, no filter was used, and a voltage of 6 kV and current of 200 mA were used and samples were run for 200 sec. A 0.05-mm-thick Ti filter was used in front of the source for Mn, Fe, Cu and Zn, with an applied voltage of 14 kV and a current 900 mA and samples were run for 400 sec. For higher *Z* elements such as Se, Br, Rb and Sr Fe filter of 0.05 mm thickness was used at a voltage of 23 kV and 200 mA current and samples were run for 600 sec. The X-rays were detected using a liquid-nitrogen-cooled 12.5 mm^2 Si (Li) semiconductor detector (resolution 150 eV at 5.9KeV). The X-ray fluorescence spectra were quantitatively analyzed by the software next integrated with the system. This software uses the Fundamental Parameter Method approach, which combines a theoretical basis of X-ray emission and absorption with experimental measurements for unknown sample analyses. Here, all matrix corrections etc. are taken into account. The experimental results were subject to statistical analysis using Excel 2007 and SPSS package v.17.0. Values are present in the standard error of mean (SEM).

2.3. CHARACTERIZATION OF THE CHEMICAL COMPONENTS IN THE MEAT FOODSTUFFS

Since majority of people in the urban area of Mizoram are non-vegetarians, it is imperative to analyse the meat samples to determine their chemical content for any etiologic factors of causing urolithiasis.

2.3.1 List of meat foodstuffs samples collected

The scientific name, common and local name of three (3) meat foodstuffs samples collected were given below. They were either purchased or collected from the Central markets of urban area of Mizoram.

Sl. No.	Local Name	Common Name	Scientific Name
1.	Arsa	Chicken	*Gallus gallus domesticus*
2.	Bawngsa	Beef	*Bos taurus*
3.	Sangha	Rohu(Fish)	*Labeo rohita*

2.3.2 Preparation of meat foodstuff samples for analysis

Three meat foodstuffs samples mentioned *per se* were selected for analysis. They were purchased from the Central Markets of Aizawl, Mizoram. The samples were thoroughly washed with triple distilled water (TDW) to eliminate contamination due to dust and environmental pollution, air-dried and then oven dried at 60^0C and stored in plastic bags until needed. Drying takes about 3-4 days depending upon the meat foodstuffs. After drying, they were grounded into fine powder using mortar and pestle, and stored in a well labelled air tight container for analysis. The powdered samples were then formed into pellets (150mg each) by using a KBr press pelletizer. The die used was 13mm.

2.3.3 Analysis using ED-XRF Spectrometer

The elemental analysis of meat samples was carried out using a Xenematrix Ex-3600 Energy dispersive X-ray fluorescence ED-XRF spectrometer which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV). The measurements were carried out in vacuum using different filters (between the source and sample) for optimum detection of elements. For example, for P, S, Cl, K and Ca, no filter was used, and a voltage of 6 kV and

current of 200 mA were used and samples were run for 200 sec. A 0.05-mm-thick Ti filter was used in front of the source for Mn, Fe, Cu and Zn, with an applied voltage of 14 kV and a current 900 mA and samples were run for 400 sec. For higher *Z* elements such as Se, Br, Rb and Sr Fe filter of 0.05mm thickness was used at a voltage of 23 kV and 200 mA current and samples were run for 600 sec. The X-rays were detected using a liquid-nitrogen-cooled 12.5 mm^2 Si (Li) semiconductor detector (resolution 150 eV at 5.9KeV). The X-ray fluorescence spectra were quantitatively analyzed by the software next integrated with the system. This software uses the Fundamental Parameter Method approach, which combines a theoretical basis of X-ray emission and absorption with experimental measurements for unknown sample analyses. Here all matrix corrections etc. are taken into account. The experimental results were subject to statistical analysis using Excel 2007 and SPSS package v.17.0.

2.4. ASSESSMENT OF FLUORIDE CONTENT IN THE DRINKING WATER

Drinking water samples were collected from the different places of the urban areas of Mizoram in clean plastic bottles and labelled them properly like place and date of collection. The bottles wered kept secure to minimise contamination till used. For the analysis of these samples, SPADNS method was employed.

2.4.1 List of locality in Aizawl from where drinking water samples were collected:

The water samples were collected from the following places/area.

Sl. No.	Places/Area of Collection	Type of water
1	Bawngkawn	Spring
2	Republic (Lungli)	Spring
3	Ramhlun Venglai	Spring
4	Durtlang	Spring
5	Dawrpui vengthar	Spring
6	Zohnuai	Spring

7	Vaivakawn	Spring
8	Tuikual South	Spring
9	Hunthar	Spring
10	Edenthar	Spring
11	Saron Veng	Spring
12	Tuivamit	Spring
13	Chawlhhmun	Spring
14	Zotlang	Spring
15	Zotlang	Pump
16	Tuikual	Pump
17	Zonuam	Spring
18	Govt. Complex (Midumtui)	Spring
19	Chhangur kawn	Pump
20	Govt. Complex (Field tui)	Spring
21	Tuikual	Spring
22	Zotlang Tlangte	Spring
23	Republic (Khurpui)	Spring
24	Mizoram University (MZU)	
25	Govt. Water Supply (PHE)	
26	Rain water	

2.4.2 Preparation of water samples for analysis

The water samples were processed to remove any residual chlorine present by adding 1 drop (0.05ml) $NaAsO_2$ solution/ 0.1 mg residual chlorine and mix before the analysis.

2.4.3 Preparation of reagents:

i. Stock fluoride solution:

221 mg anhydrous sodium fluoride, NaF_2 was dissolved in distilled water and diluted to 1000ml.

ii. Standard fluoride solution:

100ml of stock fluoride solution was diluted to 1000ml with distilled water.

iii. SPADNS solution:

958 mg SPADNS was dissolved in distilled water and diluted to 500 ml. this solution was protected from light.

iv. Zirconyl acid reagent:

133 mg Zirconyl chloride octahydrate, $ZrOCl_2.8H_2O$ was dissolved in about 25 ml distilled water, 350 ml conc. HCL was added and the solution was diluted to 500 ml.

v. Mixed acid zirconyl-SPADNS reagent:

Equal volumes of SPADNS solution and zirconyl-acid reagent were mixed together.

vi. Reference solution:

10 ml SPADNS solution was added to 100 ml distilled water. 7 ml conc. HCL was diluted to 10 ml with distilled water and added to SPADNS solution. The instrument was set to zero with this solution.

vii. Sodium arsenite solution:

5g $NaAsO_2$ was dissolved and diluted to 1L with distilled water.

2.4.4. Analysis using SPADNS method:

i. Standard curve preparation**:**

The following volumes of standard fluoride solution were taken and dissolved to 50 ml with distilled water and the temperatures were noted down.

Standard solution, ml	F^-	0	0.1	0.2	0.5	1	2	3	5	7
µg F^-		0	1	2	5	10	20	30	50	70

ii. Prepare 10 ml of mixed acid-zirconyl-SPADNS reagent to each standard and mix well. Avoid contamination. Set photometer to zero absorbance with the reference solution and obtain absorbance readings of standards (at 570 nm). Plot a curve of µg F^- versus absorbance. Prepare a new standard curve whenever fresh reagent or a different standard temperature is used.

iii. Sample Pre-treatment: If the sample contains residual chlorine, remove it by adding 1 drop (0.05ml) $NaAsO_2$ solution/ 0.1 mg residual chlorine and mix.

iv. Colour development: Use a 50 ml sample or a portion made to diluted to 50 ml with distilled water. Adjust sample temperature to that of the standard curve. Set reference point of photometer as above. Add 10 ml acid-zirconyl-SPADNS reagent, mix well and read absorbance. If the absorbance falls beyond the range of the standard curve, repeat using a diluted sample.

Calculation:

$$\text{Fluoride, F (mg/L)} = \frac{A}{B} \times \frac{1}{R}$$

Where, A = µg F^- reading from the standard curve

B = volume of diluted or undiluted sample taken for colour development, ml.

R = Absorbance reading.

2.5. ELEMENTAL CHARACTERIZATION OF THE URINARY STONES OBTAINED FROM THE PATIENTS OF UROLITHIASIS

Renal stones were collected from the Urolithiasis patients admitted in the selected hospitals/ private clinics of urban area of Mizoram. This was carried out in collaboration with the Consultant Physicians and staffs of the concerned hospitals.

2.5.1 List of Hospitals from where urinary stone samples were collected

The stone samples were collected from the following Hospitals located in Aizawl.

Sl. No.	Name of Hospital	Locality/Place
1	Aizawl Civil Hospital	Dawrpui
2	Aizawl Hospital	Khatla
3	Bethesda Hospital	Bawngkawn
4	Aizawl Adventist Hospital	Vaivakawn
5	New Life Hospital	Chanmari

2.5.2 Preparation of urinary stone samples for analysis

The stone samples were collected from the patients admitted in different Hospitals of Aizawl mentioned *per se* after informed consent keeping in the mind that the admitted patients belong to the urban area of Mizoram. The collected stones were thoroughly washed with triple distilled water (TDW) to eliminate any contamination and waste materials, air dried and stored in plastic bags until needed. After drying, they were grounded into fine powder using mortar and pestle and then sieved, and stored in a well labelled air tight container for analysis. The powdered samples were then formed into pellets (150mg each). The die used was 13mm and pressure of 300kg/cm^2 was used.

2.5.3 Analysis using ED-XRF Spectrometer

The elemental analysis of stone samples was carried out using a Jordan Valley Ex-3600 Energy dispersive X-ray fluorescence ED-XRF spectrometer installed at University Grant Commission-DAE Consortium Center, Kolkata, India, which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV, current 1 mA). The measurements were carried out in vacuum using different filters (between the source and sample) for optimum detection of elements. For example, for Na, Si and Al, no filter was used, and a voltage of 8 kV and current of 85 mA were used. A 0.05-mm-thick Ti filter was used in front of the source for K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu and Zn, with an applied voltage of 20 kV and a current 400 mA. For higher *Z* elements such as Pb, Bi, Ag and As, an Fe filter of 0.05mm thickness was used at a voltage of 35 kV and 500 mA current. All measurements were carried out for 1200 s. The X-rays were detected using a liquid-nitrogen-cooled 12.5 mm2 Si (Li) semiconductor detector (resolution 150 eV at 5.9 KeV). The X-ray fluorescence spectra were quantitatively analyzed by the software ExWIN integrated with the system. This software uses the Fundamental Parameter Method approach, which combines a theoretical basis of X-ray emission and absorption with experimental measurements for unknown sample analyses. Here all matrix corrections etc are taken into account.

2.6. Assessment of Oxidative Stress Status in the healthy control and Urolithiasis patients:

The present objective was carried out in collaboration with Consultant Physicians of different Government and Private Hospitals of Aizawl District, Mizoram, which included a total of 90 subjects. These subjects were divided into two groups. Group I included control subjects and Group II included patients with urolithiasis (stone formers). The group II for this study included 50 stone forming patients having obstruction at the ureteropelvic junction and/or vesico-ureteric junction between the age group of 22 – 72 years irrespective of sex.

Out of these, 20 subjects were recurrent stone formers and the rest had first episode of stone formation. The presence of stone was diagnosed and confirmed by the urologist with the help of either ultrasonography, KUB, IVP or spiral CT report depending upon the size, shape, position, radiolucence and radiopacity of the stone. 40 age matched healthy subjects were selected as control. These subjects were selected after careful screening for any history of diseases which may lead to increased oxidative stress such as diabetes mellitus, cardiovascular diseases, infectious diseases, inflammatory diseases etc. and were strictly excluded. None of the subjects from Group I and Group II were on vitamin supplementation or used medications that could alter the study parameters.

5 ml of venous blood was collected in a plain bulb from group-I control and group-II patients subjects who were placed in reclining position for a minimum period of 10 minutes before sampling. EDTA vials were used to collect blood samples for estimation of plasma MDA (malonylaldehyde) as an index of lipid peroxidation, vitamin-E and vitamin-C. Remaining 1 ml blood was collected in a heparinized bulb for the assessment of erythrocyte SOD and CAT.

2.6.1. List of Hospitals from where blood samples were collected

The blood samples were collected from the following Hospitals located in Aizawl.

Sl. No.	Name of Hospital	Locality/Place
1	Aizawl Civil Hospital	Dawrpui
2	Aizawl Hospital	Khatla
3	Presbyterian Hospital	Durtlang
4	Aizawl Adventist Hospital	Vaivakawn
5	New Life Hospital	Chanmari

2.6.2. Preparation of Blood Plasma:

The collected blood samples were processed as soon as possible for the preparation of blood plasma. Blood plasma is the liquid component of blood, in which the blood cells are suspended. It makes up about 60% of total blood volume. It is composed of mostly water (90% by volume), and contains dissolved proteins, glucose, clotting factors, mineral ions, hormones and carbon dioxide (plasma being the main medium for excretory product transportation). Plasma is the supernatant fluid obtained when anti-coagulated blood has been centrifuged.

2.6.3. Materials and Equipment ·

a) Human blood samples.

b) Vacutainer tubes containing anticoagulant (e.g. Vacutainer plastic EDTA tube, 10 ml, lavender top) Serological pipettes of appropriate volumes (sterile)

c) Centrifuge tubes ·

2.6.4. Procedure

The blood samples were centrifuged for a minimum of 10 minutes at 1000-2000 RCF (generally 1500 RCF) at room temperature. This resulted three layers: (from top to bottom) plasma, leucocytes (buffy coat), erythrocytes. After carefully aspirated the supernatant (plasma) at room temperature, it was pooled in a centrifuge tube. The blood plasma was the aliquoted into adequately labelled cryovials and store at –20°C.

2.6.5. Assay of Lipid Peroxidation: (Beuge and Aust, 1978)

i. Reagents:

(i) 150Mm Tris HCL Buffer (pH 7.1): 1.8125g Tris –base was dissolved in 50ml DDW, pH was adjusted to 7.1 using HCl and volume was raised to 100ml.

(ii) 1.5mM ascorbic acid: 26.4 mg of accorbic acid was dissolved in 100ml DDW.

(iii) 1mM ferrous sulphate ($FeSO_4$): 27.802mg of $FeSO_4$ was dissolved in 100ml DDW.

(iv) 10% Trichloroacetic acid (TCA): 10 gm of TCA was dissolved in 100ml DDW.

(v) 0.375% Thiobarbutyric acid (TBA): 0.375gm of TBA was dissolved in 100ml DDW.

ii. Principle:

The method is based on the formation of red chromophore which absorbs at 532nm following the reaction of thiobarbutyric acid (TBA). The breakdown products of lipids peroxidized also called thiobarbutyric acid reactive substances (TBARS) releasing malonaldehyde (MDA).

iii. Procedure:

100µl tris-buffer was added to 200 µl blood plasma followed by 100 µl ascorbic acid and 100 µl ferros sulphate solution. The volume of reaction mixture was raised to 1.0 ml with DDW and mixed thoroughly. The above reaction mixture was incubated at 37ºC for 15 minutes followed by addition of 1.0 ml TCA and 2.0 ml TBA. It was mixed thoroughly and kept in boiling water bath for 15 minutes. Then, reaction mixture was centrifuged at 2000 rpm for 10 minutes and OD was taken at 532 nm.

iv. Calculations:

$$\frac{\text{O.D.} \times \text{Total volume of the reaction mixture}}{1.56 \times 10^3 \times \text{Volume of sample} \times \text{time of incubation}}$$

The results were expressed as n mol MDA /ml.

2.6.6. Assay of Superoxide Dismutase: (Nishikimi *et al.* 1972)

i. Reagents:

(i) 20 mM Tris-HCL buffer, pH 8.3 (0.1 mM EDTA): 0.0029225 gm EDTA was dissolved in 100 ml to make 0.1 mM EDTA solution. 0.24228 gm Tris-base was disolved in 50 ml 0.1 mM EDTA solution. pH was adjusted to 8.3 using HCl and volume as raised to 100 ml.

(ii) 2.34 mM NADH: 0.01659 gm of NADH was dissolved in 10 ml DDW.

(iii) 0.750 mM NBT: 0.006132 gm of NBT was dissolved in 10 ml DDW.

(iv) 0.0465 mM Phenazine methosulfate: 0.0001424 gm 0f phenazine methosulfate was dissolved in 10 ml DDW.

ii. Principle:

Superoxide dismutase is a family of metalloproteins that catalyze the dismutation of two molecules of O_2 to form H_2O_2 (Mc Cord and Fridovich, 1969)

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The reduction of nitro blue tetrazolium (NBT) with NADH mediated by phenazine methosulfate (PMS) under aerobic conditions initiated by addition of SOD. $NADH_2$ then is reduced to PMS, which on reoxidation with O_2 produces superoxide anion $O_2^{\cdot-}$. This $O_2^{\cdot-}$ anion reduce NBT forming blue formazon that can be measured at 560 nm.

iii. Procedure:

Non-enzymatic reaction mixture consisted of 20 mM Tris-HCl buffer, pH 8.3 (with 0.1 mM EDTA) in a volume of 3.0 ml, 0.2 ml NBT, 0.1 ml NADH and 0.05 ml phenazine methosulfate. Change in OD per minute was noted at 560 nm.

Enzymatic reaction mixture consisted of 2.6 ml buffer, 0.2 ml NBT, 0.1 ml NADH, 0.05 ml blood sample. Reaction was initiated by addition of 0.05 ml phenazine methosulfate. Change in OD per minute was noted at 560 nm.

iv. Calculation:

Enzyme activity was calculated by using the millimolar extinction coefficient of H_20_2 which is at 240nm is 0.071. The results were expressed as **Unit/mg Hb**.

2.6.7. Assay of Catalase: (Aebi, 1983)

i. Reagents:

(i) Phosphate buffer (50 mM, pH 7.0): 2.89 gm of di-Sodium hydrogen phosphate and 2.12 gm of Sodium di-Hydrogen phosphate were dissolved in 50 ml DDW. pH was fixed to 7.0 with NaOH and volume was raised to 100 ml with DDW.

(ii) 30 mM H_2O_2 in phosphate buffer: 0.34 ml 0f 30% H_2O_2 was diluted with phosphate buffer to 100 ml. The optical density of diluted H_2O_2 at 240 nm should be 1.5. This solution was prepared fresh each day.

ii. Principle:

Catalase is enzyme that catalyzes the breakdown of H_2O_2.

$$2\ H_2O_2 \xrightarrow{Catalase} 2\ H_2O + O_2$$

The rate of decomposition of H_2O_2 by catalase is measured spectrophotometrically at 240 nm, since H_2O_2 absorbs light at this wavelength.

iii. Procedure:

To 2 ml of phosphate buffer, 20 µl of blood sample was added. The reaction was started by the addition of 1 ml of 30 mM H_2O_2. Initial absorbance should be 0.500 (approx). The decrease in absorbance was followed at 240 nm for one minute after 10 seconds each.

iv. Calculation:

Enzyme activity was calculated by using the millimolar extinction coefficient of H_20_2 which is at 240nm is 0.071. The results were expressed as **µmol/l**.

2.6.8. Assay of Protein Content: (Lowry *et. al.*, 1951)

i. Reagents:

(i) Reagent A (1% Copper sulphate): 1 gm $CuSO_4$ in 100ml DDW.

(ii) Reagent B (2% Sodium potassium tartarate): 2 gm Sodium potassium tartarate in 100 ml DDW.

(iii) Reagent C (2% anhydrous sodium carbonate in 0.1 N NaOH): 2 gm of Sodium carbonate and 0.4 gm of Sodium Hydroxide in 100 ml DDW.

(iv) Lowry's Reagent: Mix reagents A,B and C in the ratio of 1:1:98. It was prepared just before use.

(v) Folin-Ciocalteau Reagent: Prepared fresh before use by diluting the commercial 2N Folin's Reagent with DDW (1:1 V/V)

(vi) Standard protein solution: 20 mg BSA (bovine serum albumin) dissolved in 100ml DDW carefully to avoid denaturation and frothing and sored at 4^{o}C.

ii. Principle:

This method is based on the colour of the reactions of amino acids and tryptophan and tyrosine with the Folin's phenol reagent. By the reaction of these amino acids with phosphomolybdic acid and phosphotungstic acid (present in Folin's reagent), a blue colour is formed which is calometrically estimated. The colour is the result of reduction of phosphomolybdic acid and phosphotungstic acid and Birue reaction of proteins with Cu^{++} ions in alkaline medium.

iii. Procedure:

50 µl of blood sample was taken and volume was made to 1 ml with DDW. For blank, i ml of distilled water was taken. To all these, 3 ml of Lowry's reagent was added, vortexed and allowed to stand for 10 minutes at room temperature. 0.3 ml Folin's reagent was added to each tube, vortexed and allowed to stand for 30 minutes. The OD was taken at 750 nm. A standard curve was also made by taking different concentration of BSA ranging from 10 to 100 µg/ml. Protein content was measured by taking standard curve as

$$\frac{OD\ of\ test - OD\ of\ blank}{OD\ of\ standard - OD\ of\ blank} \times \text{concentration of standard} \times \text{dilution factor.}$$

2.6.9. Assay of Vitamin-E: (Taylor *et al.*, 1976)

i. Reagents:

(i) Methanol

(ii) Alpha-tocopherol: (2 mg/ml was prepared in absolute ethanol). An aliquot was dried under incubation at temperature not more than 40^{o}C and was reconstructed in mobile phase (methanol: water= 96:4), working standard contained 2 mg/ml of alpha-tocopherol.

(iii) Ascorbic acid: (a) ascorbic acid- 300 mg; (b) ethanol- 10 ml ("a" is dissolved in "b" to form a solution).

(iv) Petroleum ether (Boiling point- 40^{o}C-60^{o}C)

(v) Ethanol (absolute

ii. Procedure:

1 ml of blood plasma was mixed with 1 ml of ethanol containing 3% ascorbic acid. To above solution 1 ml of petroleum ether was added. The solution was vortexed for 5 minutes and then kept in ice and salt mixture for 10 minutes. Ether layer was decanted and petroleum ether was added again and the process was repeated 2-3 times. Decanted layer was stored in an amber colored tube and dried at 40oC. The dried residue was diluted with 5 ml of methanol: chloroform in the ratio of 96:4. Optical density of blank, standard and test solutions was read by UVIKON spectrophotometer at 297 nm. Accordingly, concentration of vitamin-E in the solutions was calculated.

2.6.10. Assay of Vitamin-C: (Nino and Shah, 1986)

i. Principle:

For determination of plasma ascorbic acid by 2,4-dinitrophenyl hydrazine (Roe and Keuther, 1943., Roe, 1954., Henry, 1964). A slight modification was done as described by Nino and Shah (1986).

The chemical reaction is as follows:

Ascorbic acid + Copper sulphate → Dehydro ascorbic acid.

Dehydro ascorbic acid + DNPH → Bis 2,4 Dinitrophenyl hydrazone- ascorbate.

The ascorbic acid was converted to dehydro ascorbic acid by shaking with cupric sulphate solution and then coupled with 2,4- dinitrophenyl hydrazine in the presence of thiourea as a mild reducing agent. Sulphuric acid then converted dinitrophenyl hydrazone into a red colored compound, which was assayed calorimetrically. The thiourea is added to prevent oxidation of dinitrophenyl hydrazine reagent by interfering substances.

ii. Reagents:

(i) Meta phosphoric acid 6%

(ii) Sulphuric acid 4.5 molar

(iii) Sulphuric acid 12 molar

(iv) 2,4- dinitrophenyl hydrazine

(v) Thiourea 5% solution

(vi) Cupric sulphate 0.6% solution.

(vii) Dinitrophenyl hydrazine thiourea copper sulphate solution (DTCS): Five ml of thiourea and 5ml of copper sulphate add into 100 ml of DNPH solution, mix and store at 4C for a maximum period of 1 week. Standard solution 50 mg in 6% meta phosphoric acid, always prepared fresh. Intermediate standard solution 5mg . For working standard, took 0.5, 1, 2, 4, 6,10, 5.0 and 20.0 ml of IS made volume upto 25 ml by meta phosphoric acid giving final concentrations of 0.1, 0.4, 0.8, 1.2, 2.0, 3.0 and 4.0 mg per 100 ml.

iii. Procedure:

Take 2.0 ml of freshly prepared meta-phosphoric acid in test tube. Add 0.5 ml of sample or standard. Mix and centrifuge at 2500 rpm for 15 minutes. Filter and take supernatant for blank, sample and standard. Add 0.4 ml of DTCS in each and wait for 3 hrs at 37ºC. Add 2

ml of 12 molar sulphuric acid in each and read the absorbance at 520 nm against the reagent blank.

2.7. STATISTICS

The data collected after carrying out experiments were subjected to statistical analysis by employing 'Student's t-test' (Ipsen and Feigl, 1970) and Pearson's correlation coefficient.

2.7.1. Standard deviation (SD)

Each set of analytical results should be accompanied by an indication of the precision of the analysis. Various ways of indicating precision are acceptable. The standard deviation σ of an infinite set of experimental data is theoretically given by

$$\sigma = \sqrt{\frac{\Sigma(x_i - \mu)^2}{N}}$$

Where x_i represents the individual measurements and μ the mean of the infinite number of measurements (which should represent the true value). This equation holds strictly only as N $\rightarrow\infty$, where N is the number of measurements. In practice, we must calculate the individual deviations from the mean of a limited number of measurements, $\bar{x}$, in which it is anticipated that $\bar{x} \rightarrow \mu$, although we have no assurance this will be so; $\bar{x}$ is given by $\Sigma (x_i/N)$.

For a set of N measurements, it is possible to calculate N independently. But if the reference number chosen is the estimated mean, $\bar{x}$, the sum of the individual deviations must necessarily add up to zero, and so values of N-1 deviations are adequate to define the N^{th} value. That is, there are only N-1 independent deviations from the mean; when N-1 values have been selected, the last is predetermined. We have, in effect, used one degree of freedom of the data in calculating the mean, leaving N-1 degrees of freedom for calculating the precision.

As a result, the estimated standard deviation '**s'** of a finite set of experimental data (generally N< 30) more nearly approximates σ if the number of degrees of freedom is substituted for N (N-1 adjusts for the difference between $\bar{x}$ and μ).

$$s = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{N-1}}$$

The value of '**s**' is only an estimate of σ, then, and will more nearly approach σ as the number of measurements increases. Since we deal with small numbers of measurements in an analysis, the precision is necessarily represented by 's'.

The standard deviation may be calculated also using the following equivalent equation:

$$s = \sqrt{\frac{\Sigma x_i^2 - (\Sigma x_i)^2/2}{N-1}}$$

2.7.2. Standard error of mean (S.E.M.)

It is the measure of confidence with which statistical result is interpreted.

The precision of the mean of a series of N measurements is inversely proportional to the square root of N of the deviation of the individual values. Thus,

Standard deviation of the mean = $s_{mean} = \frac{s}{\sqrt{N}}$

The standard deviation of the mean is sometimes referred to as the standard error.

The standard deviation is sometimes expressed as the relative standard deviation (rsd), which is just the standard deviation expressed as a fraction of the mean; usually it is given as the percentage of the mean (% rsd), which is often called the coefficient of variation.

2.7.3. Student's t-test (t):

A *t*-test is any statistical hypothesis test in which the test statistic follows a Student's *t*-distribution if the null hypothesis is supported. It can be used to determine if two sets of data are significantly different from each other, and is most commonly applied when the test

statistic would follow a normal distribution if the value of a scaling term in the test statistic were known. When the scaling term is unknown and is replaced by an estimate based on the data, the test statistic (under certain conditions) follows a Student's *t* distribution.

$$t = \frac{(Mean1 - Mean2)}{\sqrt{\{(S.E.M_1)^2 + (S.E.M_2)^2\}}}$$

Degree of freedom (df) $= n_1 + n_2 - 2$

$Mean_1$ = Higher mean

$Mean_2$ = Lower mean

$S.E.M._1$ = Standard error of higher data

$S.E.M._2$ = Standard error of lower data

2.7.4. Significance

The‘t’ values were used to identify the significance by employing Ipsen and Fiegl (1970) formula for significant test. The probability ‘p’ for obtaining‘t’ value for a degree of freedom (df) was determined by comparing the ‘t’ values with the probability for a given degree of freedom. ‘p’ values were considered significant to the following convention:

$p > 0.05$ insignificant

$p < 0.05$ almost significant

$p < 0.01$ significant

$p < 0.001$ highly significant.

2.7.5. Pearson’s Correlation:

The Pearson correlation coefficient is a measure of the *linear* correlation between two variables *X* and *Y*, giving a value between +1 and −1 inclusive, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation. It is widely used in the sciences as a measure of the degree of linear dependence between two variables.

The Pearson correlation coefficient is given by

$$r = \sum \frac{(x_i - \bar{x})(y_i - \bar{y})}{n s_x s_y}$$

where r is the correlation coefficient, n is the number of observations, s_x is the standard deviation of x, s_y is the standard deviation of y, x_i and y_i are the individual values of the variables x and y, respectively, and $\bar{x}$ and $\bar{y}$ are their means.

The data collected were subjected for Pearson's Correlation using Microsoft Excel v. 10 as well as SPSS v.20

3. RESULTS

In the present investigation for the characterization of the etiologic chemical components of urolithiasis present in the vegetables and meat foodstuffs commonly consumed by natives of urban regions of Mizoram, various micro and macro elements were analyzed by using Energy Dispersive X-ray fluorescence (ED-XRF). Subsequently, chemical characterization of the urinary stones collected from the patients with urolithiasis is also carried out using ED-XRF. In ED-XRF spectrometers, all of the elements in the sample are excited simultaneously, and an energy dispersive detector in combination with a multi-channel analyzer is used to simultaneously collect the fluorescence radiation emitted from the sample and then separate the different energies of the characteristic radiation from each of the different sample elements.

Moreover, estimation of fluoride in drinking water samples collected from the various water sources of the urban region of Mizoram was also carried out using standard method. For the determination of the oxidative stress and antioxidant vitamins status in the patients with urolithiasis of the urban region of Mizoram, parameters like malonildialdehyde (MDA) level, activities of antioxidant enzymes namely SOD and CAT and antioxidant vitamins like vitamin-E and C were estimated in the blood samples collected from the patients with urolithiasis and compared with their healthy control counterpart.

3.1. ELEMENTS PRESENT IN THE VEGETABLE FOODSTUFFS

A total of twenty three (23) vegetables foodstuffs commonly available during summer and winter season in a year were collected from the central markets of Aizawl and analyzed for the major and minor elements present in them. The pictures of the selected vegetables are given in figure 2 (a, b and c). The following observations were recorded**.**

3.1.1. Major Elements Present in the vegetables foodstuffs

Five (5) major elements i.e Ca, K, Cl, P and S concentrations were recorded from the 23 vegetables foodstuffs samples were given below:

Calcium (Ca) (mg/Kg): The results of the present study reveals that all the vegetable samples were found to be containing notable amount of Ca. The mean concentration of Ca were estimated as 20536.7 ± 312.3mg/Kg, 14380.5 ± 148.0 mg/Kg, 5411.6 ± 233.3 mg/Kg, 8145.8 ±105.2 mg/Kg, 18914.2 ±289.6 mg/Kg, 3917.4 ± 95.6 mg/Kg, 34730.4 ±1443.9 mg/Kg, 7058.5 ± 246.0 mg/Kg, 5837.9±317.7 mg/Kg, 2483.2±59.1 mg/Kg, 4169.2±11.5 mg/Kg, 3338.3±106.5 mg/Kg, 3885.9±42.9 mg/Kg, 3221.4 ±139.5 mg/Kg, 6192.5±47.1 mg/Kg, 5637.5±86.4 mg/Kg, 16659.7±329.5 mg/Kg, 5088.8±171.2 mg/Kg, 3493.8±162.9 mg/Kg, 2608.9±195.1 mg/Kg, 12542.9±346.2 mg/Kg and 16635±516.5 mg/Kg in *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum Acmella oleracea/ Spilanthes acmella and Passiflora edulis L* respectively. The highest content of Ca was recorded in *Clerodendron colebrookianum* (20536.7 ± 312.3 mg/Kg) while *Acacia gagaena* contained lowest level of Ca (2483.2±59.1mg/Kg). However, in *Bambusa vulgaris* no Ca was detected (Table- 3.1.1).

Potassium (K) (mg/Kg): The results obtained from the present study revealed high and notable amount of K in all the vegetables food samples studied. The mean concentration of K were estimated as14360.5 ±74.7 mg/Kg, 22392.5 ±322.3 mg/Kg, 22143.4 ± 247.7 mg/Kg, 22614.6 ± 494.4 mg/Kg, 25057.9 ± 394.7 mg/Kg, 24150.5 ±433.2 mg/Kg, 21376.1 ± 441.0 mg/Kg, 42883.6 ± 2215.4 mg/Kg, 38785.6±983.8 mg/Kg, 34157.2±413.7 mg/Kg, 12549.1±111.1 mg/Kg, 23284.6±174.7 mg/Kg, 19124.1±519.1 mg/Kg, 26183.8±351.2

mg/Kg, 16007.2±226.5 mg/Kg, 8701.1±41.7 mg/Kg, 36540.5±201.4 mg/Kg, 42180.9±789.3 mg/Kg, 46151.5±326.8 mg/Kg, 38609.5±809.7 mg/Kg, 30730.6±1177.6 mg/Kg, 46224.4±690.6 mg/Kg and 24233±781.4 mg/Kg in *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and Passiflora edulis L* respectively. The highest and lowest concentration of K were recorded in *Colocasis esculenta L.* (42883.6 ± 2215.4 mg/Kg) and *Parkia timoriana* (8701.1±41.7 mg/Kg) respectively (Table- 3.1.1).

Chlorine (Cl) (mg/Kg): The results of the present study also revealed that the vegetables foodstuffs samples commonly consumed ny the natives of urban population of Mizoram contained appreciable amount of Cl. The mean concentration of Cl were estimated as 3363.3 ± 39.2 mg/Kg, 10410.2 ±405.5 mg/Kg, 6853.2 ±138.4 mg/Kg, 5194.3 ± 113.8 mg/Kg, 9648.5 ±408.4 mg/Kg, 5004.2 ± 258.6 mg/Kg, 9965.1 ± 254.5 mg/Kg, 20434.5 ± 2373.6 mg/Kg, 9264.3±701.6 mg/Kg, 17587.4±587.9 mg/Kg, 579±1.0 mg/Kg, 580.4±1.4 mg/Kg, 8098.6±488.5 mg/Kg, 20292.6±1090.6 mg/Kg, 579±0 mg/Kg and 579±0 mg/Kg in *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr and Parkia timoriana* respectively. The highest content of Cl was observed in *Colocasis esculenta L.* (20434.5 ± 2373.6 mg/Kg) while *Acacia gagaena, Glycine max merr and Parkia timoriana contained* lowest concentration of Cl (579±0 mg/Kg). No Cl content were recorded in the vegetables foodstuffs sample namely *Brassica*

oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and *Passiflora edulis L* respectively (Table- 3.1.1).

Phosphorus (P)(mg/Kg): The mean concentration of P recorded in the vegetable foodstuffs samples recorded in the present study were estimated as 3100.2 ±76.3 mg/Kg, 3599.4 ± 230.3 mg/Kg, 3048.1 ± 18.9 mg/Kg, 4140.7 ±167.5 mg/Kg, 3965.9 ±178.9 mg/Kg, 3967.6 ± 475.0 mg/Kg, 4755.1 ± 302.4 mg/Kg, 4444.6 ±123.6 mg/Kg, 4858.8±300.6 mg/Kg, 5010.7±183.1 mg/Kg, 7833.6±143.7 mg/Kg, 5141.7±164.5 mg/Kg, 3152.5±181.1 mg/Kg, 4764.6±262.9 mg/Kg, 8257.8±325.7 mg/Kg, 2027.4±19.1 mg/Kg, 9180.6±212.3 mg/Kg, 9816.7±125.1 mg/Kg, 13272.4±82.3 mg/Kg, 8288.3±127.6 mg/Kg, 75861.1±436.6 mg/Kg, 75861.1±436.6 mg/Kg, 12310.1±312.8 mg/Kg, and 7034.4±236.6 mg/Kg in *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and Passiflora edulis L* respectively. The highest and lowest concentration of P were recorded in *Brassica oleracea* (13272.4±82.3 mg/Kg) and *Parkia timoriana* (2027.4±19.1 mg/Kg) respectively (Table- 3.1.1).

Sulphur (S)(mg/Kg): In the present study, the results of the ED-XRF analysis reveals that S is also detected in the vegetables foodstuffs samples studied. The mean S concentration recorder were 3481.2 ±103.1 mg/Kg, 5625.0 ± 16.7 mg/Kg, 2626.0 ± 46.3 mg/Kg, 2383.5 ±35.3 mg/Kg, 2758.2 ±136.1 mg/Kg, 4189.2 ±113.8 mg/Kg, 2041.4 ±16.3 mg/Kg, 1705.5 ± 287.1 mg/Kg, 2944.8±143.8 mg/Kg, 1828.5±19.4 mg/Kg, 28913.9±976.3 mg/Kg, 11107.8±266.4 mg/Kg, 1750.4±121.6 mg/Kg, 1953.5±67.9 mg/Kg, 3556.3±83.9 mg/Kg, 31870.8±631.8 mg/Kg, 8884.7±196.1 mg/Kg, 7577.9±333.1 mg/Kg, 9889.9±62.7 mg/Kg, 3112.2±67.3 mg/Kg, 2538±127.7 mg/Kg, 5608.2±116.1 mg/Kg, and 6637.2±14.7 mg/Kg in

the *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and Passiflora edulis L* respectively. *Parkia timoriana* was found to be highest concentration of S(31870.8±631.8 mg/Kg) while the minimum concentration was recorded in *Solanum anguivi(*1750.4±121.6 mg/Kg) (Table- 3.1.1).

3.1.2. Minor elements recorded in the vegetable foodstuffs:

The minor elements namely Fe, Mn, Zn, Cu and Se were detected from the vegetable foodstuffs samples while analyzed using ED-XRF. The results of the minor element in mg/L present in the vegetables foodstuffs samples are as follows:

Iron (Fe)(mg/Kg): The present stydy revelas that all the vegetables foodstuffs generally contained high and notable amount of Fe. The mean Fe content were estimated as 246.6 ± 41.7 mg/Kg, 316.7 ± 15.7 mg/Kg, 161.9 ± 10.3 mg/Kg, 158.9 ± 10.47 mg/Kg, 272.9 ± 13.5 mg/Kg, 161.7 ± 16.8 mg/Kg, 821.5 ± 55.9 mg/Kg, 109.7 ± 7.4 mg/Kg, 64.75±9.61 mg/Kg, 53.95±4.49 mg/Kg, 237.67±20.14 mg/Kg, 92.70±4.49 mg/Kg, 74.2±0.92 mg/Kg, 65.51±4.68 mg/Kg, 149.40±29.94 mg/Kg, 54.00±1.6 mg/Kg, 181.1±77.6 mg/Kg, 577.2±110.6 mg/Kg, 1377.2±7.7 mg/Kg, 710.9±153.7 mg/Kg, 444.3±121.3 mg/Kg, 736.1±90.8 mg/Kg and 828.9±26.1 mg/Kg in the *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and Passiflora edulis L* respectively. *Passiflora edulis L (*828.9±26.1 mg/Kg*) was found to be*

contained highest Fe level while Parkia timoriana contained lowest concentration of Fe(54.00±1.6 mg/Kg)(Table- 3.1.2.)

Manganese (Mn)(mg/Kg): Interestingly, the present study revealed that all the vegetable foodstuffs samples found to be contained appreciable amount of Mn. The mean concentration of Mn were estimated as 96.3 ± 6.1 mg/Kg, 67.9 ± 2.9 mg/Kg, 285.9 ± 18.7 mg/Kg, 90.8 ± 2.42 mg/Kg, 24.0 ± 1.5 mg/Kg, 241.7 ± 4.1 mg/Kg, 26.3 ± 2.07 mg/Kg, 98.8 ± 10.4 mg/Kg, 292.38±12.36 mg/Kg, 28.87±1.59 mg/Kg, 78.00±3.25 mg/Kg, 104.50±2.98 mg/Kg, 32.52±2.39 mg/Kg, 288.57±6.32 mg/Kg, 35.89±0.43 mg/Kg, 15.65±1.32 mg/Kg, 17.1±1.2 mg/Kg, 89.4±6.5 mg/Kg, 58.6±4.3 mg/Kg, 10.9±2.1 mg/Kg, 14.9±1.4 mg/Kg, 140±3.9 mg/Kg, and 59.6±4.3 mg/Kg in the *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and Passiflora edulis L* respectively. The highest level of Mn was recorded in *Calamus sp* (288.57±6.32 mg/Kg) while *Solanum melongena* (10.9±2.1 mg/Kg) found to be contained lowest level of Mn(Table- 3.1.2.).

Zinc (Zn)(mg/Kg): The ED-XRF analysis of the vegetables foodstuffs samples reveals that an appreciable amount of Zn was recorded in all the samples studied. The mean concentration of the Zn were estimated as 35.7 ± 2.6 mg/Kg, 46.8 ± 4.2 mg/Kg, 28.4 ± 1.0 mg/Kg, 31.8 ± 0.50 mg/Kg, 36.0 ± 1.40 mg/Kg, 41.2 ± 1.05 mg/Kg, 40.8 ± 0.36 mg/Kg, 104.4 ±1.7 mg/Kg, 46.35±2.06 mg/Kg, 57.92±1.2 mg/Kg, 53.98±0.48 mg/Kg, 56.21±2.39 mg/Kg, 19.05±0.06 mg/Kg, 142.51±0.26 mg/Kg, 75.46±6.23 mg/Kg, 21.63±0.31 mg/Kg, 42.5±8.1 mg/Kg, 59.1±9.9 mg/Kg, 149.7±47.5 mg/Kg, 71.3±10.3 mg/Kg, 40.4±3.5 mg/Kg, 152.5±8.9 mg/Kg and 53.1±3.1 mg/Kg in the *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum*

rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and Passiflora edulis L respectively. The highest content of Zn was estimated in *Acmella oleracea/ Spilanthes acmella* (152.5±8.9 mg/Kg) while *Vigna unguiculata L.* (31.8 ± 0.50 mg/Kg) contained low level of Zn(Table- 3.1.2.).

Copper (Cu)(mg/Kg): The result of the present study indicated that an appreciable amount of Cu was recorded when analyzed by ED-XRF in all the vegetables samples studied. The mean content of the C were estimated as 14.5 ± 0.91 mg/Kg, 4.3 ± 0.37 mg/Kg, 13.4 ± 0.72 mg/Kg, 10.6 ± 1.12 mg/Kg, 5.4 ± 0.85 mg/Kg, 5.6 ± 0.69 mg/Kg, 4.9 ± 0.13 mg/Kg, 8.7 ± 0.82 mg/Kg, 10.95±0.64 mg/Kg, 9.35±0.7 mg/Kg, 7.44±1.00 mg/Kg, 18.59±0.92 mg/Kg, 14.02±1.08 mg/Kg, 27.07±0.61 mg/Kg, 20.11±0.57 mg/Kg, 8.16±0.45 mg/Kg, 11.8±1.2 mg/Kg, 11.3±0.1 mg/Kg, 21.5±7.4 mg/Kg, 42.8±6.4 mg/Kg, 29.1±2.1 mg/Kg, 10.2±0.7 mg/Kg and 13.4±2.5 mg/Kg in *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum Acmella oleracea/ Spilanthes acmella and Passiflora edulis L* respectively. In *Solanum incanum* the highest concentration of the Cu (29.1±2.1 mg/Kg) was recorded while *Brassica juncea (*4.3 ± 0.37 mg/Kg) found to be contained lowest level of Cu (Table- 3.1.2.).

Selenium (Se) (mg/Kg): In the present study, Se concentration was detected in low level as compared to pther trace element in all the vegetables food samples studied. The mean concentration of Se were estimated as 0.31 ± 0.19 mg/Kg, 0.15 ± 0.07 mg/Kg, 0.18 ± 0.05

mg/Kg, 0.85 ± 0.42 mg/Kg, 0.24 ± 0.004 mg/Kg, 0.12 ± 0.00 mg/Kg, 0.12 ± 0.00 mg/Kg, 0.12 ± 0.00, 0.12±0 mg/Kg, 0.12±0 mg/Kg, 0.48±0 mg/Kg, 0.85±0.53 mg/Kg, 0.12±0 mg/Kg, 0.12±0 mg/Kg, 0.17±0 mg/Kg; 1.74±0.72 mg/Kg, 0.21±0.03 mg/Kg, 0.23 mg/Kg, 0.14±0.05 mg/Kg, 0.23 mg/Kg, 0.23 mg/Kg, 13.9±0.1 mg/Kg and 0.7±0.3 mg/Kg in . *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella* and *Passiflora edulis L* respectively. In *Acmella oleracea/ Spilanthes acmella*, the highest concentration of Se (13.9±0.1 mg/Kg) was recorded (Table 3.1.2.)

3.1.3. Earth elements present in the vegetable foodstuffs:

In an interesting finding, three (3) earth elements namely Sr, Rb and Br were detected from the vegetable foodstuffs samples while analyzed by using ED-XRF and their concentrations in mg/L are given below.

Strontium(Sr)(mg/Kg): The results of the present study reveals that mean concentration of Sr in the vegetables foodstuffs samples commonly consumed by the urban population of Mizoram were estimated as 63.4 ± 1.4 mg/Kg, 121.5 ± 3.54 mg/Kg, 38.1 ± 2.76 mg/Kg, 50.8 ± 2.05 mg/Kg, 67.4 ± 4.00 mg/Kg, 9.5 ± 1.34 mg/Kg, 122.5 ± 3.70 mg/Kg, 18.5 ± 1.08 mg/Kg, 32.14±0.37 mg/Kg, 5.15±1.01 mg/Kg, 7.05±0.24 mg/Kg, 16.05±0.24 mg/Kg, 13.5±0.81 mg/Kg, 42.42±2.42 mg/Kg, 8.07±1.81 mg/Kg, 24.92±0.53 mg/Kg, 21.9±2.2 mg/Kg, 91.5±5.2 mg/Kg, 10.7±3.3 mg/Kg, 6.8±2.7 mg/Kg, 8.7±3.5 mg/Kg, 58.1±2.4 mg/Kg and51.2±2.1 mg/Kg in the *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum*

gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella and *Passiflora edulis L* respectively. The higest content of Sr was recorded in C*ucurbita maxima(*122.5 ± 3.70 mg/Kg) sample while the lowest concentration detected in the *Bambusa vulgaris(*5.15±1.01 mg/Kg) (Table 3.1.3).

Rubidium (Rb) (mg/Kg): The result of the present study reveals Rb was detected in appreciable amount analyzed by ED-XRF in all the vegetables foodstuffs samples studied. The mean concentration of Rb were estimated as 5.1 ± 0.43 mg/Kg, 35.4 ± 1.27 mg/Kg, 21.7 ± 1.72 mg/Kg, 24.2 ± 0.87 mg/Kg, 13.8 ± 0.28 mg/Kg, 13.8 ± 0.28 mg/Kg, 51.2 ± 1.05 mg/Kg, 13.5 ± 1.04 mg/Kg, 33.2 ± 1.35 mg/Kg, 25.00±1.54 mg/Kg, 44.33±1.48 mg/Kg, 10.07±1.09 mg/Kg, 45.34±2.38 mg/Kg, 8.68±1.84 mg/Kg, 155.25±6.8 mg/Kg, 22.27±0.81 mg/Kg, 14.82±2.05 mg/Kg, 35.2±1.2 mg/Kg, 20.7±0.7 mg/Kg, 76.9±1.1 mg/Kg, 12.9±1.7 mg/Kg, 12.1±0.8 mg/Kg, 16.2±3.0 mg/Kg, 23.4±3.9.1 mg/Kg and 10.4±2.2 mg/Kg in *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella* and *Passiflora edulis L* respectively. It further reveals that *Calamus sp (*155.25±6.8 mg/Kg) has highest concentration of Rb while *Clerodendron colebrookianum(*5.1 ± 0.43 mg/Kg) contained lowest concentration of Rb (Table 3.1.3).

Bromine (Br) (mg/Kg): As revealed by RD-XRF study, Br was detected in all the vegetables foodstuffs samples studied. The mean concentration of Br were estimated as 12.9 ± 0.92 mg/Kg, 36.8 ± 2.0 mg/Kg, 13.0 ± 0.65 mg/Kg, 16.6 ± 0.89 mg/Kg, 9.4 ± 0.58 mg/Kg, 1.7 ± 0.70 mg/Kg, 10.5 ± 0.65 mg/Kg, 1.5 ± 0.68 mg/Kg, 5.15±0.56 mg/Kg, 13.96±2.3 mg/Kg,

2.57±0.05 mg/Kg, 3.19±0.78 mg/Kg, 13.37±5.25 mg/Kg, 70.89±3.85 mg/Kg, 3.14±0.03 mg/Kg, 1.46±0.12 mg/Kg, 1.8±0.3 mg/Kg, 15.3±1.4 mg/Kg, 6.8±1.0, 20.9±0.8 mg/Kg, 23.6±3.1 mg/Kg, 110.4±2.2 mg/Kg and 38.5±1.3 mg/Kg in the *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L., Vigna unguiculata L., Cucurbita pepo L., Gnetum gnemon L., Cucurbita maxima, Colocasis esculenta L., Musa paradisiacal, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea/ Spilanthes acmella* and *Passiflora edulis* L respectively. Further, *Acmella oleracea/ Spilanthes acmella (*110.4±2.2 mg/Kg) was found to be contained highest concentration of Br while *Colocasis esculenta L(*1.5 ± 0.68 mg/Kg) has lowest concentration of Br(Table 3.1.3).

3.2. ELEMENTS PRESENT IN THE MEAT FOODSTUFFS

A total of thirteen elements (13) were detected in the meat foodstuffs commonly consumed by the urban population of Mizoram while analyzed by ED-XRF. The following observations were recorded**.**

3.2.1. Major Elements Present in the Meat foodstuffs:

Five (5) major elements namely Ca, K, Cl, P and S concentrations were recorded from the 3 meat vegetable foodstuffs samples were given below:

Calcium (Ca) (mg/Kg): The results of the present study reveals that all the meat food samples were found to be contained notable amount of Ca. The mean concentration of Ca were estimated as 1667.6±197.5 mg/Kg, 1993.4 ± 291.5 mg/Kg and 413.1 ± 21.6 mg/Kg for Chicken, Fish and Beef respectively. The highest content of Ca was recorded in fish sample (1993.4 ± 291.5 mg/Kg) while beef sample *(*413.1 ± 21.6 mg/Kg) contained lowest level of Ca (Table- 3.2.1).

Potassium (K) (mg/Kg): The results obtained from the present study revealed high and notable amount of K in all the meat food samples studied. The mean concentrations of K were estimated as 2532.6 ± 58.2 mg/Kg, 2623.3 ± 41.4 mg/Kg and 3365.8 ± 89.6 mg/Kg in Chicken, Fish and Beef respectively. The highest content of K was recorded in beef sample (3365.8 ± 89.6 mg/Kg) while chicken (2532.6 ±58.2 mg/Kg) contained lowest level of K (Table- 3.2.1).

Chlorine (Cl) (mg/Kg): The results of the present study also revealed that the meat foodstuffs samples commonly consumed by the natives of urban population of Mizoram contained appreciable amount of Cl. The mean concentrations of Cl were estimated as 544.5 ±3.5 mg/Kg, 549.4 ±2 mg/Kg and 594.7 ± 6.2 mg/Kg in Chicken, Fish and Beef respectively. The study further reveals that highest content of Cl was recorded in beef sample (594.7 ±6.2 mg/Kg) while chicken (544.5 ± 3.5 mg/Kg) contained lowest level of Cl (Table- 3.2.1).

Phosphorus (P)(mg/Kg): The mean concentration of P recorded in the meat foodstuffs samples recorded in the present study were estimated as 788.5 ±71.4 mg/Kg, 1894.9 ± 124.6 mg/Kg and 1493.1 ± 91.9 mg/Kg in Chicken, Fish and Beef respectively. The highest and lowest concentration of P were recorded in fish (1894.9 ± 124.6 mg/Kg) and beef (1493.1 ± 91.9 mg/Kg) respectively (Table- 3.2.1).

Sulphur (S)(mg/Kg): In the present study, the results of the ED-XRF analysis reveals that S is also detected in the meat foodstuffs samples studied. The mean S concentration recorded were 1676 ±93.7 mg/Kg, 1648.8 ± 44.3 mg/Kg and 1666.2 ± 139.2 mg/Kg in Chicken, Fish and Beef respectively. Beef was found to be highest concentration of S (1666.2 ± 139.2 mg/Kg) while the minimum concentration was recorded in fish (1648.8 ± 44.3 mg/Kg) (Table- 3.1.1).

3.2.2. Minor elements detected in the meat foodstuffs:

The minor elements namely Fe, Mn, Zn, Cu and Se were detected from the meat foodstuffs samples while analyzed using ED-XRF. The results of the minor element in mg/L present in the meat foodstuffs samples are as follows:

Iron (Fe)(mg/Kg): The present study reveals that all the meat foodstuffs generally contained high and notable amount of Fe. The mean Fe content were estimated as 72.9 ± 5.1 mg/Kg, 47.9 ± 2.3 mg/Kg and 161.2 ± 6.2 mg/Kg in Chicken, Fish and Beef respectively. Beef *(*161.2 ± 6.2 mg/Kg*)* was found to be contained highest Fe level while fish*(*47.9 ± 2.3 mg/Kg) contained lowest concentration of Fe (Table- 3.2.2.)

Manganese (Mn)(mg/Kg): Interestingly, the present study revealed that all the vegetable foodstuffs samples found to be contained appreciable amount of Mn. The mean concentrations of Mn were estimated as 0.3 ± 0.1 mg/Kg and 1.1 ± 0.3 mg/Kg in chicken and fish respectively. No Mn was detected in the beef. The highest level of Mn was recorded in fish *(1.1* ± 0.3 mg/Kg (Table- 3.2.2.).

Zinc (Zn)(mg/Kg): The ED-XRF analysis of the meat foodstuffs samples reveals that an appreciable amount of Zn was recorded in all the samples studied. The mean concentration of the Zn were estimated as 64.9 ± 7.3 mg/Kg, 46.2 ± 0.7 mg/Kg and 198.1 ± 4.2 mg/Kg in Chicken, Fish and Beef respectively. The highest content of Zn was estimated in Beef (198.1 ± 4.2 mg/Kg) while fish(46.2 ± 0.7 mg/Kg) found to be contained low level of Zn(Table- 3.2.2.).

Copper (Cu)(mg/Kg): The result of the present study indicated that an appreciable amount of Cu was recorded when analyzed by ED-XRF in all the meat samples studied. The mean content of the C were estimated as 3.4 ± 0.4 mg/Kg, 4.8 ± 0.6 mg/Kg and 6.8 ± 1.4 mg/Kg in Chicken, Fish and Beef respectively. In beef (6.8 ± 1.4 mg/Kg) the highest concentration of

the Cu was recorded while *Chicken* 3.4 ± 0.4 mg/Kg) found to be contained lowest level of Cu (Table- 3.2.2.).

Selenium (Se) (mg/Kg): In the present study, Se concentration was detected in low level as compared to other trace element in all the meat food samples studied. The mean concentrations of Se were estimated as 1.8 ± 0.1 mg/Kg, 0.12 mg/Kg and 0.17 mg/Kg in Chicken, Fish and Beef respectively. In chicken (1.8 ± 0.1 mg/Kg), the highest concentration of the Se (13.9±0.1 mg/Kg) was recorded while fish (0.12 mg/Kg) contained lowest concentration of Se (Table 3.2.2.).

3.2.3. Earth elements present in the meat foodstuffs

In an interesting finding, three (3) earth elements namely Sr, Rb and Br were detected from the vegetable foodstuffs samples while analyzed by using ED-XRF and their concentrations in mg/L are given below.

Strontium (Sr) (mg/Kg): The results of the present study reveal that mean concentration of Sr in the meat foodstuffs samples commonly consumed by the urban population of Mizoram were estimated as 11.4 ± 1.8 mg/Kg and 91.8 ± 5.9 mg/Kg in chicken and fish respectively. No Sr was detected in the beef food samples in the present study. The highest content of Sr was recorded in fish (91.8 ± 5.9 mg/Kg) (Table 3.2.3).

Rubidium (Rb) (mg/Kg): The result of the present study reveals Rb was detected in appreciable amount analyzed by ED-XRF in all the vegetables foodstuffs samples studied. The mean concentration of Rb were estimated as43.2 ± 3.2 mg/Kg, 8.6 ± 1.7 mg/Kg and 26.2 ± 1.7 mg/Kg in chicken, fish and beef respectively. It further reveals that chicken (43.2 ± 3.2 mg/Kg) has highest concentration of Rb while fish (8.6 ± 1.7 mg/Kg) contained lowest concentration of Rb (Table 3.2.3).

Bromine (Br) (mg/kg): As revealed by RD-XRF study, Br was detected in all the vegetables foodstuffs samples studied. The mean concentration of Br were estimated as 3.4 ± 0.7

mg/Kg, 10.2 ± 0.9 mg/Kg and 17.5 ± 0.8 mg/Kg in chicken, fish and beef respectively. Further, beef (17.5 ± 0.8 mg/Kg) was found to be contained highest concentration of Br while chicken (3.4 ± 0.7 mg/Kg) has lowest concentration of Br(Table 3.2.3).

3.3. FLUORIDE CONTENT IN THE DRINKING WATER

Water samples from 26 different sources around Aizawl were collected and analysed for their fluoride content using SPADNS method. The results of the analysis are given in Table 3.3.

Among the water samples analysed, the highest content of fluoride was found in Edenthar (which is located near a highway) with a fluoride concentration of 1.362 mg/L. This was followed by the water sample from Chhangurkawn which has a fluoride content of 1.192 mg/L and the third highest content of fluoride was found in Tuikual having 1.161 mg/L.

On the other hand, the lowest content of fluoride was found in Govt, Complex (Midumtui) having a fluoride content of 0.287 mg/L followed by Hunthar having 0.288 mg/L. The third lowest fluoride content was found in Govt Complex (Field) having 0.290 mg/L fluoride.

The water sample procured from PHE water supply and rain water was also measured and the fluoride content was found to be 0.776 mg/L and 0.572 mg/L respectively.

3.4. ELEMENTS PRESENT IN THE UROLITHIATIC STONES

In the present study, 19 (Nineteen) urinary stones were collected from patients with urolithiasis and the chemical compositions were analyzed using ED-XRF. The photographs of some urolithiatic stones are given in figure 3.

3.4.1. Major elements recorded in the stones:

The quantitative estimation of the major elements present in the urolithiatic stones collected from patients was as follows:

Phosphorus (P) (mg/Kg): The results of the present study reveal that a notable concentration of P was recorded in the all the stones samples analyzed using ED-XRF. The mean P concentration estimated was in the ranged between 61.9±10.5 mg/Kg to 35923.5±1580 mg/Kg (Table 3.4.1).

Sulphur (S) (mg/Kg): The results of the present study reveal that an appreciable amount of S was detected in the all the stones samples analyzed. The mean S concentration estimated was in the ranged between minimum of 30.5±3.3 mg/Kg to maximum of 1298.3±364.7 mg/Kg. However, in some stones no S levels were detected (Table 3.4.1).

Chlorine (Cl)(mg/Kg): The ED-XRF results reveals that a good amount of Cl was detected in the all the urinary stones samples analyzed. The mean Cl concentration recorded was in the ranged between minimum of 87.4±4.5 mg/Kg to maximum of 579±0 mg/Kg. However, in some stones no Cl level were detected (Table 3.4.1).

Pottasium (K)(mg/Kg): The results of the study reveals that a high concentration of K was detected in the all the urinary stones samples analyzed. The mean P concentration recorded was in the ranged between minimum of 725.96±0 mg/Kg to maximum of 2584.1±1394.1 mg/Kg. (Table 3.4.1).

Calcium (Ca)(mg/Kg): The ED-XRF analysis results of the present study reveals that generally high and appreciable amount of Ca was detected in the all the urinary stones samples analyzed as compared to other major elements recorded. The mean Ca concentration recorded was in the ranged between minimum of 939.4±30.1 mg/Kg to maximum of 118809.4±1968.7 mg/Kg. (Table 3.4.1).

3.4.2. Minor elements present in the urinary stones

The quantitative estimation of the minor elements present in the urolithiatic stones collected from patients was carried out with the help of ED-XRF. The following minor elements were recorded in the present study.

Manganese (Mn)(mg/Kg): The ED-XRF analysis results of the present study reveals that generally high and appreciable amount of Mn was detected in the all the urinary stones samples analyzed. The mean Mn concentration recorded was in the ranged between minimum of 9.4±0.6 mg/Kg to maximum of 580.3±13.2 mg/Kg. In some stone samples, no Mn level were detected (Table 3.4.2).

Iron (Fe)(mg/Kg): The results of the present study reveals notable amount of Fe in the all the urinary stones samples analyzed using ED-XRF. The mean Fe concentration recorded was in the ranged between minimum of 27.7±2.1 mg/Kg to maximum of 110.7±3.7 mg/Kg. (Table 3.4.2).

Copper (Cu)(mg/Kg): The results of the present study reveals appreciable amount of Cu recorded in the all the urinary stones samples analyzed. The mean Cu concentration recorded was in the ranged between minimum of 0.19 1 mg/Kg to maximum of 364.1±18.7 mg/Kg. However, sin some stone samples no Cu was detected (Table 3.4.2).

Zinc (Zn)(mg/Kg): The results of the present study reveals appreciable amount of Zn is also recorded in the all the urinary stones samples analyzed. The mean Zn concentration recorded was in the ranged between minimum of 1.4±1.1 mg/Kg to maximum of 107.8±2.3 mg/Kg. However, in one stone sample no Zn was detected (Table 3.4.2).

Selenium (Se)(mg/Kg): A low level of Se was recorded in all the urinary stones samples analyzed. The mean Se concentration recorded was in the ranged between minimum of 0.12±0 mg/Kg to maximum of 1.0±0.6 mg/Kg (Table 3.4.2).

3.4.3. Earth elements present in the stones:

ED-XRF analysis reveals the following earth elements present in the urolithiatic stones collected from patients from the urban area of Mizoram.

Bromine (Br) (mg/Kg): The results of the present study reveals the mean Br concentration recorded was in the ranged between minimum of 1.1±0.2 mg/Kg to maximum of 6.4±2.1 mg/Kg. However, in some stone samples no Br was detected (Table 3.4.3).

Rubidium (Rb)(mg/Kg): The results of the present study reveals that no Rb was detected in all the urinary stone samples analyzed using ED-XRF.

Strontium (Sr) (mg/Kg): The results of the present study reveals the mean Sr concentration recorded was in the ranged between minimum of 0.1±0 mg/Kg to maximum of 88.3±1.3 mg/Kg. However, in one stone samples no Sr was detected (Table 3.4.3).

3.5. STATISTICAL ANALYSIS OF FOODSTUFFS WITH UROLITHIATIC STONES BY KARL PEARSON CORRELATION COEFFICIENT

The concentrations of elements measured from the selected vegetable foodstuffs and meat foodstuffs were analysed along with the concentrations of elements present in the urolithiatic stones using Pearson's correlation coefficient. The results of correlation of vegetable foodstuffs with urolithiatic stones are given in table 3.5.1., 3.5.2. and 3.5.3. The results of correlation of meat foodstuffs with urolithiatic stones are given in table 3.5.4., 3.5.5. and 3.5.6.

3.5.1. Statistical analysis of major elements in vegetable foodstuffs with urolithiatic stones

***Clerodendron colebrookianum**:* The results of the study reveals that a negative correlation was observed between P, S, Cl, and Ca concentration in the *Clerodendron colebrookianum* to that of their concentration in the urinary stone(r= -0.935, -0.815, -0.880, -0.179 for P, S, Cl and Ca respectively). However, K concentration in the *Clerodendron colebrookianum* is found to be positively correlated to their urinary stone counterpart (p=0.294, r= 0.895)(Table No- 3.5.1)

Brassica juncea: From the Karl Pearson's correlation analysis, it was found that a negative correlation was established between P, Cl, K and Ca concentration in *Brassica juncea* to that of their concentration in the urinary stone(r= -0.861,-0.848,-0.838,-0.348 for P, Cl, K and Ca respectively). However the S concentration was found to be positively correlated (p=0.696, r=0.459). (Table No-3.5.1)

***Zanthoxylum rhetsa L.*:** From the present investigation, it was revealed that a positive correlation was observed in all major elements namely S, Cl, K and Ca recorded in *Zanthoxylum rhetsa L* except P concentration to that of their concentration in the urinary stone(p=0.145,r= 0.974; p=0.809, r= 0.295; p=0.243, r=0.928; p=0.635, r=0.543 for S, Cl, K and Ca respectively and r=-0.990 for P). (Table No- 3.5.1).

Vigna unguiculata L: The result of the present study reveals that a negative correlation was observed in the P, S, K and Ca concentration (r= -0.185, -0.998, -0.624, -0.402 for P,S, K and Ca respectively) while a positive correlation was recorded in the Cl content(p=0.832, r= 0.260) recorded in *Vigna unguiculata L* to that of their concentration in the urinary stones (Table No-3.5.1).

Cucurbita pepo L: The result of the study reveals that a negative correlation was observed between P, K and Ca concentration in the *Cucurbita pepo L* to that of their concentration in the urinary stone(r= -0.780, -0.411, -0.216) for P, K and Ca respectively). However, S and Cl concentration in the *Cucurbita pepo L* is found to be positively correlated to their urinary stone counterpart (p=0.499, r= 0.708; p=0.749, r=0.384 for S and Cl respectively) (Table No-3.5.1)

Gnetum gnemon L: From the Karl Pearson's correlation analysis, it was found that a positive correlation was established between P, K and Ca concentration in *Gnetum gnemon L* to that of their concentration in the urinary stone (p=0.570, r=0.625; p=0.391, r=0.817; p=0.565,

r=0.632 for P, K and Ca respectively). However the S and Cl concentration was found to be negatively correlated (r= -0.959, -0.603 for S and Cl respectively) (Table No-3.5.1)

Cucurbita maxima: The result of the study reveals that a negative correlation was observed in Cl, K and Ca concentration in the *Cucurbita maxima* to that of their concentration in the urinary stone (r= -0.751, -0.995, -0.413 for Cl, K and Ca respectively). However, P and S concentration in the *Cucurbita maxima* is found to be positively correlated to their urinary stone counterpart (p=0.548, r=0.652; p=0.683, r=0.478 for P and S respectively) (Table No-3.5.1)

***Colocasis esculenta L*:** From the present investigation, it was revealed that a positive correlation was observed in all major elements namely P, S, Cl, K and Ca recorded in *Colocasis esculenta L* to that of their concentration in the urinary stone (p=0.179,r= 0.961 ; p=0.080, r= 0.992; p=0.908, r=0.144; p=0.436, r=0.774; p=0.868, r=0.205 for P, S, Cl, K and Ca respectively). (Table No- 3.5.1).

***Musa paradisiaca*:** From the present investigation, it was revealed that a positive correlation was observed in P, K and Ca recorded in *Musa paradisiaca* to that of their concentration in the urinary stone (p=0.081,r= 0.992; p=0.482, r= 0.726; p=0.936, r=0.100 for P, K and Ca respectively). However the S and Cl concentration was found to be negatively correlated (r= -0.914, -0.363 for S and Cl respectively) (Table No- 3.5.1).

***Bambusa vulgaris*:** From the present investigation, it was revealed that a negative correlation was observed in P, Cl and K recorded in *Bambusa vulgaris* to that of their concentration in the urinary stone (r= -0.717, - 0.680, -0.070 for P, Cl and K respectively). However the S concentration was found to be positively correlated (p=0.412, r=0.798). No correlation was found in the case of Ca.(Table No- 3.5.1).

***Acacia gagaena*:** From the present investigation, it was revealed that a negative correlation was observed in S, K and Ca recorded in *Acacia gagaena* to that of their concentration in the

urinary stone (r= -0.215, -0.941 and -0.976 for P, K and Ca respectively). However the P concentration was found to be positively correlated (p=0.948, r=0.081). No correlation was found in the case of Cl. (Table No- 3.5.1).

***Dysoxylum gobara merr*:** From the present investigation, it was revealed that a negative correlation was observed in S, Cl and Ca recorded in *Dysoxylum gobara merr* to that of their concentration in the urinary stone (r= -0.884, -0.688 and -0.286 for S, Cl and Ca respectively). However the P and K concentration was found to be positively correlated (p=0.709, r=0.441; p=0.416, r=0.794) (Table No- 3.5.1).

***Solanum anguivi*:** From the present investigation, it was revealed that a positive correlation was observed in P, Cl and K recorded in *Solanum anguivi* to that of their concentration in the urinary stone (p=0.806,r= 0.301; p=0.663, r= 0.505; p=0.035, r=0.999 for P, Cl and K respectively). However the S and Ca concentration was found to be negatively correlated (r= -0.838, -0.641 for S and Ca respectively) (Table No- 3.5.1).

***Calamus sp*:** From the present investigation, it was revealed that a positive correlation was observed in all major elements namely P, S, Cl and Ca recorded in *Calamus sp* to that of their concentration in the urinary stone (p=0.589,r= 0.601; p=0.392, r= 0.816; p=0.566, r=0.630 and p=0.911, r=0.140 for P, S, Cl and Ca respectively) except for K which shows a negative correlation (r=-0.388) (Table No- 3.5.1).

***Glycine max merr*:** From the present investigation, it was revealed that a positive correlation was observed in P, S and K recorded in *Glycine max merr* to that of their concentration in the urinary stone (p=0.086, r=0.991; p=0.284, r=0.902 and p=0.944, r=0.088 for P, S and K respectively) while Ca shows negative correlation (r=-0.636). No correlation was observed in the case of Cl. (Table No- 3.5.1).

***Parkia timoriana*:** From the present investigation, it was revealed that a positive correlation was observed in P, S, K and Ca recorded in *Parkia timoriana* to that of their concentration in

the urinary stone (p=0.536, r=0.666; p=0.771, r=0.352; p=627, r=0.553 and p=0.383, r=0.825 for P,S, K and Ca respectively). No correlation was observed in the case of Cl. (Table No- 3.5.1).

Brassica oleracea: From the present investigation, it was revealed that a positive correlation was observed in K and Ca recorded in *Brassica oleracea* to that of their concentration in the urinary stone (p=0.556, r=0.641 and p=0.848, r=0.237 for K and Ca respectively) while P and S shows negative correlation (r=-0.273 and -0.657 for P and S respectively). No correlation was observed in the case of Cl. (Table No- 3.5.1).

***Solanum nigrum linn*:** From the present investigation, it was revealed that a negative correlation was observed in P, S and Ca recorded in *Solanum nigrum linn* to that of their concentration in the urinary stone (r= -0.993, -0.472 and -0.991 for P, S and Ca respectively) while K shows positive correlation (p=0.159, r=0.969). No correlation was observed in the case of Cl. (Table No- 3.5.1).

***Brassica oleracea*:** From the present investigation, it was revealed that a positive correlation was observed in P, S and Ca recorded in *Brasicca oleracea* to that of their concentration in the urinary stone (p=0.398, r=0.810; p=0.659, r=0.510 and p=0.011, r=1.000 for P,S and Ca respectively) while K shows negative correlation (r=-0.578). No correlation was observed in the case of Cl. (Table No- 3.5.1).

***Solanum melongena*:** From the present investigation, it was revealed that a negative correlation was observed in P, S and Ca recorded in *Brasicca oleracea* to that of their concentration in the urinary stone (r= -0.456, -0.824 and -0.052 for P,S and Ca respectively) while K shows positive correlation (p=0.649, r=0.523). No correlation was observed in the case of Cl. (Table No- 3.5.1).

***Solanum incanum*:** From the present investigation, it was revealed that a positive correlation was observed in P and K recorded in *Solanum incanum* to that of their concentration in the

urinary stone (p=0.291, r=0.897 and p=0.294, r=0.895 for P and K respectively) while S and Ca shows negative correlation (r=-0.806 and -0.222 for S and Ca respectively). No correlation was observed in the case of Cl. (Table No- 3.5.1).

***Acmella oleracea*:** From the present investigation, it was revealed that a negative correlation was observed in S, K and Ca recorded in *Acmella oleracea* to that of their concentration in the urinary stone (r= -0.960, -0.494 and -0.731 for S, K and Ca respectively) while P shows positive correlation (p=0.287, r=0.900). No correlation was observed in the case of Cl. (Table No- 3.5.1).

***Passiflora edulis L*:** From the present investigation, it was revealed that a positive correlation was observed in P, S, K and Ca recorded in *Passiflora edulis L* to that of their concentration in the urinary stone (p=0.090, r=0.990; p=0.563, r=0.634; p=0.119, r=0.982 and p=0.507, r=0.699 for P, S, K and Ca respectively). No correlation was observed in the case of Cl. (Table No- 3.5.1).

3.5.2. Statistical analysis of minor elements in vegetable foodstuffs with urolithiatic stones

***Clerodendron colebrookianum**:* The result of the study reveals that a negative correlation was observed in Mn, Fe and Cu concentration in the *Clerodendron colebrookianum* to that of their concentration in the urinary stone (r= -0.099, -0.862 and -0.253 respectively). However, Zn and Se concentration in the *Clerodendron colebrookianum* was found to be positively correlated to their urinary stone counterpart (p=0.952, r= 0.075 and p=0.538, r=0.664) (Table No- 3.5.2).

Brassica juncea: The result of the study reveals that a negative correlation was observed in Zn and Se concentration in the *Brassica juncea to* that of their concentration in the urinary stone (r= -0.866 and -0.419 respectively). However, Mn, Fe and Cu concentration in the

Brassica juncea was found to be positively correlated to their urinary stone counterpart (p=0.914, r= 0.135; p=0.361, r=0.843 and p=0.560, r=0.637) (Table No- 3.5.2).

***Zanthoxylum rhetsa L.*:** The result of the study reveals that a negative correlation was observed in Fe, Cu, Zn and Se concentration in the *Zanthoxylum rhetsa L* to that of their concentration in the urinary stone (r= -0.508, -0.978, -0.600 and -0.506 respectively). However, Mn concentration in the *Zanthoxylum rhetsa L* was found to be positively correlated to its urinary stone counterpart (p=0.267, r= 0.913) (Table No- 3.5.2).

Vigna unguiculata L: The result of the study reveals that a negative correlation was observed in Mn, Fe, Cu and Se concentration in the *Vigna unguiculata L* to that of their concentration in the urinary stone (r= -0.249, -0.749, -0.115 and -0.708 respectively). However, Zn concentration in the *Vigna unguiculata L* was found to be positively correlated to its urinary stone counterpart (p=0.464, r= 0.746) (Table No- 3.5.2).

Cucurbita pepo L: The result of the study reveals that a negative correlation was observed in Mn, Zn and Se concentration in the *Cucurbita pepo L* to that of their concentration in the urinary stone (r= -0.146, -0.303 and -0.866 respectively). However, Fe and Cu concentration in the *Cucurbita pepo L* was found to be positively correlated to their urinary stone counterpart (p=0.616, r= 0.568 and p=0.333, r=0.866) (Table No- 3.5.2).

Gnetum gnemon L: The result of the study reveals that a negative correlation was observed in Mn, Cu and Zn concentration in the *Gnetum gnemon L* to that of their concentration in the urinary stone (r= -0.139, -0.736 and -0.926 respectively). However, Fe and Se concentration in the *Gnetum gnemon L* was found to be positively correlated to their urinary stone counterpart (p=0.453, r= 0.757 and p=0.000, r=1) (Table No- 3.5.2).

Cucurbita maxima: The result of the study reveals that a negative correlation was observed in all the elements Mn, Fe, Cu, Zn and Se concentration in the *Cucurbita maxima* to that of

their concentration in the urinary stone (r= -0.430, -0.010, -0.995, -0.955 and -0.5 respectively) (Table No- 3.5.2).

***Colocasis esculenta L*:** The result of the study reveals that a negative correlation was observed in Fe, Cu and Zn concentration in the *Colocasis esculenta L* to that of their concentration in the urinary stone (r= -0.249, -0.562 and -0.042 respectively). However, Mn and Se concentration in the *Colocasis esculenta L* was found to be positively correlated to their urinary stone counterpart (p=0.736, r= 0.403 and p=0.000, r=1) (Table No- 3.5.2).

***Musa paradisiaca*:** The result of the study reveals that a positive correlation was observed in Fe, Cu, Zn and Se concentration in the *Musa paradisiaca* to that of their concentration in the urinary stone (p=0.311, r=0.883; p=0.198, r=0.952; p=0.552, r=0.648 and p=0.000, r=1 respectively). However, Mn concentration in the *Musa paradisiaca* was found to be negatively correlated to its urinary stone counterpart (r=-0.020) (Table No- 3.5.2).

***Bambusa vulgaris*:** The result of the study reveals that a positive correlation was observed in Mn, Fe, Cu and Zn concentration in the *Bambusa vulgaris* to that of their concentration in the urinary stone (p=0.898, r=0.159; p=0.378, r=0.829; p=0.385, r=0.823 and p=0.594, r=0.596 respectively). However, Se concentration in the *Bambusa vulgaris* was found to be negatively correlated to its urinary stone counterpart (r=-0.945) (Table No- 3.5.2).

***Acacia gagaena*:** The result of the study reveals that a positive correlation was observed in Fe, Cu and Zn concentration in the *Acacia gagaena* to that of their concentration in the urinary stone (p=0.785, r=0.331; p=0.404, r=0.806 and p=0.836, r=0.254 respectively). However, Mn and Se concentration in the *Acacia gagaena* was found to be negatively correlated to their urinary stone counterpart (r=-0.751 and -0.774) (Table No- 3.5.2).

***Dysoxylum gobara merr*:** The result of the study reveals that a positive correlation was observed in Mn, Zn and Se concentration in the *Dysoxylum gobara merr* to that of their concentration in the urinary stone (p=0.552, r=0.647; p=0.299, r=0.892 and p=0.077, r=0.993

respectively). However, Fe and Cu concentration in the *Dysoxylum gobara merr* was found to be negatively correlated to their urinary stone counterpart (r=-0.082 and -0.042) (Table No- 3.5.2).

***Solanum anguivi*:** The result of the study reveals that a positive correlation was observed in Mn, Cu, Zn and Se concentration in the *Solanum anguivi* to that of their concentration in the urinary stone (p=0.999, r=0.001; p=0.472, r=0.737; p=0.338, r=0.863 and p=0.000, r=1 respectively). However, Fe concentration in the *Solanum anguivi* was found to be negatively correlated to its urinary stone counterpart (r=-0.004) (Table No- 3.5.2).

***Calamus sp*:** The result of the study reveals that a positive correlation was observed in Cu and Se concentration in the *Calamus sp* to that of their concentration in the urinary stone (p=0.449, r=0.762 and p=0.000, r=1 respectively). However, Mn, Fe and Zn concentration in the *Calamus sp* was found to be negatively correlated to their urinary stone counterpart (r=-0.996, -0.846 and -0.448) (Table No- 3.5.2).

***Glycine max merr*:** The result of the study reveals that a positive correlation was observed in Mn, Fe and Cu concentration in the *Glycine max merr* to that of their concentration in the urinary stone (p=0.758, r=0.371; p=0.801, r=0.307 and p=0.686, r=0.474 respectively). However, Zn and Se concentration in the *Glycine max merr* was found to be negatively correlated to their urinary stone counterpart (r=-0.827 and -0.721) (Table No- 3.5.2).

***Parkia timoriana*:** The result of the study reveals that a positive correlation was observed in Se concentration in the *Parkia timoriana* to that of its concentration in the urinary stone (p=0.601, r=0.587). However, Mn, Fe, Cu and Zn concentration in the *Parkia timoriana* was found to be negatively correlated to their urinary stone counterpart (r=-0.289, -0.965,-0.619 and -0.250 respectively) (Table No- 3.5.2).

Brassica oleracea: The result of the study reveals that a positive correlation was observed in Zn and Se concentration in the *Brassica oleracea* to that of their concentration in the urinary

stone (p=0.852, r=0.230 and p=0.534, r=0.668). However, Mn, Fe and Cu concentration in the *Brassica oleracea* was found to be negatively correlated to their urinary stone counterpart (r=-0.703, -0.973 and -0.374 respectively) (Table No- 3.5.2).

***Solanum nigrum linn*:** The result of the study reveals that a positive correlation was observed in Cu and Se concentration in the *Solanum nigrum linn* to that of their concentration in the urinary stone (p=0.530, r=0.673 and p=0.000, r=1). However, Mn, Fe and Zn concentration in the *Solanum nigrum linn* was found to be negatively correlated to their urinary stone counterpart (r=-0.804, -0.420 and -0.020 respectively) (Table No- 3.5.2).

***Brassica oleracea*:** The result of the study reveals that a positive correlation was observed in Zn concentration in the *Brassica oleracea* to that of its concentration in the urinary stone (p=0.847, r=0.238). However, Mn, Fe, Cu and Se concentration in the *Brassica oleracea* was found to be negatively correlated to their urinary stone counterpart (r=-0.595, -0.855, -0.290 and -0.998 respectively) (Table No- 3.5.2).

***Solanum melongena*:** The result of the study reveals that a positive correlation was observed in Zn and Se concentration in the *Solanum melongena* to that of their concentration in the urinary stone (p=0.837, r=0.254 and p=0.000, r=1). However, Mn, Fe and Cu concentration in the *Solanum melongena* was found to be negatively correlated to their urinary stone counterpart (r=-0.987, -0.573 and -0.618 respectively) (Table No- 3.5.2).

***Solanum incanum*:** The result of the study reveals that a positive correlation was observed in Fe, Cu and Zn concentration in the *Solanum incanum* to that of their concentration in the urinary stone (p=0.751, r=0.381; p=0.019, r=1 and p=0.991, r=0.014 respectively). However, Mn and Se concentration in the *Solanum incanum* was found to be negatively correlated to their urinary stone counterpart (r=-0.983 and -0.500) (Table No- 3.5.2).

***Acmella oleracea*:** The result of the study reveals that a positive correlation was observed in Cu and Zn concentration in the *Acmella oleracea* to that of their concentration in the urinary

stone (p=0.252, r=0.922 and p=0.276, r=0.907). However, Mn, Fe and Se concentration in the *Acmella oleracea* was found to be negatively correlated to their urinary stone counterpart (r=-0.933, -0.806 and -1 respectively) (Table No- 3.5.2).

***Passiflora edulis L*:** The result of the study reveals that a positive correlation was observed in Cu, Zn and Se concentration in the *Passiflora edulis L* to that of their concentration in the urinary stone (p=0.098, r=0.988; p=0.222, r=0.940 and p=0.092, r=0.990 respectively). However, Mn and Fe concentration in the *Passiflora edulis L* was found to be negatively correlated to their urinary stone counterpart (r=-0.172 and -0.148) (Table No- 3.5.2).

3.5.3. Statistical analysis of earth elements in vegetable foodstuffs with urolithiatic stones

***Clerodendron colebrookianum**:* From the study, it was found that Br in *Clerodendron colebrookianum* shows a positive correlation with its concentration in the urinary stone (p=0.105, r=0.986) while Sr shows a negative correlation (r=-0.998). No correlation was observed in the case of Rb. (Table No- 3.5.3)

Brassica juncea: From the study, it was found that Br in *Brassica juncea* shows a positive correlation with its concentration in the urinary stone (p=0.527, r=0.677) while Sr shows a negative correlation (r=-0.226). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Zanthoxylum rhetsa L.*:** From the study, it was found that Br and Sr in *Zanthoxylum rhetsa L* shows a positive correlation with their concentration in the urinary stone (p=0.289, r=0.899 and p=0.708, r=0.443). No correlation was observed in the case of Rb. (Table No- 3.5.3)

Vigna unguiculata L: From the study, it was found that Br and Sr in *Vigna unguiculata L* shows a negative correlation with their concentration in the urinary stone (r= -0.997 and r= -0.999). No correlation was observed in the case of Rb. (Table No- 3.5.3).

Cucurbita pepo L: From the study, it was found that Br and Sr in *Cucurbita pepo L* shows a negative correlation with their concentration in the urinary stone (r= -0.895 and r= -0.310). No correlation was observed in the case of Rb. (Table No- 3.5.3)

Gnetum gnemon L: From the study, it was found that Sr in *Gnetum gnemon L* shows a positive correlation with its concentration in the urinary stone (p=0.936, r=0.101) while Br shows a negative correlation (r=-0.616). No correlation was observed in the case of Rb. (Table No- 3.5.3)

Cucurbita maxima: From the study, it was found that Br and Sr in *Cucurbita maxima* shows a negative correlation with their concentration in the urinary stone (r= -0.407 and r= -0.936). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Colocasis esculenta L*:** From the study, it was found that Br and Sr in *Colocasis esculenta L* shows a positive correlation with their concentration in the urinary stone (p=0.041, r=0.998 and p=0.908, r=0.145). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Musa paradisiaca*:** From the study, it was found that Sr in *Musa paradisiaca* shows a positive correlation with its concentration in the urinary stone (p=0.028, r=0.999) while Br shows a negative correlation (r=-0.630). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Bambusa vulgaris*:** From the study, it was found that Br and Sr in *Bambusa vulgaris* shows a positive correlation with their concentration in the urinary stone (p=0.769, r=0.356 and p=0.989, r=0.017). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Acacia gagaena*:** From the study, it was found that Br in *Acacia gagaena* shows a positive correlation with its concentration in the urinary stone (p=0.567, r=0.629) while Sr shows a negative correlation (r=-0.210). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Dysoxylum gobara merr*:** From the study, it was found that Br and Sr in *Dysoxylum gobara merr* shows a positive correlation with their concentration in the urinary stone (p=0.870, r=0.203 and p=0.696, r=0.459). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Solanum anguivi*:** From the study, it was found that Sr in *Solanum anguivi* shows a positive correlation with its concentration in the urinary stone (p=0.686, r=0.473) while Br shows a negative correlation (r=-0.066). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Calamus sp*:** From the study, it was found that Sr in *Calamus sp* shows a positive correlation with its concentration in the urinary stone (p=0.567, r=0.628) while Br shows a negative correlation (r=-0.904). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Glycine max merr*:** From the study, it was found that Sr in *Glycine max merr* shows a positive correlation with its concentration in the urinary stone (p=0.697, r=0.459) while Br shows a negative correlation (r=-0.985). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Parkia timoriana*:** From the study, it was found that Br and Sr in *Parkia timoriana* shows a negative correlation with their concentration in the urinary stone (r= -0.709 and r= -0.999). No correlation was observed in the case of Rb. (Table No- 3.5.3)

Brassica oleracea: From the study, it was found that Br and Sr in *Brassica oleracea* shows a positive correlation with their concentration in the urinary stone (p=0.747, r=0.387 and p=0.461, r=0.749). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Solanum nigrum linn*:** From the study, it was found that Br and Sr in *Solanum nigrum linn* shows a negative correlation with their concentration in the urinary stone (r= -0.858 and r= -0.999). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Brassica oleracea*:** From the study, it was found that Br in *Brassica oleracea* shows a positive correlation with its concentration in the urinary stone (p=0.983, r=0.026) while Sr shows a negative correlation (r=-0.105). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Solanum melongena*:** From the study, it was found that Br and Sr in *Solanum melongena* shows a negative correlation with their concentration in the urinary stone (r= -0.071 and r= -0.298). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Solanum incanum*:** From the study, it was found that Br in *Solanum incanum* shows a positive correlation with its concentration in the urinary stone (p=0.638, r=0.538) while Sr shows a negative correlation (r=-0.450). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Acmella oleracea*:** From the study, it was found that Br and Sr in *Acmella oleracea* shows a positive correlation with their concentration in the urinary stone (p=0.925, r=0.118 and p=0.963, r=0.059). No correlation was observed in the case of Rb. (Table No- 3.5.3)

***Passiflora edulis L*:** From the study, it was found that Sr in *Passiflora edulis L* shows a positive correlation with its concentration in the urinary stone (p=0.221, r=0.940) while Br shows a negative correlation (r=-0.311). No correlation was observed in the case of Rb. (Table No- 3.5.3)

3.5.4. Statistical analysis of major elements in meat foodstuffs with urolithiatic stones

***Gallus gallus domesticus*:** Using Karl Pearson's correlation coefficient, it was found that P, Cl and K in *Gallus gallus domesticus* shows a positive correlation with their concentration in the urinary stone (p=0.780, r=0.339; p=0.808, r=0.296 and p=0.622, r=0.560 respectively) while S and Ca shows a negative correlation (r=-0.362 and r=-0.487) (Table No- 3.5.4).

***Labeo rohita*:** Using Karl Pearson's correlation coefficient, it was found that K and Ca in *Labeo rohita* shows a positive correlation with their concentration in the urinary stone

(p=0.329, r=0.870 and p=0.376, r=0.831 respectively) while P, S and Cl shows a negative correlation (r=-0.980, r=-0.755 and r=-0.365) (Table No- 3.5.4).

***Bos Taurus*:** Using Karl Pearson's correlation coefficient, it was found that P, S, Cl and K in *Bos taurus* shows a positive correlation with their concentration in the urinary stone (p=0.067, r=0.994, p=0.997, r=0.005, p=0.180, r=0.960 and p=0.310, r=0.884 respectively) while Ca shows a negative correlation (r=-0.962) (Table No- 3.5.4).

3.5.5. Statistical analysis of minor elements in meat foodstuffs with urolithiatic stones:

***Gallus gallus domesticus*:** Using Karl Pearson's correlation coefficient, it was found that Mn, Fe, Cu and Zn in *Gallus gallus domesticus* shows a negative correlation with their concentration in the urinary stone (r= -0.988, r=-0.027, r=-0.092 and r=-0.700 respectively) while Se shows a positive correlation (p=0.842, r=0.246) (Table No- 3.5.5).

***Labeo rohita*:** Using Karl Pearson's correlation coefficient, it was found that Mn, Fe and Cu in *Labeo rohita* shows a negative correlation with their concentration in the urinary stone (r=-0.258, r=-0.926 and r=-0.952 respectively) while Zn and Se shows a positive correlation (p=0.715, r=0.433 and p=0.000, r=1) (Table No- 3.5.5).

***Bos Taurus*:** Using Karl Pearson's correlation coefficient, it was found that Fe, Zn and Se in *Bos taurus* shows a negative correlation with their concentration in the urinary stone (r=-0.986, r=-0.262 and r=-0.480 respectively) while Cu shows a positive correlation (p=0.657, r=0.513). No correlation was observed in the case of Mn. (Table No- 3.5.5).

3.5.6. Statistical analysis of earth elements in meat foodstuffs with urolithiatic stones

***Gallus gallus domesticus*:** Using Karl Pearson's correlation coefficient, it was found that Br and Rb in *Gallus gallus domesticus* shows a negative correlation with their concentration in the urinary stone (r=-0.984 and r=-0.230 respectively). No correlation was observed in the case of Rb. (Table No- 3.5.6).

***Labeo rohita*:** Using Karl Pearson's correlation coefficient, it was found that Br and Rb in *Labeo rohita* shows a negative correlation with their concentration in the urinary stone (r=-0.994 and r=-0.974 respectively). No correlation was observed in the case of Rb. (Table No-3.5.6).

***Bos Taurus*:** Using Karl Pearson's correlation coefficient, it was found that Br and Rb in *Bos taurus* shows a positive correlation with their concentration in the urinary (p=0.446, r=0.765 and p=097, r=935). No correlation was observed in case of Rb. (Table No- 3.5.6).

3.5.7. Correlation among elements in vegetable foodstuffs:

***Clerodendron colebrookianum**:* P&Cl, P&Cu, Zn&Br and K&Sr correlation is significant at 0.05 level. Ca shows positive correlation with P, Cl, Cu, Zn, Br and Sr while Cl shows positive correlation with P, Ca, Cu, Se, Rb and Sr. On the other hand, K shows negative correlation with P, Cl, Ca, Cu and Sr. (Table No-3.5.7)

Brassica juncea: P&Cu correlation is significant at 0.05 level. S&Se and Zn&Fe correlation is significant at 0.01 level. Ca shows positive correlation with P and Cu while Cl shows positive correlation with P, S, K, Mn Fe, Cu, Zn and Se. On the other hand, K shows negative correlation with Ca, Rb and Sr. (Table No-3.5.8)

***Zanthoxylum rhetsa L.*:** P&Zn, P&Br, K&Zn and K&Br correlation is significant at 0.05 level. Ca&Fe and Zn&Br correlation is significant at 0.01 level. Ca shows positive correlation with P, Cl, Mn and Zn while Cl shows positive correlation with K, Ca, Se, br, Rb and Sr. On the other hand, K shows negative correlation with P, S, Ca, Mn and Zn. (Table No-3.5.9)

Vigna unguiculata L: S&Cl and Cl&K correlation is significant at 0.05 level. S&K correlation is significant at 0.01 level. Ca shows positive correlation with S, Zn and Rb while Cl shows positive correlation with P, K, Mn, Fe, Cu, Se and Sr. On the other hand, K shows negative correlation with S, Ca, Zn and Br. (Table No-3.5.10)

Cucurbita pepo L: Cl&K, Cl&Ca, Cl&Mn, Ca&K and Zn&Br correlation is significant at 0.05 level. Ca&Mn and K&Mn correlation is significant at 0.01 level. Ca shows positive correlation with P, S, Cl, K, Mn, Fe, Zn, Se and Rb while Cl shows positive correlation with P, S, K, Ca, Mn, Fe, Zn, Se and Rb. On the other hand, K shows negative correlation with Cu, Br and Sr. (Table No-3.5.11)

Gnetum gnemon L: P&Cu and S&K correlation is significant at 0.05 level. Ca shows positive correlation with P, S, Cl, K, Mn, Fe, Se and Rb while Cl shows positive correlation with P, S, K, Ca, Mn, Fe and Se. On the other hand, K shows negative correlation with Cu, Zn, Br and Sr. (Table No-3.5.12)

Cucurbita maxima: P&Zn, S&Se and K&Zn correlation is significant at 0.05 levels. Fe&Cu correlation is significant at 0.01 level. Ca shows positive correlation with P and Br while Cl shows positive correlation with K, Fe, Cu, Zn, Rb and Sr. On the other hand, K shows negative correlation with P, S, Ca Se and Br. (Table No-3.5.13)

***Colocasis esculenta L*:** P&Br, K&Fe, Ca&K and Br&Sr correlation is significant at 0.05 levels. Cl&Mn and Fe&Ca correlation is significant at 0.01 level. Ca shows positive correlation with P, S, Mn, Cu, Zn, Br, Rb and Sr while Cl shows positive correlation with P, K, Fe, Se and Br. On the other hand, K shows negative correlation with Cl, Fe and Se. (Table No-3.5.14)

***Musa paradisiaca*:** Cl&Ca, Ca&Mn and Mn&Se correlation is significant at 0.05 level. K&Cu, Ca&Se and Zn&Br correlation is significant at 0.01 levels. Ca shows positive correlation with S, Cl, K, Mn, Cu, Zn, Se, Br and Sr while Cl shows positive correlation with S, K, Ca, Mn, Cu, Zn, Se, Br and Sr. On the other hand, K shows negative correlation with Fe and Rb. (Table No-3.5.15)

***Bambusa vulgaris*:** P&K, P&Cu, K&Cu and Zn&Br correlation is significant at 0.05 level. Rb&Sr correlation is significant at 0.01 level. Cl shows positive correlation with P, K, Cu, Zn

and Rb. On the other hand, K shows negative correlation with S, Mn, Se, Br and Sr. (Table No-3.5.16)

***Acacia gagaena*:** Mn&Zn correlation is significant at 0.05 level. Ca shows positive correlation with P, Mn, Cu, Se and Br. On the other hand, K shows negative correlation with P, Ca, Mn, Cu, Se and Sr. (Table No-3.5.17)

***Dysoxylum gobara merr*:** P&S, P&Br and S&Br correlation is significant at 0.01 level. P&Rb, K&Ca, Fe&Zn and Br&Rb correlation is significant at 0.05 level. Ca shows positive correlation with P, S, K, Mn, Fe, Zn, Se, Br, Rb and Sr while Cl shows positive correlation with Mn. On the other hand, K shows negative correlation with Cl and Cu. (Table No-3.5.18)

***Solanum anguivi*:** S&Cu, Cl&Ca, Mn&Fe, Fe&Zn and Fe&Se correlation is significant at 0.05 level. Mn&Zn, Mn&Se and Zn&Se correlation is significant at 0.01 level. Ca shows positive correlation with P, S, Cl, K, Mn, Fe, Cu, Zn, Se, Br and Sr while Cl shows positive correlation with P, S, Ca, K, Mn, Fe, Cu, Zn, Se, Br and Sr. On the other hand, K shows negative correlation with Rb. (Table No-3.5.19)

***Calamus sp*:** P&Zn, P&Rb, S&Rb and Zn&Rb correlation is significant at 0.05 level. P&S and S&Zn correlation is significant at 0.01 level. Ca shows positive correlation with P, S, Cl, K, Fe and Zn while Cl shows positive correlation with P, S, K, Ca, Mn, Fe and Zn. On the other hand, K shows negative correlation with Cu, Se, Br, Rb and Sr. (Table No-3.5.20)

***Glycine max merr*:** S&Sr and Ca&K correlation is significant at 0.05 level. Ca shows positive correlation with P, S, K, Mn, Cu, Zn, Se and Rb. On the other hand, K shows negative correlation with Fe, Br, Rb and Sr. (Table No-3.5.21)

***Parkia timoriana*:** Fe&Zn and Rb&Sr correlation is significant at 0.05 level. Ca shows positive correlation with S, Cu, Zn, Se, Rb and Sr. On the other hand, K shows negative correlation with S, Ca, Mn, Cu, Zn, Se, Br, Rb and Sr. (Table No-3.5.22)

Brassica oleracea: P&Ca, S&Mn, S&Se, Mn&Se and Cu&Se correlation is significant at 0.05 level. Ca shows positive correlation with P, S, K, Fe, Se, Br and Sr. On the other hand, K shows negative correlation with Mn, Cu and Zn. (Table No-3.5.23)

***Solanum nigrum linn*:** K&Cu and Br&Rb correlation is significant at 0.05 level. Ca shows positive correlation with P, S, K, Mn, Fe, Cu, Zn, Se and Rb. On the other hand, K shows negative correlation with P, Rb and Sr. (Table No-3.5.24)

***Brassica oleracea*:** P&Sr and Cu&Zn correlation is significant at 0.05 level. Ca shows positive correlation with Br. On the other hand, K shows negative correlation with Ca, Mn and Fe. (Table No-3.5.25)

***Solanum melongena*:** K&Se and Zn&Sr correlation is significant at 0.05 level. S&Fe correlation is significant at 0.01 level. Ca shows positive correlation with P, S, K, Se, Br and Sr. On the other hand, K shows negative correlation with Mn, Fe, Cu, Zn and Rb. (Table No-3.5.26)

***Solanum incanum*:** P&Rb correlation is significant at 0.05 level. Ca shows positive correlation with P, S, K, Mn, Cu, Rb and Sr. On the other hand, K shows negative correlation with Fe, Zn and Se. (Table No-3.5.27)

***Acmella oleracea*:** K&Rb, Ca&Fe, Ca&Zn and Fe&Zn correlation is significant at 0.05 level. P&Mn correlation is significant at 0.01 level. Ca shows positive correlation with P, S, Mn, Fe, Cu, Zn, Br and Sr. On the other hand, K shows negative correlation with P, Ca, Mn, Fe, Zn, Se and Sr. (Table No-3.5.28)

***Passiflora edulis L*:** S&Ca and Mn&Se correlation is significant at 0.05 level. Fe&Zn correlation is significant at 0.01 level. Ca shows positive correlation with P, S, Mn and Br. On the other hand, K shows negative correlation with S, Ca, Mn and Br. (Table No-3.5.29)

3.5.8. Correlation among elements in meat foodstuffs

Gallus gallus domesticus: K&Fe correlation is significant at 0.05 level. Mn&Cu and Zn&Se correlation is significant at 0.01 level. Ca shows positive correlation with P, S, Mn, Se and Sr while Cl shows positive correlation with S, K, Mn, Fe, Se and Rb. On the other hand, K shows negative correlation with P, Ca, Cu, Zn and Sr. (Table No-3.5.30)

Labeo rohita: Ca shows positive correlation with P, Mn, Cu, Br and Sr while Cl shows positive correlation with S, K, Fe, Se, Br and Rb. On the other hand, K shows negative correlation with P, Ca, Mn, Cu, Br and Sr. (Table No-3.5.31)

Bos Taurus: S&Cl, K&Fe, Cu&Se, Cu&Sr and Se&Sr correlation is significant at 0.05 level. Ca shows positive correlation with P, S, Cl, K, Fe, Zn, Se, Br, Rb and Sr while Cl shows positive correlation with all the other elements. On the other hand, K shows negative correlation with none of the elements. (Table No-3.5.32)

3.6. OXIDATIVE STRESS AND ANTIOXIDANT VITAMINS STATUS IN THE PATIENTS WITH UROLITHIASIS FROM THE URBAN AREA OF MIZORAM

In order to provide a clinical data and moreover confirmed whether oxidative stress is a bearing in the patients with urolithiasis, a clinical study was carried out to estimate oxidative stress parameters like malondialdehyde(MDA) as an indicator of Lipid peroxidation(LPO), antioxidant enzymes like SOD and CAT and antioxidant vitamins namely vitamin-E and C in the blood samples collected from the patients with urolithiasis. The same parameters mentions *per se* were also estimated in the healthy control counterpart.

3.6.1. Study Groups of the oxidative stress and antioxidant vitamins status: The study group i.e Group-I healthy control and Group-II patients with urolthiais comprised a total number of 90 people. The group II of this study included 50 stone forming patients having obstruction at the ureteropelvic junction and/or vesico-ureteric junction between the age group of 22 – 62 years irrespective of sex. 40 age matched healthy subjects were selected as

control. The study group (Both patients and healthy control) have average age of 40.27 ± 12.91, of which 51 were men and 39 women.

3.6.2. Oxidative stress status

Lipid Peroxidation or Plasma MDA status in blood plasma (nmol/ml): The results of the present study reveals that plasma MDA level estimated as an indicator of LPO was found to be significantly higher in group-II patients with urolithiasis as compared to group-I control. The mean plasma MDA level was estimated as 1.94 ± 0.456nmol/ml in group-I control and. 4.06 ± 0.693nmol/ml in group-II patients with urolithiasis (p<0.001; Table- **3.6.1.)**

Superoxide Dismutase (SOD) status in the blood erythrocytes (Unit/mg Hb): The mean erythrocyte superoxide dismutase activity was recorded as 12.17 ± 1.21 Unit/mg Hb in group-I control and 7.25 ± 1.87 Unit/mg Hb in group-II patients with urolithiasis. There was a significant increase in SOD activity in Group I control than that of the group-II patients with urolithiasis ((p<0.001; Table- **3.6.1.)** .

Catalase(CAT) status in the blood erythrocyte(µmol/l): The mean erythrocyte catalse activity was recorded as 71.4 ± 6.23 µmol/l in group-I control and 56.5 ± 4.98 µmol/l in group-II patients with urolithiasis. There was a significant increase in SOD activity in Group I control than that of the group-II patients with urolithiasis ((p<0.001; Table- **3.6.1.)**.

3.6.3. Antioxidant Vitamins Status:

Vitamin-E (α-tocopherol) status (mg/dl): The mean plasma vitamin-E level was recorded as 1.66 ± 0.23mg/dl in group-I control and 1.59 ± 0.24mg/dl in group-II patients with urolithiasis. There was decrease in vitamin-E level in group-II patients with urolithiasis than that of Group I control. However decrease in the vitamin-E level in the group-II is found to be non significant. A negative correlation was observed between MDA and vitamin-E (r= -0.46, p>0.05), however, the correlation is not significant (p>0.05; Table- 3.6.1 and 3.6.2.).

Vitamin-C (ascorbate) status (mg/dl): The mean plasma vitamin-c level was recorded as 0.47 ± 0.22 mg/dl in group-I control and 0.44 ± 0.24 mg/dl in group-II patients with urolithiasis. There was decrease in vitamin-c level in group-II patients with urolithiasis than that of Group I control. However decrease in the vitamin-c level in the group-II is also found to be non significant. A negative correlation was observed between MDA and vitamin-c (($r = -0.116$, $p>0.05$), however, the correlation is not significant ($p>0.05$; Table- 3.6.1 and 3.6.2).

Table 3.1.1. Ca, K, Cl, P and S concentration (mg/Kg) of the Selected Vegetable Foodstuffs of Mizoram. Values are mean ± SEM, 6 observations each.

Name of the Vegetable Foodstuffs analyzed	Calcium (Ca)	Potassium (K)	Chlorine (Cl)	Phosphorus (P)	Sulphur (S)
Clerodendron colebrookianum	20536.7 ± 312.3	14360.5 ±74.7	3363.3 ± 39.2	3100.2 ±76.3	3481.2 ±103.1
Brassica juncea	14380.5 ± 148.0	22392.5 ±322.3	10410.2 ±405.5	3599.4 ± 230.3	5625.0 ± 16.7
Zanthoxylum rhetsa L.	5411.6 ± 233.3	22143.4 ± 247.7	6853.2 ±138.4	3048.1 ± 18.9	2626.0 ± 46.3
Vigna unguiculata L.	8145.8 ±105.2	22614.6 ± 494.4	5194.3 ± 113.8	4140.7 ±167.5	2383.5 ±35.3
Cucurbita pepo L.	18914.2 ±289.6	25057.9 ± 394.7	9648.5 ±408.4	3965.9 ±178.9	2758.2 ±136.1
Gnetum gnemon L.	3917.4 ± 95.6	24150.5 ±433.2	5004.2 ± 258.6	3967.6 ± 475.0	4189.2 ±113.8
Cucurbita maxima	34730.4 ±1443.9	21376.1 ± 441.0	9965.1 ± 254.5	4755.1 ± 302.4	2041.4 ±16.3
Colocasis esculenta L.	7058.5 ± 246.0	42883.6 ± 2215.4	20434.5 ± 2373.6	4444.6 ±123.6	1705.5 ± 287.1
Musa paradisiaca	5837.9±317.7	38785.6±983.8	9264.3±701.6	4858.8±300.6	2944.8±143.8
Bambusa vulgaris	ND*	34157.2±413.7	17587.4±587.9	5010.7±183.1	1828.5±19.4
Acacia gagaena	2483.2±59.1	12549.1±111.1	579 ± 1.0	7833.6±143.7	28913.9±976.3
Dysoxylum gobara merr	4169.2±11.5	23284.6±174.7	580.4±1.4	5141.7±164.5	11107.8±266.4
Solanum anguivi	3338.3±106.5	19124.1±519.1	8098.6±488.5	3152.5±181.1	1750.4±121.6
Calamus sp	3885.9±42.9	26183.8±351.2	20292.6±1090.6	4764.6±262.9	1953.5±67.9
Glycine max merr	3221.4±139.5	16007.2±226.5	579±0	8257.8±325.7	3556.3±83.9
Parkia timoriana	6192.5±47.1	8701.1±41.7	579±0	2027.4±19.1	31870.8±631.8
Brassica oleracea	5637.5±86.4	36540.5±201.4	ND*	9180.6±212.3	8884.7±196.1
Solanum nigrum linn	16659.7±329.5	42180.9±789.3	ND*	9816.7±125.1	7577.9±333.1
Brassica oleracea	5088.8±171.2	46151.5±326.8	ND*	13272.4±82.3	9889.9±62.7
Solanum melongena	3493.8±162.9	38609.5±809.7	ND*	8288.3±127.6	3112.2±67.3
Solanum incanum	2608.9±195.1	30730.6±1177.6	ND*	75861.1±436.6	2538±127.7
Acmella oleracea/ Spilanthes acmella	12542.9±346.2	46224.4±690.6	ND*	12310.1±312.8	5608.2±116.1
Passiflora edulis L	16635±516.5	24233±781.4	ND*	7034.4±236.6	6637.2±14.7

*ND: Not detected

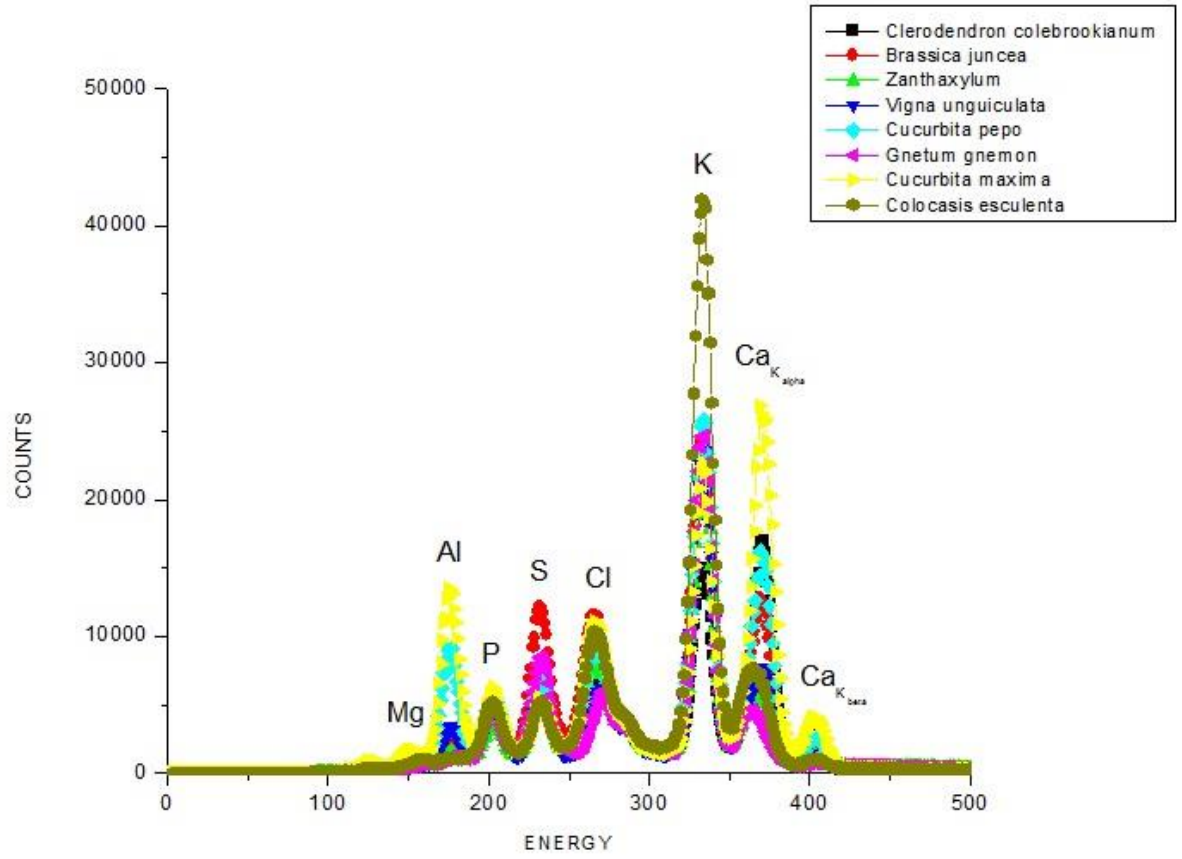

Fig. 3.1.1(a). ED-XRF spectra of *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L, Vigna unguiculata L, Cucurbita pepo L, Gnetum gnemon L, Cucurbita maxima, Colocasis esculenta L*.

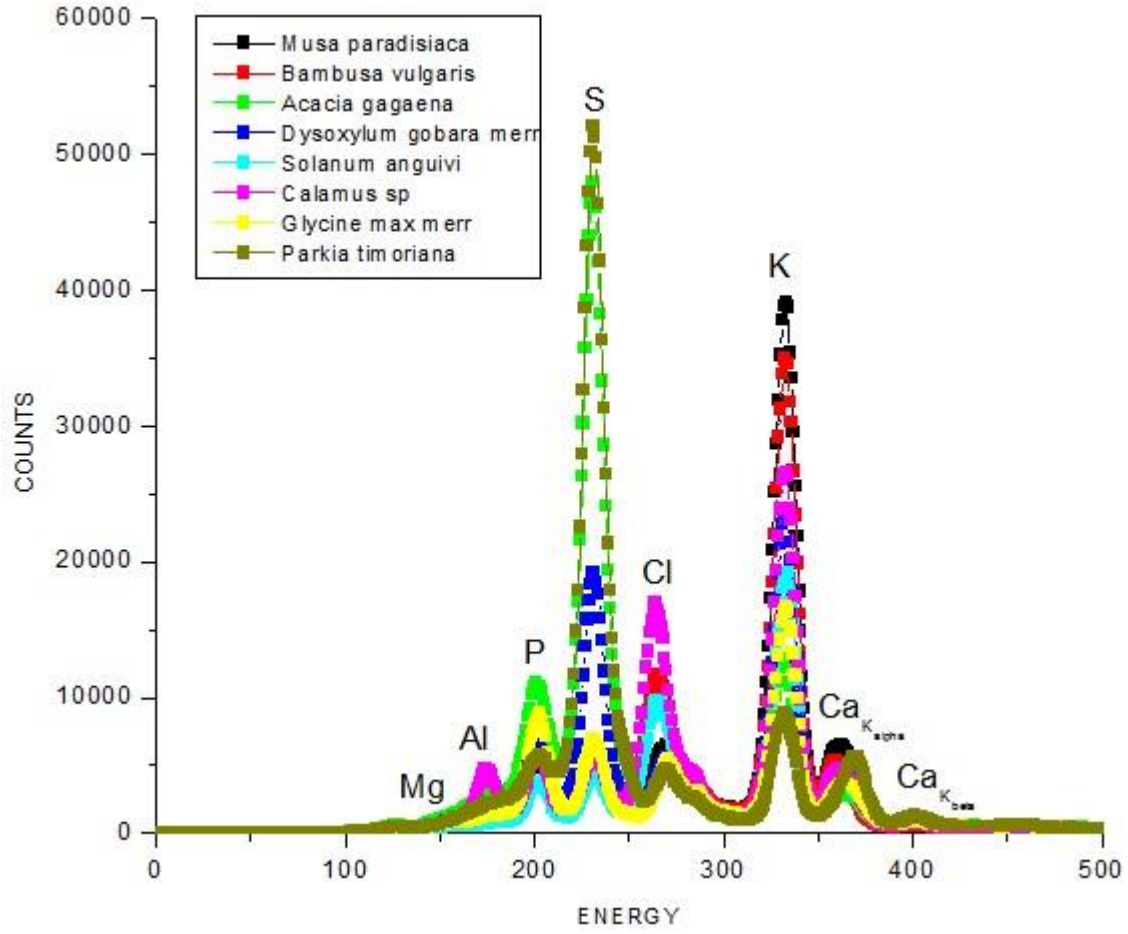

Fig. 3.1.1(b). ED-XRF spectra of *Musa paradisiaca, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana.*

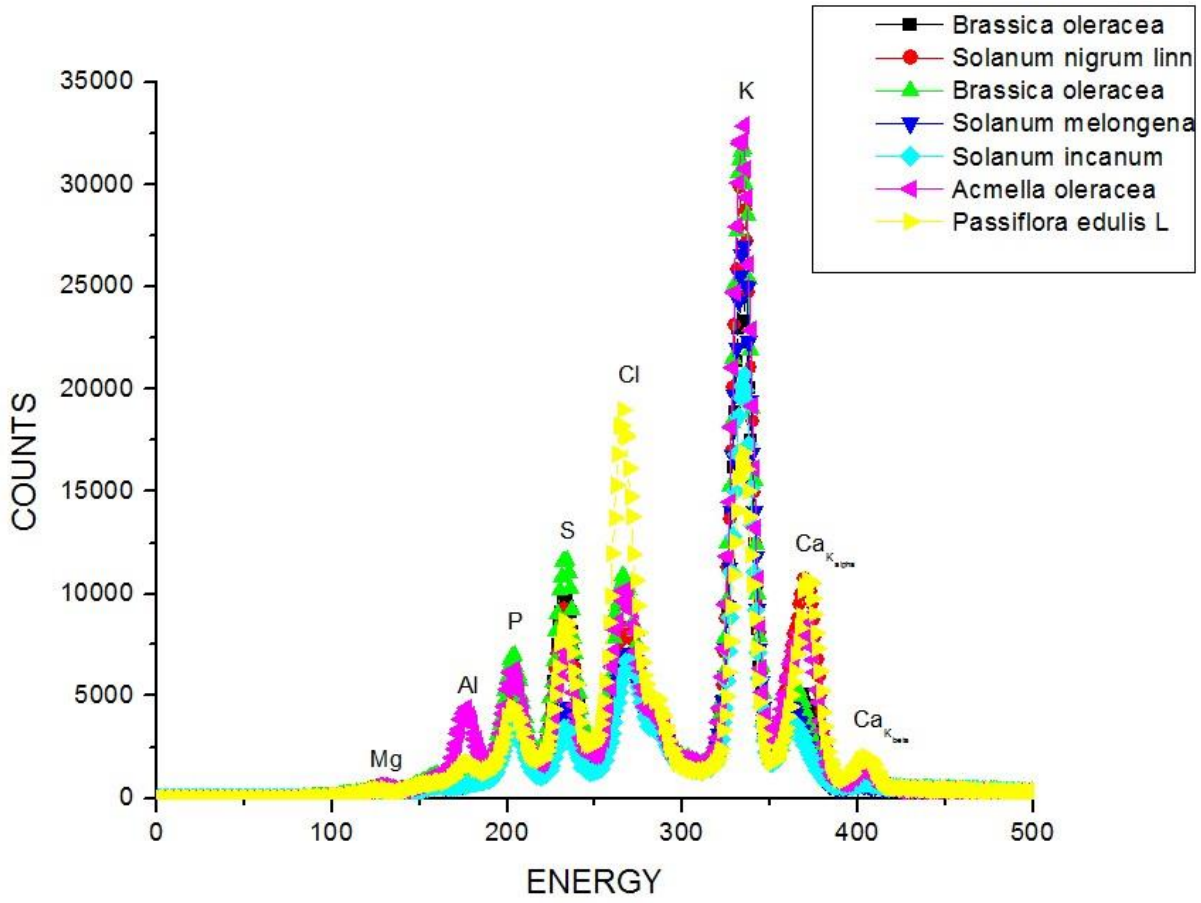

Fig. 3.1.1(c). ED-XRF spectra of *Brassica oleracea, Solanum nigrum linn, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea, Passiflora edulis L.*

Table 3.1.2. Fe, Mn, Zn, Cu and Se concentration (mg/Kg) of the Selected Vegetable Foodstuffs of Mizoram. Values are mean ± SEM, 6 observations each.

Name of the Vegetable Foodstuffs analyzed	Iron (Fe)	Manganese (Mn)	Zinc (Zn)	Copper (Cu)	Selenium (Se)
Clerodendron colebrookianum	246.6 ± 41.7	96.3 ± 6.1	35.7 ± 2.6	14.5 ± 0.91	0.31 ± 0.19
Brassica juncea	316.7 ± 15.7	67.9 ± 2.9	46.8 ± 4.2	4.3 ± 0.37	0.15 ± 0.07
Zanthoxylum rhetsa L.	161.9 ± 10.3	285.9 ± 18.7	28.4 ± 1.0	13.4 ± 0.72	0.18 ± 0.05
Vigna unguiculata L.	158.9 ± 10.47	90.8 ± 2.42	31.8 ± 0.50	10.6 ± 1.12	0.85 ± 0.42
Cucurbita pepo L.	272.9 ± 13.5	24.0 ± 1.5	36.0 ± 1.40	5.4 ± 0.85	0.24 ± 0.004
Gnetum gnemon L.	161.7 ± 16.8	241.7 ± 4.1	41.2 ± 1.05	5.6 ± 0.69	0.12 ± 0.00
Cucurbita maxima	821.5 ± 55.9	26.3 ± 2.07	40.8 ± 0.36	4.9 ± 0.13	0.12 ± 0.00
Colocasis esculenta L.	109.7 ± 7.4	98.8 ± 10.4	104.4 ±1.7	8.7 ± 0.82	0.12 ± 0.00
Musa paradisiaca	64.75±9.61	292.38±12.36	46.35±2.06	10.95±0.64	0.12±0
Bambusa vulgaris	53.95±4.49	28.87±1.59	57.92±1.2	9.35±0.7	0.12±0
Acacia gagaena	237.67±20.14	78.00±3.25	53.98±0.48	7.44±1.00	0.48±0
Dysoxylum gobara merr	92.70±4.49	104.50±2.98	56.21±2.39	18.59±0.92	0.85±0.53
Solanum anguivi	74.2±0.92	32.52±2.39	19.05±0.06	14.02±1.08	0.12±0
Calamus sp	65.51±4.68	288.57±6.32	142.51±0.26	27.07±0.61	0.12±0
Glycine max merr	149.40±29.94	35.89±0.43	75.46±6.23	20.11±0.57	0.17±0
Parkia timoriana	54.00±1.6	15.65±1.32	21.63±0.31	8.16±0.45	1.74±0.72
Brassica oleracea	181.1±77.6	17.1±1.2	42.5±8.1	11.8±1.2	0.21±0.03
Solanum nigrum linn	577.2±110.6	89.4±6.5	59.1±9.9	11.3±0.1	0.23
Brassica oleracea	1377.2±7.7	58.6±4.3	149.7±47.5	21.5±7.4	0.14±0.05
Solanum melongena	710.9±153.7	10.9±2.1	71.3±10.3	42.8±6.4	0.23
Solanum incanum	444.3±121.3	14.9±1.4	40.4±3.5	29.1±2.1	0.23
Acmella oleracea/ Spilanthes acmella	736.1±90.8	140±3.9	152.5±8.9	10.2±0.7	13.9±0.1
Passiflora edulis L	828.9±26.1	59.6±4.3	53.1±3.1	13.4±2.5	0.7±0.3

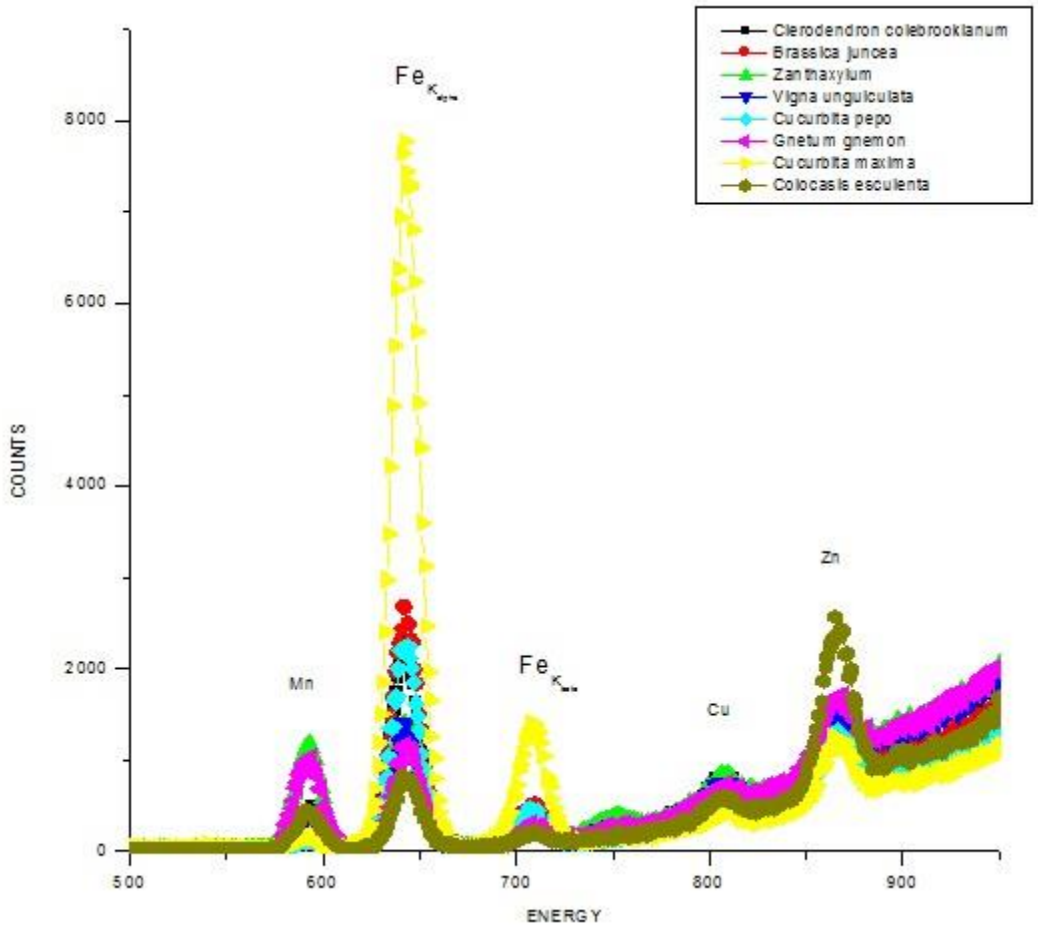

Fig. 3.1.2(a). ED-XRF spectra of *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L, Vigna unguiculata L, Cucurbita pepo L, Gnetum gnemon L, Cucurbita maxima, Colocasis esculenta L*.

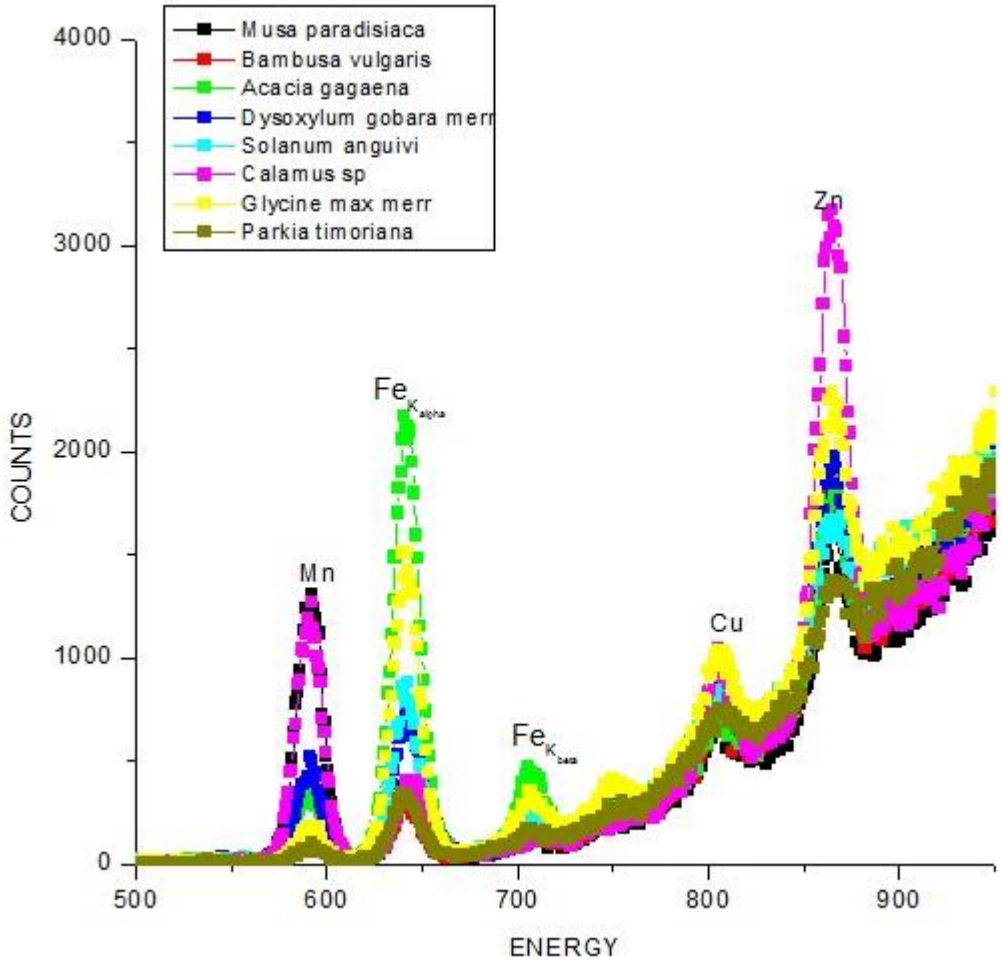

Fig. 3.1.2(b). ED-XRF spectra of *Musa paradisiaca, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana.*

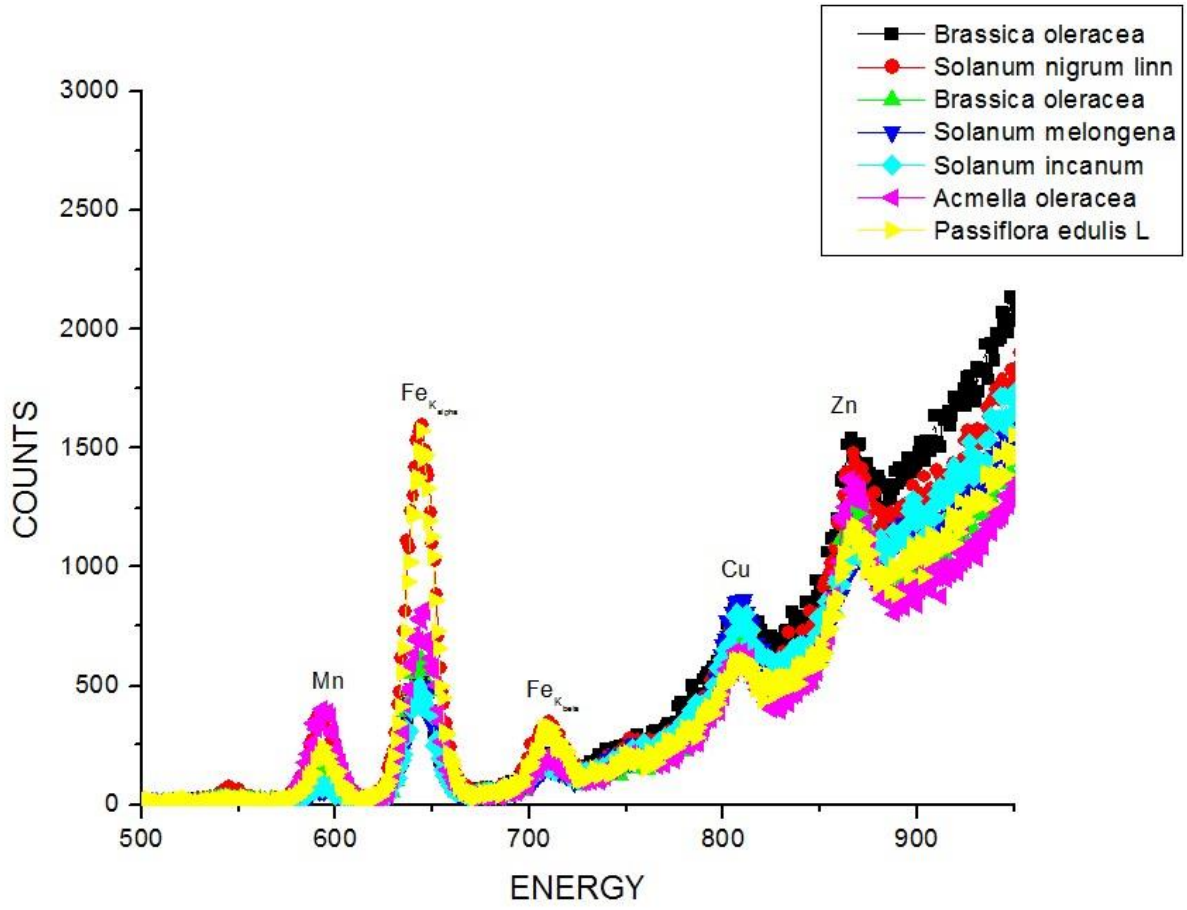

Fig. 3.1.2(c). ED-XRF spectra of *Brassica oleracea, Solanum nigrum linn, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea, Passiflora edulis L.*

Table 3.1.3. Sr, Rb and Br concentration (mg/Kg) of the Selected Vegetable Foodstuffs of Mizoram. Values are mean ± SEM, 6 observations each.

Name of the Vegetable Foodstuffs analyzed	Strontium (Sr)	Rubidium (Rb)	Bromine (Br)
Clerodendron colebrookianum	63.4 ± 1.4	5.1 ± 0.43	12.9 ± 0.92
Brassica juncea	121.5 ± 3.54	35.4 ± 1.27	36.8 ± 2.0
Zanthoxylum rhetsa L.	38.1 ± 2.76	21.7 ± 1.72	13.0 ± 0.65
Vigna unguiculata L.	50.8 ± 2.05	24.2 ± 0.87	16.6 ± 0.89
Cucurbita pepo L.	67.4 ± 4.00	13.8 ± 0.28	9.4 ± 0.58
Gnetum gnemon L.	9.5 ± 1.34	51.2 ± 1.05	1.7 ± 0.70
Cucurbita maxima	122.5 ± 3.70	13.5 ± 1.04	10.5 ± 0.65
Colocasis esculenta L.	18.5 ± 1.08	33.2 ± 1.35	1.5 ± 0.68
Musa paradisiaca	32.14±0.37	25.00±1.54	5.15±0.56
Bambusa vulgaris	5.15±1.01	44.33±1.48	13.96±2.3
Acacia gagaena	7.05±0.24	10.07±1.09	2.57±0.05
Dysoxylum gobara merr	16.05±0.24	45.34±2.38	3.19±0.78
Solanum anguivi	13.5±0.81	8.68±1.84	13.37±5.25
Calamus sp	42.42±2.42	155.25±6.8	70.89±3.85
Glycine max merr	8.07±1.81	22.27±0.81	3.14±0.03
Parkia timoriana	24.92±0.53	14.82±2.05	1.46±0.12
Brassica oleracea	21.9±2.2	35.2±1.2	1.8±0.3
Solanum nigrum linn	91.5±5.2	20.7±0.7	15.3±1.4
Brassica oleracea	10.7±3.3	76.9±1.1	6.8±1.0
Solanum melongena	6.8±2.7	12.9±1.7	20.9±0.8
Solanum incanum	8.7±3.5	12.1±0.8	23.6±3.1
Acmella oleracea/ Spilanthes acmella	58.1±2.4	16.2±3.0	110.4±2.2
Passiflora edulis L	51.2±2.1	23.4±3.9	38.5±1.3

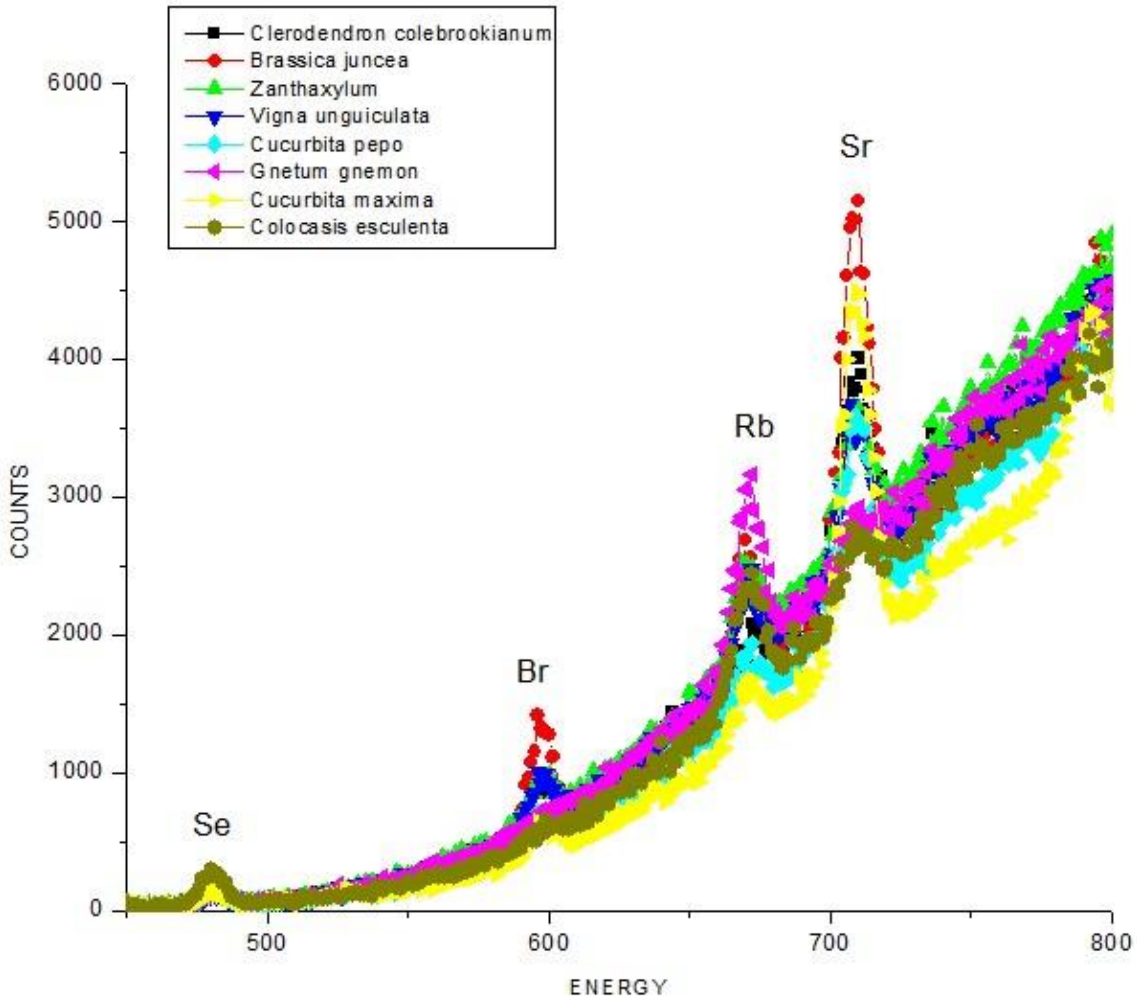

Fig. 3.1.3(a). ED-XRF spectra of *Clerodendron colebrookianum, Brassica juncea, Zanthoxylum rhetsa L, Vigna unguiculata L, Cucurbita pepo L, Gnetum gnemon L, Cucurbita maxima, Colocasis esculenta L*.

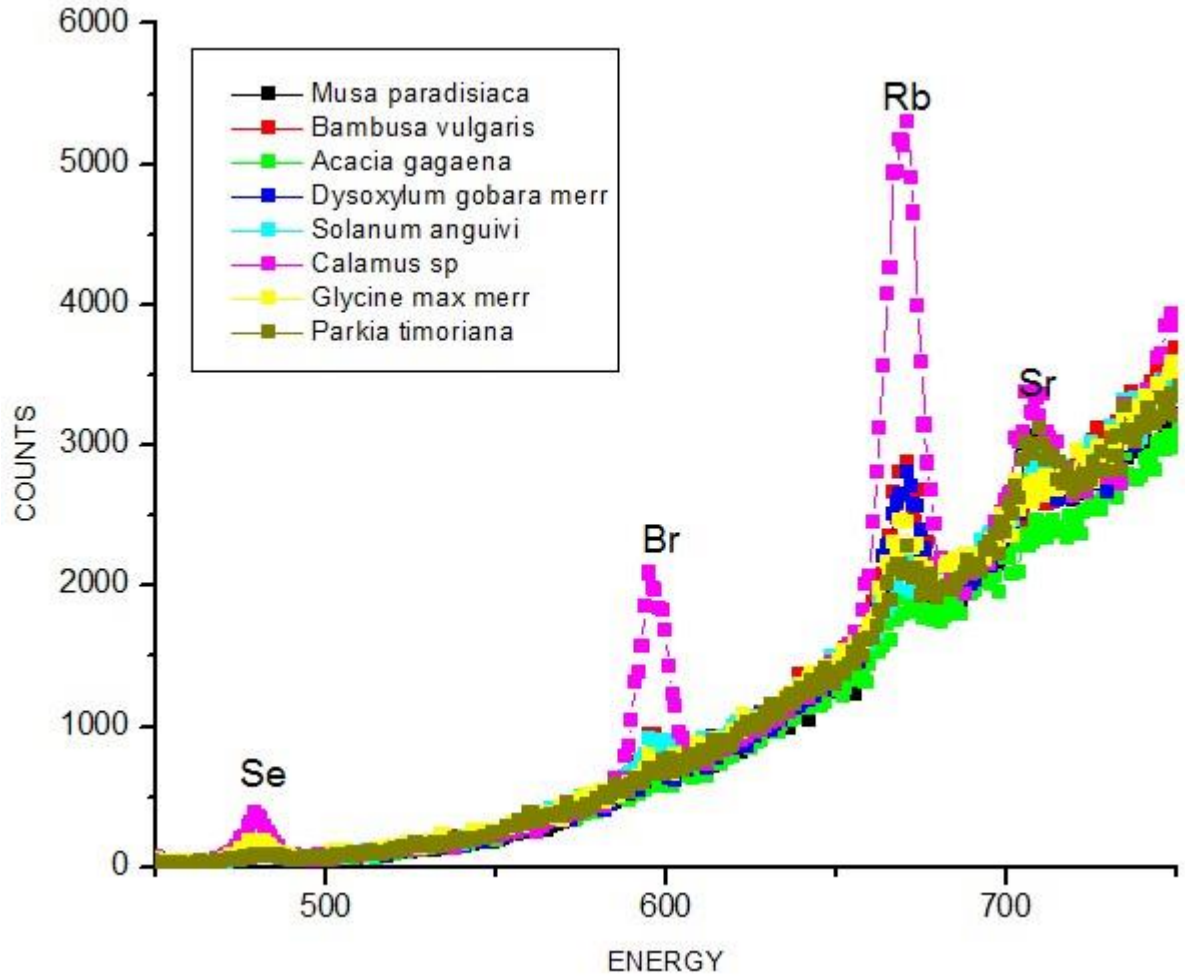

Fig. 3.1.3(b). ED-XRF spectra of *Musa paradisiaca, Bambusa vulgaris, Acacia gagaena, Dysoxylum gobara merr, Solanum anguivi, Calamus sp, Glycine max merr, Parkia timoriana.*

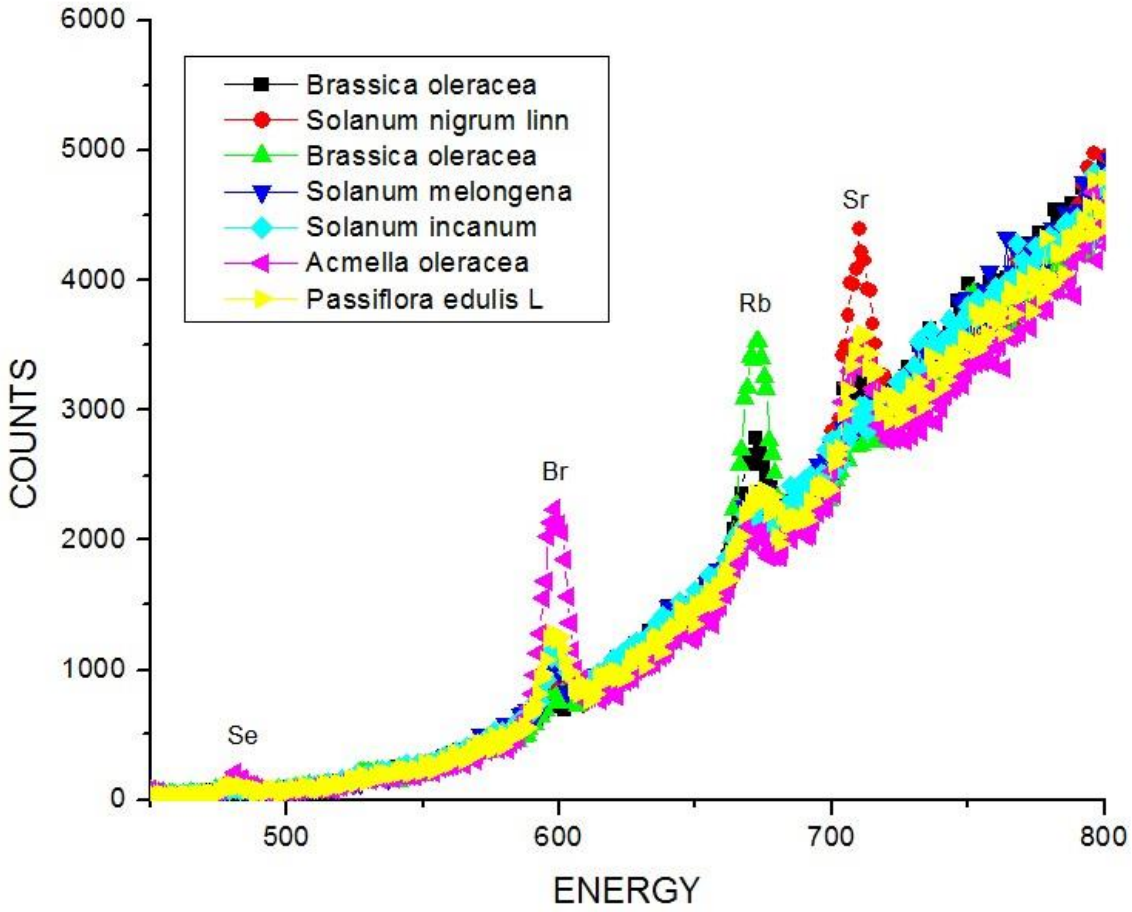

Fig. 3.1.3(c). ED-XRF spectra of *Brassica oleracea, Solanum nigrum linn, Brassica oleracea, Solanum melongena, Solanum incanum, Acmella oleracea, Passiflora edulis L.*

Table 3.2.1 Ca, K, Cl, P and S concentration (mg/Kg) of the meat samples of Aizawl.

Values are mean ± SEM, 6 observations each.

Name of the meat sample analyzed	Calcium (Ca)	Potassium (K)	Chlorine (Cl)	Phosphorus (P)	Sulphur (S)
Gallus gallus domesticus	1667.6±197.5	2532.6 ±58.2	544.5 ±3.5	1788.5 ±71.4	1676 ±93.7
Labeo rohita	1993.4 ± 291.5	2623.3 ±41.4	549.4 ±2	1894.9 ± 124.6	1648.8 ± 44.3
Bos taurus	413.1 ± 21.6	3365.8 ± 89.6	594.7 ±6.2	1493.1 ± 91.9	1666.2 ± 139.2

Table 3.2.2. Fe, Mn, Zn and Cu concentration (mg/Kg) of the meat samples of Aizawl.

Values are mean ± SEM, 6 observations each.

Name of the meat sample	Iron (Fe)	Manganese (Mn)	Zinc (Zn)	Copper (Cu)	Selenium (Se)
Gallus gallus domesticus	72.9 ± 5.1	0.3 ± 0.1	64.9 ± 7.3	3.4 ± 0.4	1.8 ± 0.1
Labeo rohita	47.9 ± 2.3	1.1 ± 0.3	46.2 ± 0.7	4.8 ± 0.6	0.12
Bos Taurus	161.2 ± 6.2	ND*	198.1 ± 4.2	6.8 ± 1.4	0.17

ND*: Not Detected

Table 3.2.3. Sr, Rb and Br concentration (mg/Kg) of the meat samples of Aizawl. Values are mean ± SEM, 6 observations each.

Name of the meat sample analyzed	Strontium (Sr)	Rubidium (Rb)	Bromine (Br)
Gallus gallus domesticus	11.4 ± 1.8	43.2 ± 3.2	3.4 ± 0.7
Labeo rohita	91.8 ± 5.9	8.6 ± 1.7	10.2 ± 0.9
Bos Taurus	ND*	26.2 ± 1.7	17.5 ± 0.8

ND*: Not detected

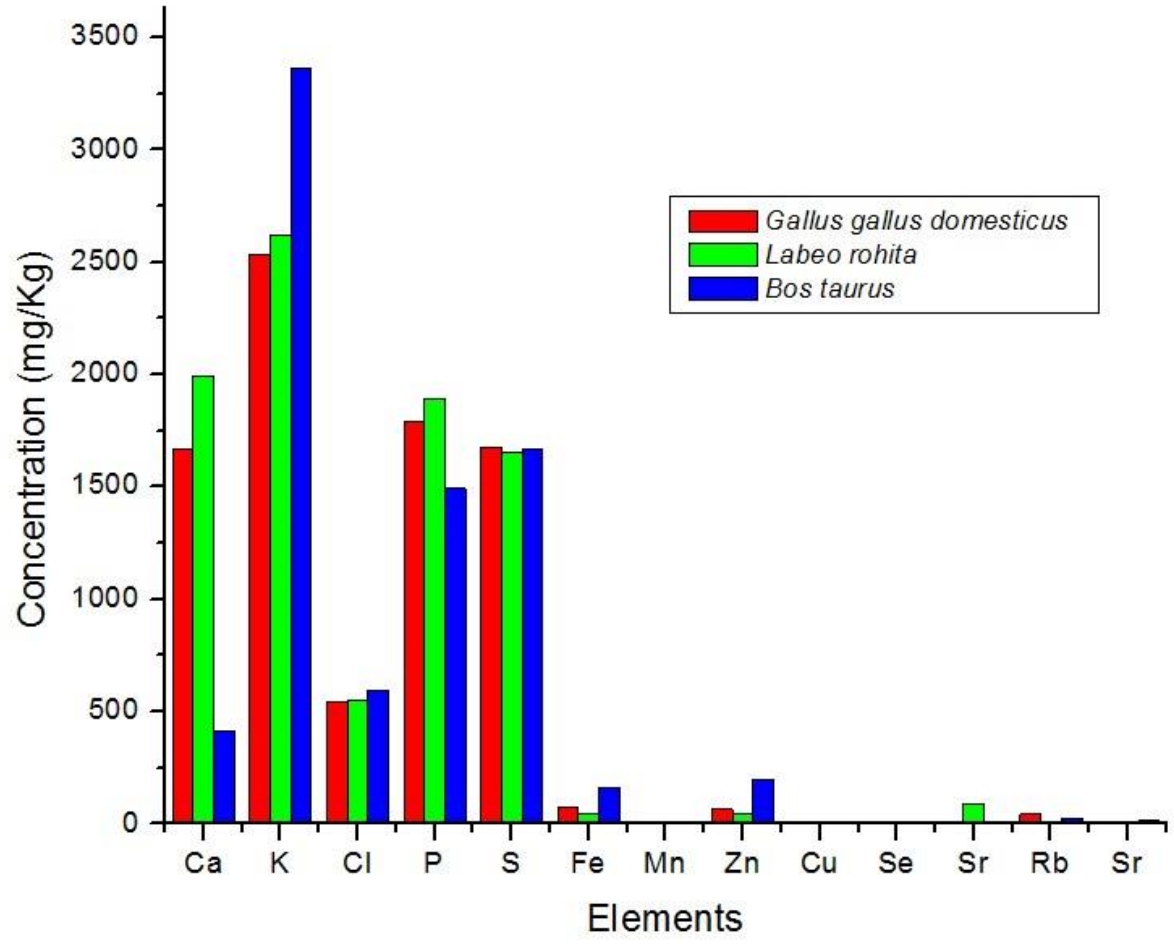

Fig. 3.2.1. Spectra of *Gallus gallus domesticus, Bos Taurus, Labeo rohita.*

Table 3.3. Fluoride content of drinking water collected from various sources.

Sl. No.	Water Source	Fluoride content (mg/L)
1	Bawngkawn	**0.762**
2	Republic (Lungli)	**0.845**
3	Ramhlun Venglai	**0.487**
4	Durtlang	**0.292**
5	Dawrpui vengthar	**0.628**
6	Zohnuai	**1.049**
7	Vaivakawn	**0.409**
8	Tuikual South	**0.626**
9	Hunthar	**0.288**
10	Edenthar	**1.362**
11	Saron Veng	**0.460**
12	Tuivamit	**0.809**
13	Chawlhhmun	**0.672**
14	Zotlang	**0.991**
15	Zotlang (pump)	**0.051**
16	Tuikual (pump)	**0.819**
17	Zonuam	**0.492**
18	Govt. Complex (Midumtui)	**0.287**
19	Chhangur kawn (pump)	**1.192**
20	Govt. Complex (Field tui)	**0.290**
21	Tuikual	**1.161**
22	Zotlang Tlangte	**0.845**
23	Republic (Khurpui)	**0.365**
24	Mizoram University (MZU)	**0.362**
25	Govt. Water Supply (PHE)	**0.776**
26	Rain water	**0.572**

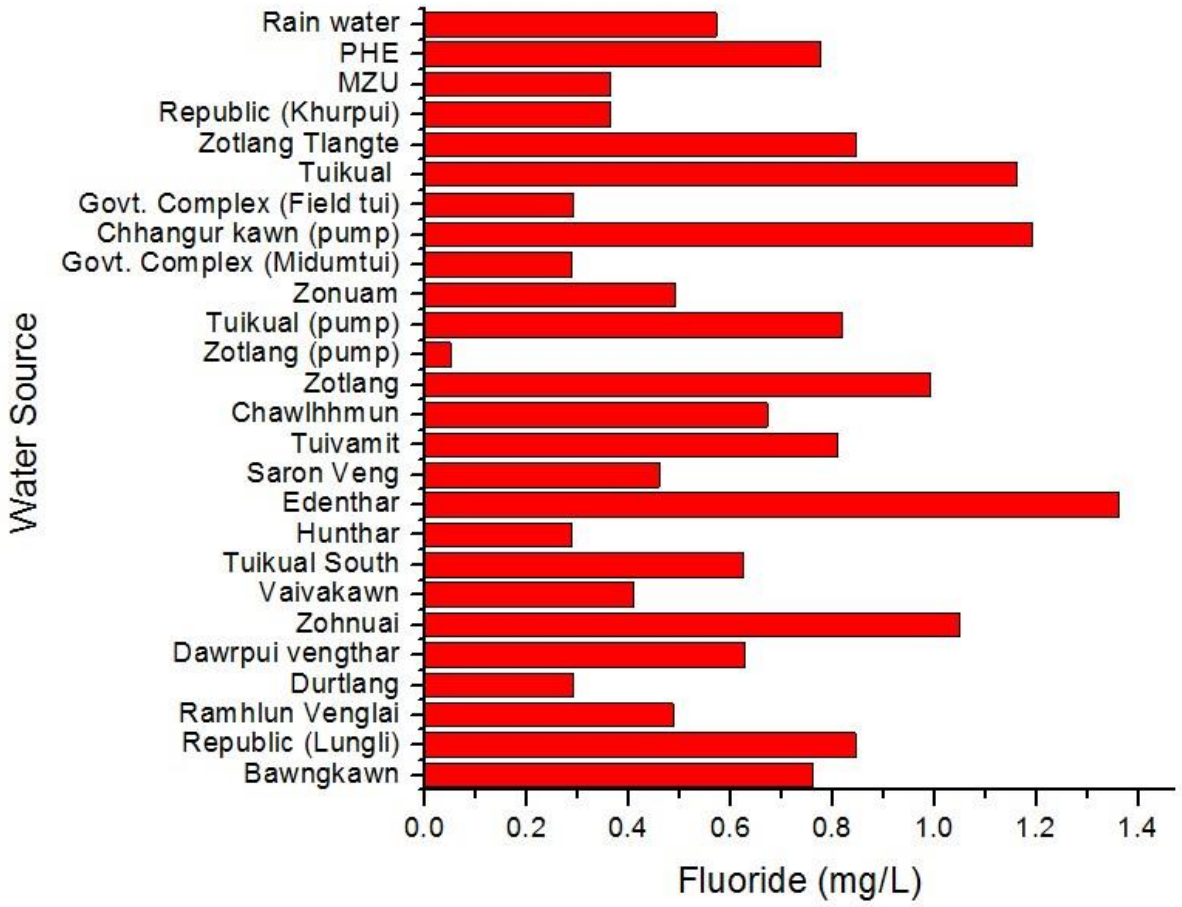

Fig. 3.3. Spectra of fluoride content in drinking water samples.

Table 3.4.1. Ca, K, Cl, P and S concentration (mg/Kg) of the Urolithiatic stones of Mizoram. Values are mean ± SEM, 6 observations each.

No of Urinary stone samples Analyzed	Phosphorus P	Sulphur S	Chlorine Cl	Potassium K	Calcium Ca
1	745.1±79.1	344.5±67.8	145.4±0.03	954.2±44.6	3110.9±184.3
2	414.8±31.1	208.2±38.5	87.4±4.5	903.0±6.9	2312.3±199.9
3	1368.4±53.4	829.5±48.3	ND*	764.1±12.1	118809.4±1968.7
4	4214.6±136.2	138.4±19.4	ND*	826.9±19.7	110823.9±1229.0
5	8919.66±675.9	ND*	ND*	804.54±13.5	106710.8±2320.1
6	14808.69±286.8	ND*	ND*	804.4±30.6	104029.2±2031.7
7	35923.5±1580	ND*	ND*	844.9±42.3	94340.2±3446.6
8	4601.5±187.1	30.5±3.3	ND*	924.8±23.8	109241.7±1824.7
9	61.9±10.5	35.7±1.3	ND*	725.96±0	939.4±30.1
10	172.9±43.1	179.9±36.5	579±0	846.7±19.8	51857.3±1711.3
11	35227.3±705.7	ND*	ND*	1278.8±30.2	87367.9±1282.4
12	53.9±28.6	399.5±19.5	ND*	725.96±0	2809.9±79.4
13	2262.2±207	1298.3±364.7	198.26±0	2584.1±1394.1	102246±5060.9
14	5718.9±318.4	472.9±15.5	57.1±22.8	853.82±49.1	17200.2±332.4
15	2067.1±143	176.2±22.6	579±0	776.2±100.6	6934.4±121.8
16	242.7±19.8	ND*	579±0	886.1±99.3	30872.2±689.2
17	445.0±45.7	ND*	579±0	807.7±8.4	30929.4±522.2
18	1302.3±114.4	ND*	579±0	770.3±12.3	30840.4±402.7
19	4372.3±83.5	ND*	579±0	745.8±17.7	28827.3±605.9

ND*: Not detected

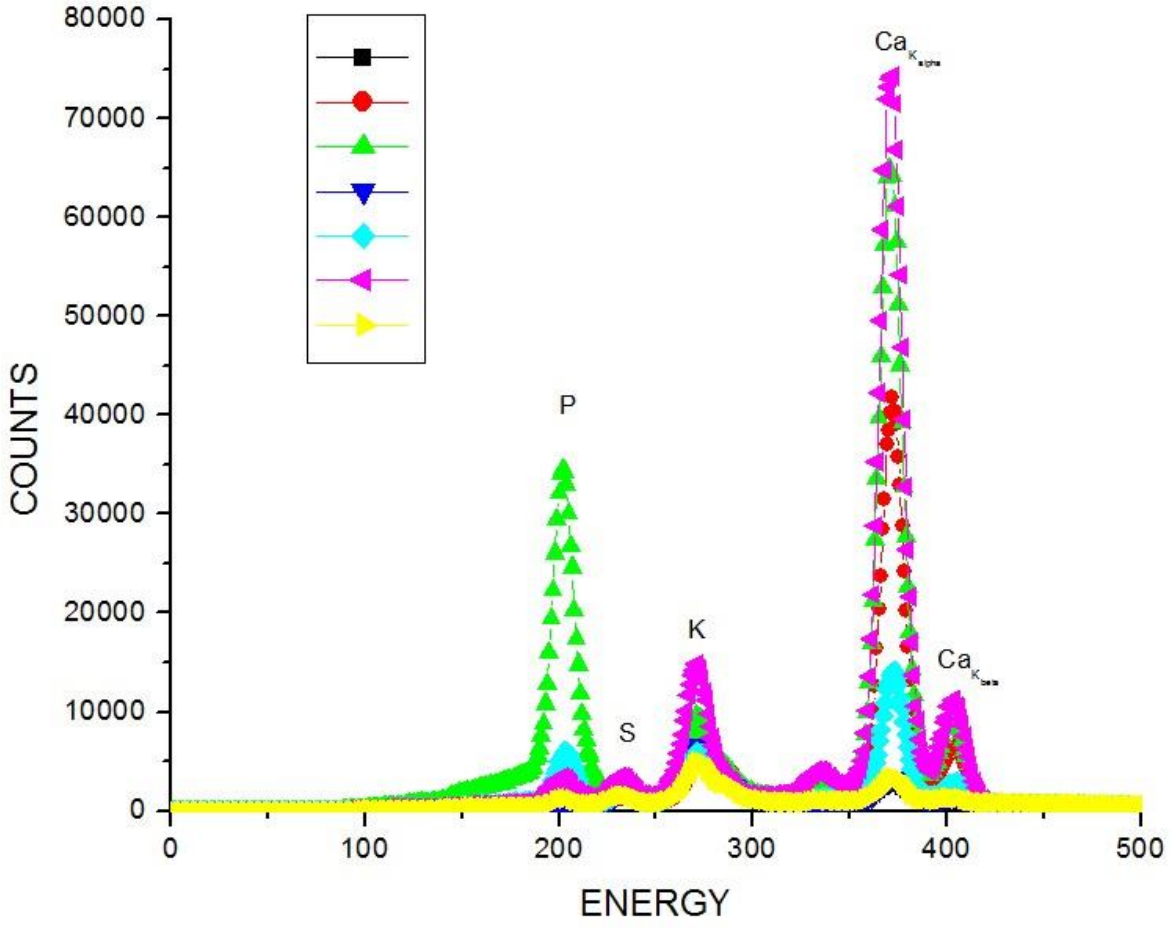

Fig. 3.4.1. (a) ED-XRF spectra of Urolithiatic stones

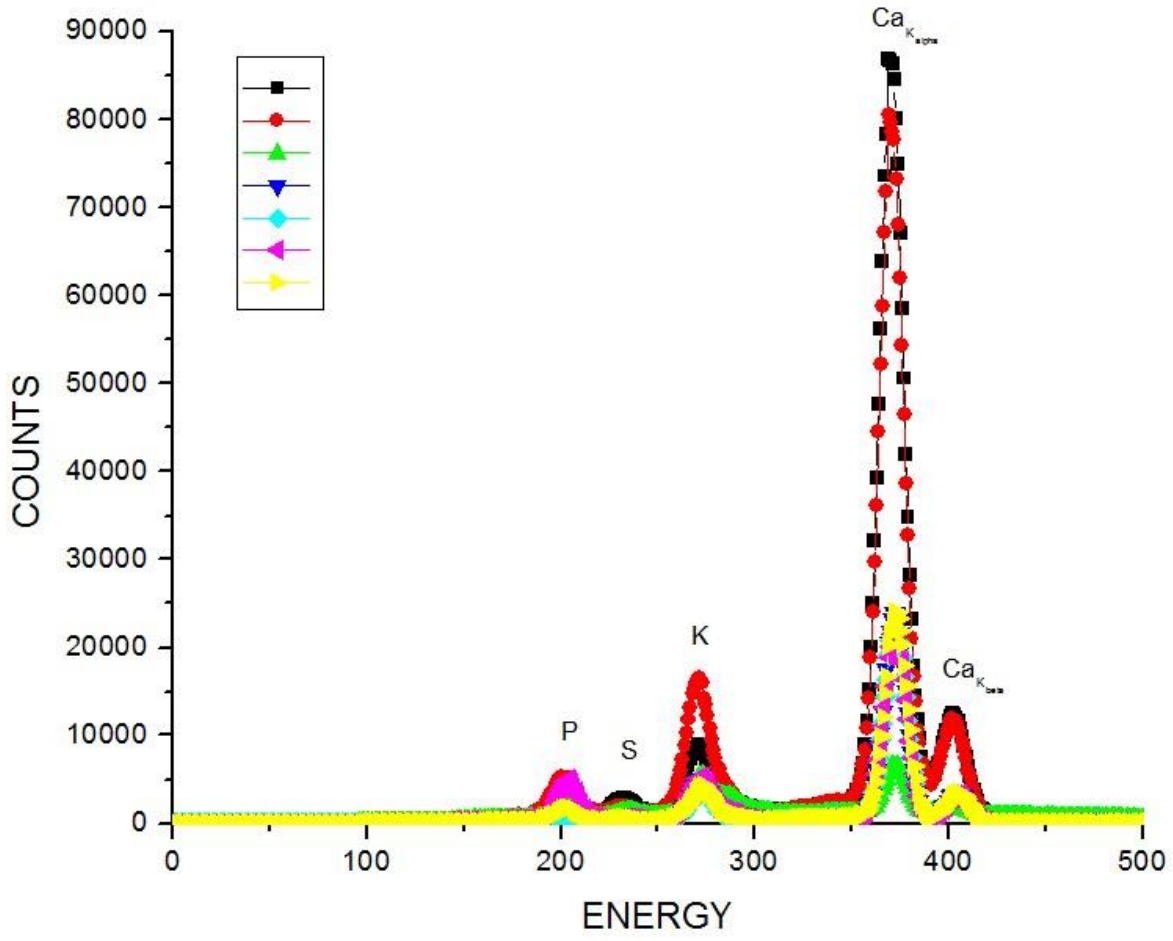

Fig. 3.4.1. (b) ED-XRF spectra of Urolithiatic stones

Table 3.4.2. Fe, Mn, Zn, Cu and Se concentration (mg/Kg) of the Urolithiatic stones of Mizoram. Values are mean ± SEM, 6 observations each.

No. of Urinary stone samples Analyzed	Manganese Mn	Iron Fe	Copper Cu	Zinc Zn	Selenium Se
1	30.3±4.2	43.1±3.6	29.7±1.5	2.1±0.4	0.12±0
2	18.7±1.2	37.8±0.1	16.5±1.5	3.3±1.7	0.12±0
3	580.3±13.2	110.7±3.7	78.4±1.8	17.7±1.1	0.12±0
4	0.12±0	37.7±1.2	ND*	66.5±1.5	0.12±0
5	ND*	41.6±3.8	ND*	32.9±1.4	0.3±0.1
6	ND*	38.3±2.6	ND*	95.4±4.5	0.12±0
7	ND*	70.7±31.2	0.9±0.2	107.8±2.3	0.4±0.2
8	ND*	33.9±2.8	ND*	18.8±0.9	0.12±0
9	9.4±0.6	38.5±10.6	13.4±2.2	1.4±1.1	1.0±0.6
10	100.3±3.2	49.6±5.4	16.7±1.0	1.8±0.7	0.3±0.1
11	ND*	35.8±4.7	4.7±0.9	212.8±0.9	0.5±0.1
12	14.6±1.4	62.7±4.4	364.1±18.7	ND*	0.4±0.1
13	ND*	30±2.9	ND*	14.7±1.2	0.1±0
14	145.6±5.1	72.5±0.7	31.1±1.4	7.4±1.4	0.12±0
15	21.5±1.3	32.3±0.1	8.8±1.3	2.3±0.1	0.2±0
16	47.2±3.3	33.7±3.2	ND*	6.3±0.6	0.7±0.3
17	ND*	27.7±2.1	ND*	7.9±0.2	0.12±0
18	ND*	28.9±1.4	0.19	20.6±0.9	0.12±0
19	ND*	30.0±2.3	ND*	127.8±2.3	0.12±0

ND*: Not detected

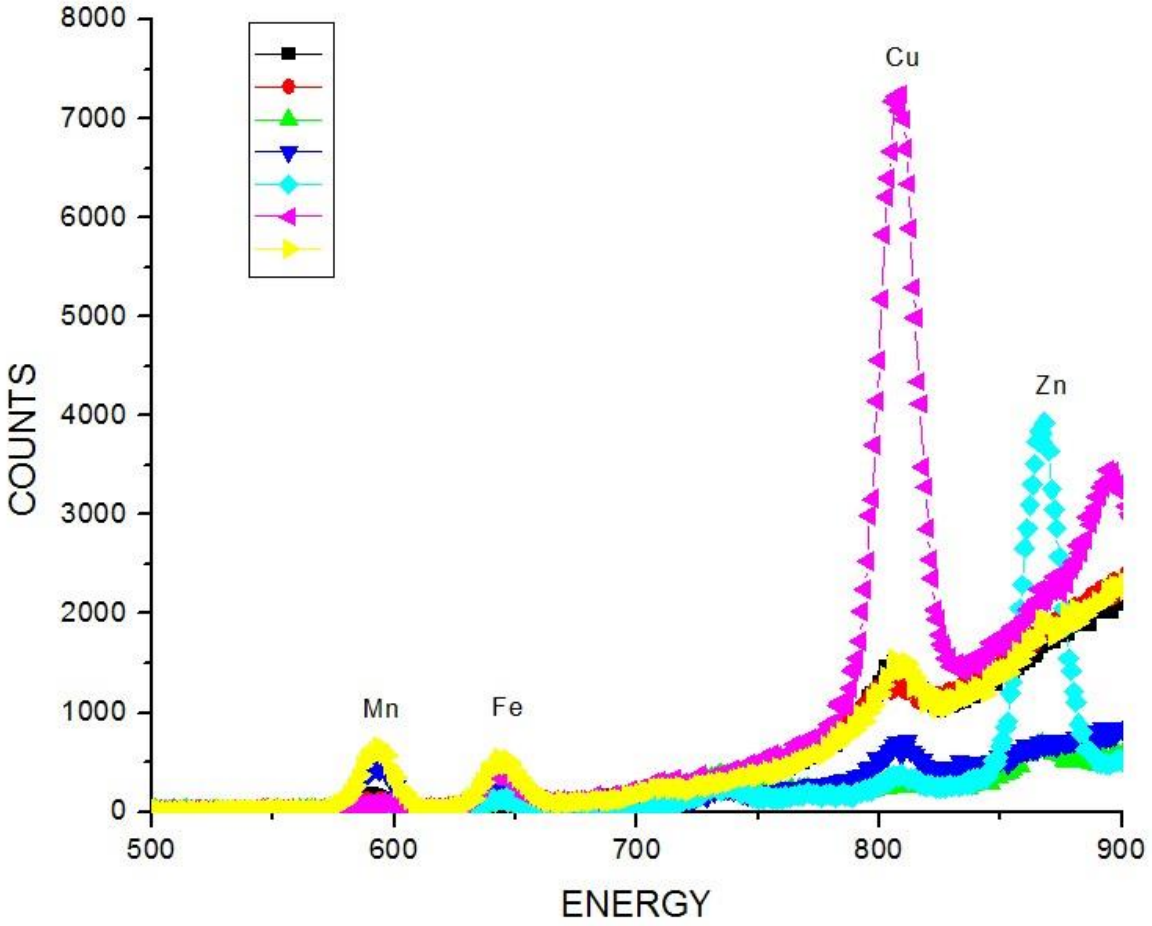

Fig. 3.4.2. (a) ED-XRF spectra of Urolithiatic stones

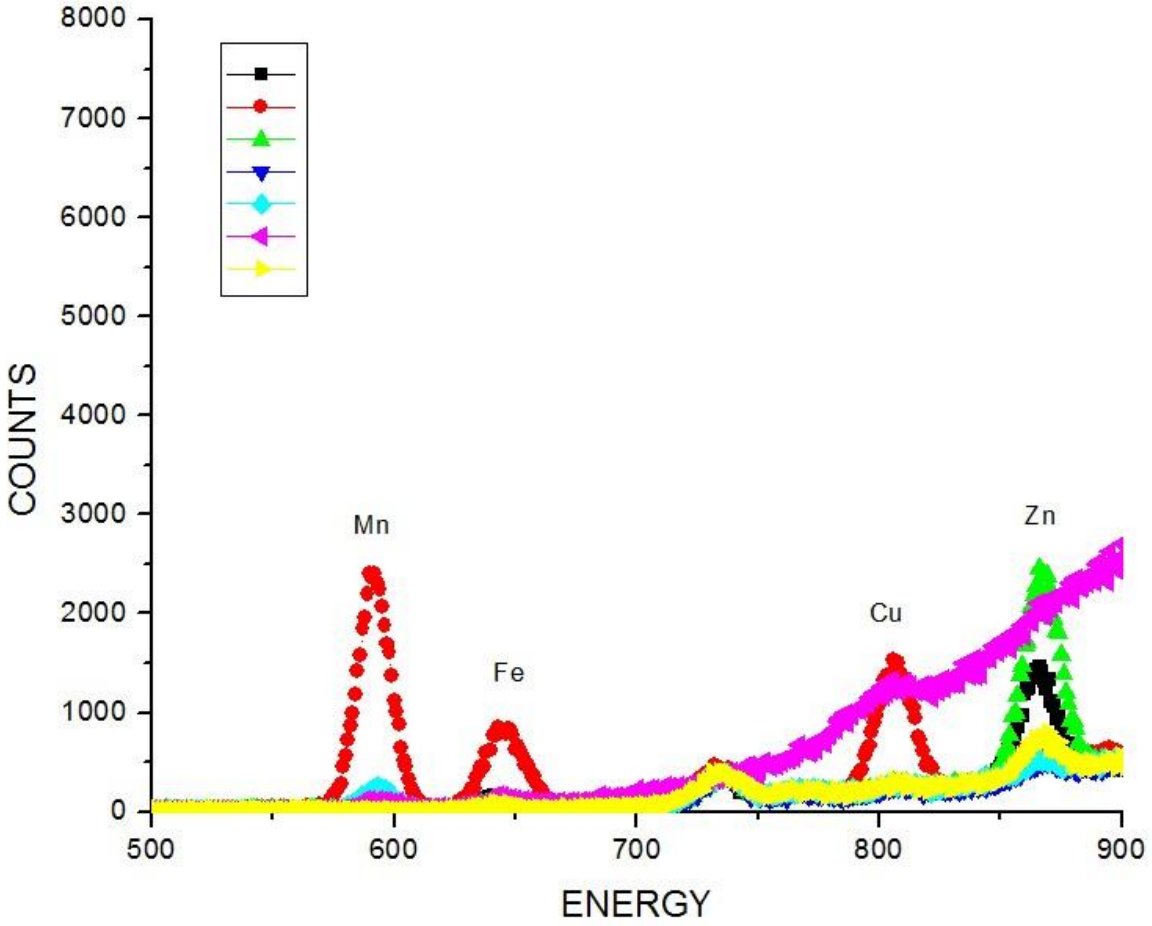

Fig. 3.4.2. (b) ED-XRF spectra of Urolithiatic stones

Table 3.4.3. Sr, Rb and Br concentration (mg/Kg) of the Urolithiatic stones of Mizoram.

Values are mean ± SEM, 6 observations each.

No. of Urinary stone samples Analyzed	Bromine Br	Rubidium Rb	Strontium Sr
1	0.9±0.2	ND*	0.12±0
2	3.5±1.7	ND*	1.75±0
3	ND*	ND*	46.7±0.5
4	1.1±0.2	ND*	29.9±2.2
5	1.1±0.9	ND*	28.3±1.2
6	2.3±1.2	ND*	44.0±0.4
7	2.4±1.1	ND*	88.1±1.6
8	2.5±0.2	ND*	26.7±1.0
9	3.7±0.3	ND*	ND*
10	ND*	ND*	62.4±2.5
11	0.8±0.2	ND*	88.3±1.3
12	2.2±0.1	ND*	ND*
13	3.1±0.4	ND*	27.1±0.6
14	6.4±2.1	ND*	4.7±1.3
15	5.5±2.0	ND*	0.1±0
16	5.8±2.7	ND*	16.9±0.9
17	2.8±0.4	ND*	47.9±6.9
18	2.2±1.0	ND*	27.8±3.3
19	3.4±0.1	ND*	35.8±1.7

ND*: Not detected

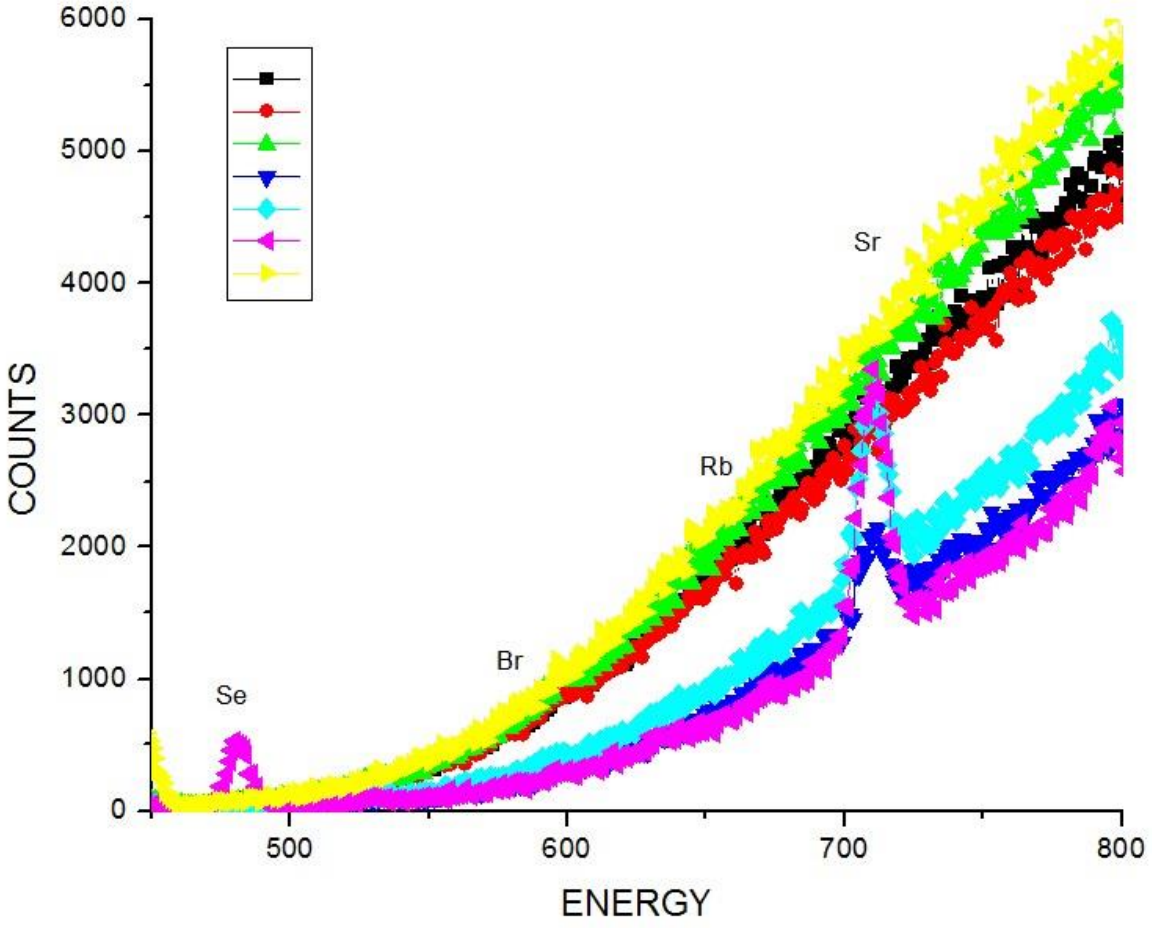

Fig. 3.4.3. (a) ED-XRF spectra of Urolithiatic stones

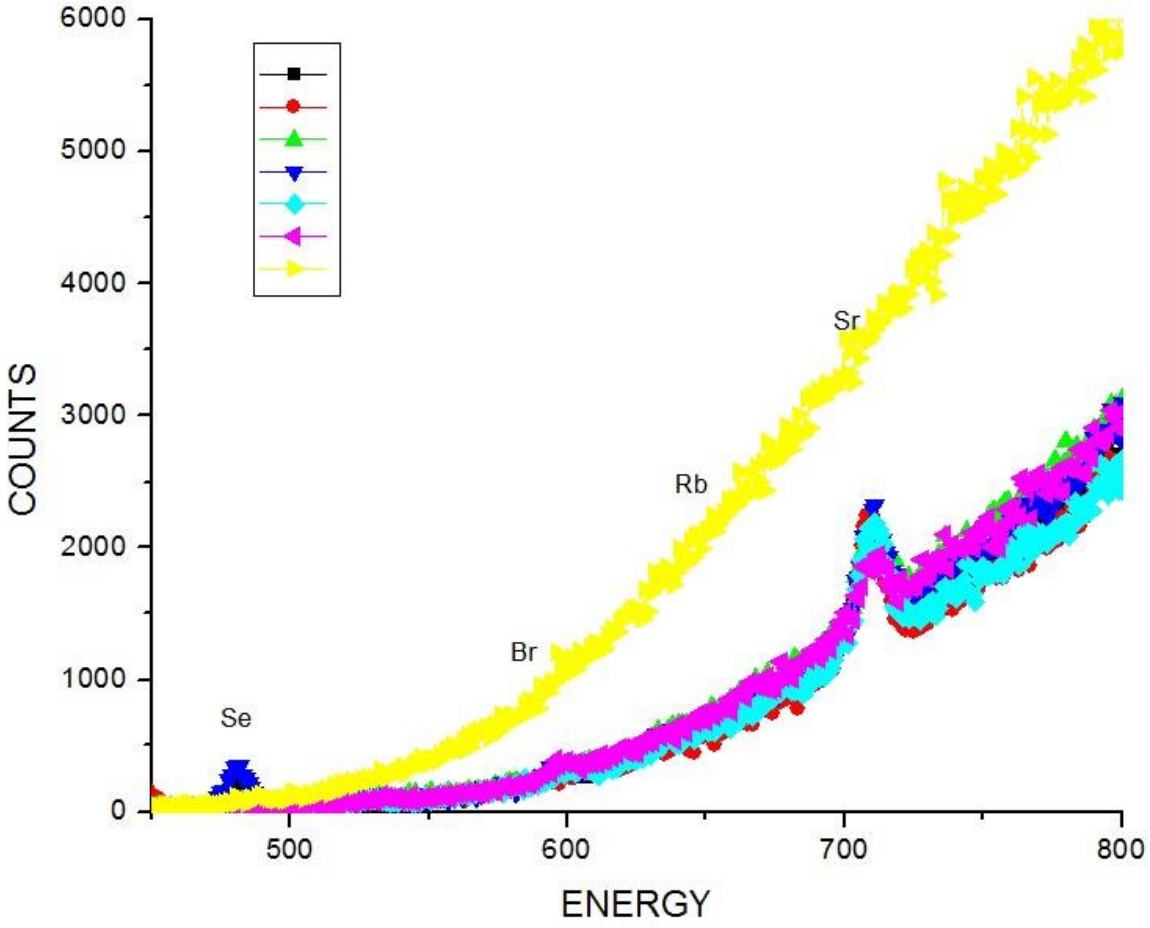

Fig. 3.4.3. (b) ED-XRF spectra of Urolithiatic stones

Table 3.5.1. Karl Pearson's correlation coefficient of different major elements (P, S, Cl, K and Ca) between vegetable foodstuffs and urinary stones

Vegetable foodstuffs samples/ urinary Stone	Phosphorus		Sulphur		Chlorine		Potassium		Calcium	
	r	p	r	p	r	p	r	p	r	p
Clerodendron colebrookianum	-0.935	0.231	-0.815	0.394	-0.880	0.315	0.895	0.294	-0.179	0.885
Brassica juncea	-0.861	0.340	0.459	0.696	-0.848	0.356	-0.838	0.367	-0.348	0.774
Zanthoxylum rhetsa L.	-0.990	0.088	0.974	0.145	0.295	0.809	0.928	0.243	0.543	0.635
Vigna unguiculata L	-0.185	0.881	-0.998	0.037	0.260	0.832	-0.624	0.571	-0.402	0.737
Cucurbita pepo L	-0.780	0.430	0.708	0.499	0.384	0.749	-0.411	0.730	-0.216	0.862
Gnetum gnemon L	0.625	0.570	-0.959	0.183	-0.603	0.588	0.817	0.391	0.632	0.565
Cucurbita maxima	0.652	0.548	0.478	0.683	-0.751	0.459	-0.995	0.063	-0.413	0.729
Colocasis esculenta L	0.961	0.179	0.992	0.080	0.144	0.908	0.774	0.436	0.205	0.868
Musa paradisiaca	0.992	0.081	-0.914	0.266	-0.363	0.764	0.726	0.482	0.101	0.936
Bambusa vulgaris	-0.717	0.491	0.798	0.412	-0.680	0.524	-0.070	0.955	-	-
Acacia gagaena	0.081	0.948	-0.215	0.862	-	-	-0.941	0.220	-0.976	0.140
Dysoxylum gobara merr	0.441	0.709	-0.884	0.310	-0.688	0.517	0.794	0.416	-0.286	0.816
Solanum anguivi	0.301	0.806	-0.838	0.368	0.505	0.663	0.999	0.035	-0.641	0.557
Calamus sp	0.601	0.589	0.816	0.392	0.630	0.566	-0.388	0.747	0.140	0.911
Glycine max merr	0.991	0.086	0.902	0.284	-	-	0.088	0.944	-0.636	0.561
Parkia timoriana	0.666	0.536	0.352	0.771	-	-	0.553	0.627	0.825	0.383
Brassica oleracea	-0.273	0.824	-0.657	0.544	-	-	0.642	0.556	0.237	0.848
Solanum nigrum linn	-0.993	0.074	-0.472	0.687	-	-	0.969	0.159	-0.991	0.085
Brassica oleracea	0.811	0.398	0.511	0.659	-	-	-0.578	0.607	1	0.011
Solanum melongena	-0.456	0.699	-0.824	0.383	-	-	0.523	0.649	-0.053	0.966
Solanum incanum	0.897	0.291	-0.806	0.403	-	-	0.895	0.294	-0.940	0.222
Acmella oleracea	0.900	0.287	-0.960	0.181	-	-	-0.494	0.671	-0.731	0.478
Passiflora edulis L	0.990	0.090	0.634	0.563	-	-	0.982	0.119	0.699	0.507

Table 3.5.2. Karl Pearson's correlation coefficient of different minor elements (Mn, Fe, Cu, Zn and Se) between vegetable foodstuffs and urinary stones

Vegetable foodstuffs samples/ urinary Stone	Manganese		Iron		Copper		Zinc		Selenium	
	r	p	r	p	r	p	r	p	R	p
Clerodendron colebrookianum	-0.099	0.937	-0.862	0.338	-0.253	0.837	0.075	0.952	0.664	0.538
Brassica juncea	0.135	0.914	0.844	0.361	0.637	0.560	-0.866	0.333	-0.419	0.725
Zanthoxylum rhetsa L.	0.913	0.267	-0.508	0.660	-0.978	0.135	-0.600	0.590	-0.506	0.662
Vigna unguiculata L	-0.249	0.840	-0.749	0.461	-0.115	0.927	0.746	0.464	-0.708	0.499
Cucurbita pepo L	-0.146	0.907	0.568	0.616	0.866	0.333	-0.303	0.804	-0.866	0.333
Gnetum gnemon L	-0.139	0.911	0.757	0.453	-0.736	0.474	-0.926	0.246	1	0.000
Cucurbita maxima	-0.430	0.717	-0.010	0.994	-0.995	0.065	-0.955	0.193	-0.5	0.667
Colocasis esculenta L	0.403	0.736	-0.249	0.840	-0.562	0.620	-0.042	0.973	1	0.000
Musa paradisiaca	-0.020	0.987	0.883	0.311	0.952	0.198	0.648	0.552	1	0.000
Bambusa vulgaris	0.159	0.898	0.829	0.378	0.823	0.385	0.596	0.594	-0.945	0.212
Acacia gagaena	-0.751	0.459	0.331	0.785	0.806	0.404	0.254	0.836	-0.774	0.437
Dysoxylum gobara merr	0.647	0.552	-0.082	0.948	-0.742	0.467	0.892	0.299	0.993	0.077
Solanum anguivi	0.001	0.999	-0.004	0.997	0.737	0.472	0.863	0.338	1	0.000
Calamus sp	-0.996	0.056	-0.846	0.359	0.762	0.449	-0.448	0.704	1	0.000
Glycine max merr	0.371	0.758	0.307	0.801	0.474	0.686	-0.827	0.381	-0.721	0.488
Parkia timoriana	-0.289	0.813	-0.965	0.168	-0.619	0.575	-0.250	0.839	0.587	0.601
Brassica oleracea	-0.703	0.503	-0.973	0.148	-0.374	0.756	0.230	0.852	0.668	0.534
Solanum nigrum linn	-0.804	0.406	-0.420	0.724	0.673	0.530	-0.020	0.987	1	0.000
Brassica oleracea	-0.595	0.594	-0.855	0.347	-0.290	0.813	0.238	0.847	-0.998	0.038
Solanum melongena	-0.987	0.102	-0.573	0.611	-0.618	0.576	0.254	0.837	1	0.000
Solanum incanum	-0.983	0.117	0.381	0.751	1	0.019	0.014	0.991	-0.500	0.667
Acmella oleracea	-0.933	0.234	-0.806	0.403	0.922	0.252	0.907	0.276	-1.000	0.013
Passiflora edulis L	-0.172	0.890	-0.148	0.905	0.988	0.098	0.940	0.222	0.990	0.092

Table 3.5.3. Karl Pearson's correlation coefficient of different earth elements (Br, Rb and Sr) between vegetable foodstuffs and urinary stones

Vegetable foodstuffs samples/ urinary Stone	Bromine		Rubidium		Strontium	
	r	p	r	p	r	p
Clerodendron colebrookianum	0.986	0.105	-	-	-0.998	0.035
Brassica juncea	0.677	0.527	-	-	-0.226	0.855
Zanthoxylum rhetsa L.	0.899	0.289	-	-	0.443	0.708
Vigna unguiculata L	-0.997	0.051	-	-	-0.999	0.010
Cucurbita pepo L	-0.895	0.294	-	-	-0.310	0.799
Gnetum gnemon L	-0.616	0.577	-	-	0.101	0.936
Cucurbita maxima	-0.407	0.733	-	-	-0.936	0.230
Colocasis esculenta L	0.998	0.041	-	-	0.144	0.908
Musa paradisiaca	-0.630	0.566	-	-	0.999	0.028
Bambusa vulgaris	0.356	0.769	-	-	0.017	0.989
Acacia gagaena	0.629	0.567	-	-	-0.211	0.865
Dysoxylum gobara merr	0.203	0.870	-	-	0.459	0.696
Solanum anguivi	-0.066	0.958	-	-	0.473	0.686
Calamus sp	-0.904	0.281	-	-	0.628	0.567
Glycine max merr	-0.985	0.111	-	-	0.459	0.697
Parkia timoriana	-0.709	0.498	-	-	-0.999	0.022
Brassica oleracea	0.386	0.747	-	-	0.749	0.461
Solanum nigrum linn	-0.858	0.344	-	-	-0.999	0.022
Brassica oleracea	0.026	0.983	-	-	-0.105	0.933
Solanum melongena	-0.071	0.954	-	-	-0.298	0.807
Solanum incanum	0.538	0.638	-	-	-0.450	0.703
Acmella oleracea	0.118	0.925	-	-	0.059	0.963
Passiflora edulis L	-0.311	0.799	-	-	0.940	0.221

Table 3.5.4. Karl Pearson's co-efficient of correlation of different major elements(P, S, Cl, K and Ca) between meat foodstuffs and urinary stones

Meat foodstuffs samples/ urinary Stone	Phosphorus		Sulphur		Chlorine		Potassium		Calcium	
	r	p	r	p	r	p	r	p	r	p
Chicken	0.339	0.780	-0.362	0.764	0.296	0.808	0.560	0.622	-0.487	0.677
Fish	-0.980	0.129	-0.755	0.455	-0.365	0.762	0.870	0.329	0.831	0.376
Beef	0.994	0.067	0.005	0.997	0.960	0.180	0.884	0.310	-0.962	0.176

Table 3.5.5. Karl Pearson's co-efficient of correlation of different minor elements(Mn, Fe, Cu, Zn and Se) between meat foodstuffs and urinary stones

Meat foodstuffs samples/ urinary Stone	Manganese		Iron		Copper		Zinc		Selenium	
	r	p	r	p	r	p	r	p	r	p
Chicken	-0.988	0.099	-0.027	0.983	-0.092	0.941	-0.700	0.506	0.246	0.842
Fish	-0.258	0.834	-0.926	0.247	-0.952	0.199	0.433	0.715	1	0.000
Beef	-		-0.986	0.103	0.513	0.657	-0.262	0.831	-0.480	0.682

Table 3.5.6. Karl Pearson's co-efficient of correlation of different earth elements(Br, Rb and Sr) between meat foodstuffs and urinary stones

Meat foodstuffs samples/ urinary Stone	Bromine		Rubidium		Strontium	
	r	p	r	p	r	p
Chicken	-0.984	0.114	-	-	-0.230	0.852
Fish	-0.994	0.069	-	-	-0.974	0.145
Beef	0.765	0.446	-	-	0.097	0.938

Table 3.5.7. Karl Pearson's correlation co-efficient of different elements in *Clerodendron colebrookianum*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.810	.997*	-.730	.190	-.381	-.978	.999*	-.744	.532	-.710	.648	.750
S		1	-.763	.992	-.729	.851	.915	-.829	.211	.065	.162	-.078	-.995
Cl			1	-.676	.114	-.309	-.959	.994	-.793	.596	-.762	.705	.697
K				1	-.809	.910	.857	-.752	.087	.189	.037	.047	-1.000*
Ca					1	-.980	-.391	.222	.515	-.730	.557	-.625	.792
Mn						1	.566	-.411	-.334	.580	-.381	.457	-.898
Fe							1	-.984	.588	-.344	.547	-.474	-.872
Cu								1	-.722	.504	-.686	.623	.771
Zn									1	-.962	.999*	-.991	-.116
Se										1	-.974	.990	-.161
Br											1	-.996	-.066
Rb												1	-.018
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.8. Karl Pearson's correlation co-efficient of different elements in *Brassica juncea*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.501	.635	.821	.168	-.451	.416	1.000*	.421	.502	-.853	-.994	-.972
S		1	.987	.906	-.769	.547	.995	.485	.996	1.000**	.024	-.399	-.284
Cl			1	.962	-.655	.403	.967	.621	.968	.987	-.139	-.543	-.436
K				1	-.426	.140	.861	.810	.864	.906	-.402	-.750	-.664
Ca					1	-.955	-.826	.186	-.823	-.768	-.657	-.279	-.394
Mn						1	.624	-.467	.619	.545	.850	.550	.648
Fe							1	.400	1.000**	.995	.119	-.310	-.192
Cu								1	.405	.486	-.863	-.995	-.976
Zn									1	.996	.113	-.315	-.197
Se										1	.023	-.400	-.285
Br											1	.907	.952
Rb												1	.993
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.9. Karl Pearson's correlation co-efficient of different elements in *Zanthoxylum rhetsa*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.325	-.603	-.991	.413	.884	-.423	-.212	.998*	-.831	-.998*	-.992	-.976
S		1	-.950	-.447	-.727	-.156	.720	.856	.381	.256	-.381	-.205	-.110
Cl			1	.703	.478	-.159	-.468	-.652	-.650	.057	.650	.500	.414
K				1	-.289	-.814	.299	.080	-.997*	.750	.997*	.967	.938
Ca					1	.791	-1.000**	-.978	.357	-.850	-.357	-.522	-.602
Mn						1	-.798	-.645	.854	-.995	-.854	-.935	-.965
Fe							1	.975	-.367	.856	.367	.532	.611
Cu								1	-.152	.719	.152	.331	.420
Zn									1	-.796	-1.000**	-.983	-.961
Se										1	.796	.893	.933
Br											1	.983	.961
Rb												1	.995
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.10. Karl Pearson's correlation co-efficient of different elements in *Vigna unguiculata*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.987	.971	.987	-.972	.874	.476	.426	-.892	.869	.011	-.825	.943
S		1	-.997*	-1.000**	.921	-.941	-.613	-.273	.954	-.776	.153	.906	-.876
Cl			1	.997*	-.889	.964	.671	.199	-.974	.726	-.227	-.936	.837
K				1	-.921	.941	.613	.273	-.954	.776	-.152	-.906	.876
Ca					1	-.735	-.256	-.627	.760	-.961	-.245	.669	-.995
Mn						1	.844	-.068	-.999*	.518	-.477	-.996	.662
Fe							1	-.593	-.823	-.023	-.874	-.890	.156
Cu								1	.030	.819	.909	.159	.703
Zn									1	-.550	.443	.992	-.690
Se										1	.505	-.437	.984
Br											1	.556	.343
Rb												1	-.590
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.11. Karl Pearson's correlation co-efficient of different elements in *Cucurbita pepo*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.989	.472	.433	.451	.443	.945	-.873	-.141	.073	.177	.579	.620
S		1	.595	.559	.577	.569	.983	-.934	.004	.218	.032	.692	.499
Cl			1	.999*	1.000*	.999*	.734	-.842	.806	.914	-.784	.992	-.399
K				1	1.000*	1.000**	.703	-.818	.831	.931	-.810	.986	-.439
Ca					1	1.000**	.718	-.830	.819	.923	-.798	.989	-.420
Mn						1	.711	-.824	.825	.927	-.804	.987	-.429
Fe							1	-.984	.190	.395	-.154	.814	.330
Cu								1	-.360	-.551	.326	-.903	-.158
Zn									1	.977	-.999*	.725	-.864
Se										1	-.968	.856	-.737
Br											1	-.700	.882
Rb												1	-.281
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.12. Karl Pearson's correlation co-efficient of different elements in *Gnetum gnemon*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.912	.442	.932	.220	.822	-.005	-.998*	-.988	.729	-.970	.639	.233
S		1	.772	.999*	.601	.983	.407	-.938	-.836	.946	-.786	.267	-.188
Cl			1	.737	.972	.874	.895	-.504	-.297	.936	-.213	-.407	-.770
K				1	.559	.973	.359	-.955	-.864	.928	-.817	.317	-.136
Ca					1	.736	.975	-.288	-.065	.828	.022	-.610	-.898
Mn						1	.566	-.860	-.723	.989	-.660	.088	-.363
Fe							1	-.066	.160	.681	.246	-.772	-.974
Cu								1	.974	-.775	.951	-.584	-.164
Zn									1	-.613	.996	-.751	-.381
Se										1	-.542	-.061	-.496
Br											1	-.806	-.460
Rb												1	.897
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.13. Karl Pearson's correlation co-efficient of different elements in *Cucurbita maxima*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.245	-.939	-.994	.963	-.339	-.724	-.734	-.999*	.262	.641	-.996	-.981
S		1	-.563	-.351	-.026	.829	.491	.478	-.282	1.000*	-.587	-.158	-.427
Cl			1	.971	-.812	-.004	.444	.457	.952	-.577	-.339	.905	.988
K				1	-.927	.234	.644	.655	.997*	-.367	-.553	.980	.997
Ca					1	-.581	-.884	-.890	-.952	-.009	.825	-.983	-.893
Mn						1	.895	.888	.304	.819	-.939	.421	.153
Fe							1	1.000**	.698	.476	-.994	.782	.578
Cu								1	.708	.463	-.992	.791	.590
Zn									1	-.298	-.612	.992	.988
Se										1	-.573	-.175	-.442
Br											1	-.707	-.482
Rb												1	.961
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.14. Karl Pearson's correlation co-efficient of different elements in *Colocasis esculenta*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.191	.073	-.048	.065	-.062	-.072	-.525	.979	-.353	.999*	.728	.993
S		1	-.965	-.990	.992	.968	-.993	.735	.387	-.986	.227	.812	.302
Cl			1	.993	-.990	-1.000**	.990	-.887	-.132	.907	.036	-.630	-.042
K				1	-1.000*	-.994	1.000*	-.824	-.251	.952	-.085	-.720	-.163
Ca					1	.992	-1.000**	.815	.267	-.957	.102	.731	.179
Mn						1	-.991	.882	.142	-.912	-.026	.639	.052
Fe							1	-.811	-.274	.959	-.109	-.736	-.186
Cu								1	-.341	-.611	-.494	.201	-.424
Zn									1	-.536	.986	.853	.996
Se										1	-.387	-.898	-.458
Br											1	.753	.997*
Rb												1	.802
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.15. Karl Pearson's correlation co-efficient of different elements in *Musa paradisiaca*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.252	-.284	.034	-.217	-.176	-.792	.044	-.521	-.204	-.526	.973	.443
S		1	.856	.976	.890	.908	-.791	.978	.694	.896	.690	.020	.979
Cl			1	.948	.998*	.994	-.360	.945	.966	.997	.965	-.500	.733
K				1	.968	.978	-.637	1.000**	.835	.971	.832	-.200	.911
Ca					1	.999*	-.425	.966	.946	1.000**	.944	-.438	.779
Mn						1	-.461	.976	.932	1.000*	.930	-.401	.804
Fe							1	-.645	-.108	-.436	-.103	-.628	-.898
Cu								1	.830	.969	.826	-.190	.915
Zn									1	.942	1.000**	-.706	.534
Se										1	.940	-.427	.787
Br											1	-.710	.529
Rb												1	.222
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.16. Karl Pearson's correlation co-efficient of different elements in *Bambusa vulgaris*

	P	S	Cl	K	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.159	.966	.997*	-.630	.163	.998*	.952	-.926	-.771	.970	-.970
S		1	-.409	-.236	.867	.948	-.215	-.455	.521	.752	.086	-.086
Cl			1	.983	-.809	-.097	.979	.999*	-.992	-.909	.874	-.874
K				1	-.688	.086	1.000*	.973	-.952	-.818	.948	-.948
Mn					1	.663	-.673	-.838	.877	.980	-.422	.422
Fe						1	.108	-.148	.222	.503	.399	-.399
Cu							1	.967	-.946	-.805	.955	-.955
Zn								1	-.997*	-.929	.848	-.848
Se									1	.954	-.806	.806
Br										1	-.592	.592
Rb											1	-1.000**
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.17. Karl Pearson's correlation co-efficient of different elements in *Acacia gagaena*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Se	Br	Rb	Sr
P	1	.100	-.378	.287	-.539	.896	.916	.582	-.663	.657	.499	.246
S		1	.884	-.925	-.892	.531	-.309	.867	-.811	-.684	.912	.989
Cl			1	-.995	-.577	.073	-.718	.533	-.443	-.946	.614	.805
K				1	.653	-.168	.648	-.612	.527	.910	-.687	-.858
Ca					1	-.857	-.154	-.999*	.988	.281	-.999*	-.949
Mn						1	.642	.882	-.926	.254	.832	.651
Fe							1	.206	-.306	.905	.108	-.165
Cu								1	-.995	-.230	.995	.931
Zn									1	.128	-.980	-.889
Se										1	-.325	-.569
Br											1	.963
Rb												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.18. Karl Pearson's correlation co-efficient of different elements in *Dysoxylum gobara merr*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	1.000**	-.872	.982	.988	.333	.915	-.238	.887	.792	1.000**	.997*	.533
S		1	-.868	.983	.989	.340	.917	-.245	.889	.796	1.000**	.997	.538
Cl			1	-.763	-.784	.171	-.599	-.268	-.546	-.391	-.873	-.906	-.050
K				1	.999*	.506	.975	-.418	.958	.894	.981	.965	.683
Ca					1	.477	.967	-.388	.948	.878	.987	.973	.659
Mn						1	.686	-.995	.732	.839	.331	.263	.976
Fe							1	-.611	.998*	.971	.913	.882	.829
Cu								1	-.661	-.782	-.236	-.166	-.949
Zn									1	.985	.885	.850	.864
Se										1	.791	.745	.938
Br											1	.997*	.530
Rb												1	.468
Sr													1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.19. Karl Pearson's correlation co-efficient of different elements in *Solanum anguivi*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.972	.907	.848	.921	.930	.942	.956	.925	.927	.993	-.235	.666
S		1	.981	.948	.986	.818	.837	.998*	.810	.814	.938	-.456	.473
Cl			1	.992	.999*	.689	.713	.991	.679	.684	.852	-.622	.291
K				1	.988	.593	.620	.966	.582	.587	.780	-.715	.170
Ca					1	.712	.736	.995	.703	.707	.869	-.596	.323
Mn						1	.999*	.781	1.000**	1.000**	.966	.139	.894
Fe							1	.802	.999*	.999*	.975	.106	.878
Cu								1	.772	.776	.915	-.510	.419
Zn									1	1.000**	.963	.153	.900
Se										1	.964	.147	.897
Br											1	-.120	.749
Rb												1	.568
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.20. Karl Pearson's correlation co-efficient of different elements in *Calamus sp*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	1.000**	.985	.925	.701	.614	.919	-.353	1.000*	-.844	-.974	-.999*	-.957
S		1	.984	.922	.695	.620	.922	-.346	1.000**	-.840	-.975	-.999*	-.955
Cl			1	.976	.812	.470	.838	-.508	.982	-.924	-.920	-.992	-.992
K				1	.920	.268	.700	-.682	.918	-.985	-.813	-.940	-.995
Ca					1	-.133	.363	-.915	.689	-.974	-.519	-.730	-.878
Mn						1	.875	.522	.627	-.096	-.778	-.580	-.358
Fe							1	.044	.926	-.565	-.985	-.902	-.765
Cu								1	-.337	.799	.130	.392	.610
Zn									1	-.835	-.977	-.998*	-.952
Se										1	.699	.866	.964
Br											1	.963	.865
Rb												1	.968
Sr													1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.21. Karl Pearson's correlation co-efficient of different elements in *Glycine max merr*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.340	.569	.519	-.430	-.217	.879	.018	-.733	-.846	-.832	-.406
S		1	.967	.980	.703	-.992	-.151	.946	.391	-.789	.239	-.997*
K			1	.998*	.498	-.926	.107	.833	.143	-.920	-.017	-.982
Ca				1	.549	-.947	.047	.864	.202	-.895	.043	-.992
Mn					1	-.788	-.809	.895	.930	-.118	.859	-.651
Fe						1	.275	-.980	-.505	.705	-.361	.980
Cu							1	-.462	-.969	-.488	-.996	.080
Zn								1	.667	-.549	.540	-.921
Se									1	.256	.987	-.325
Br										1	.407	.831
Rb											1	-.170
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.22. Karl Pearson's correlation co-efficient of different elements in *Parkia timoriana*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.327	.873	-.137	-.580	.814	.077	-.796	-.957	-.981	.348	.328
S		1	-.746	.981	-.580	-.815	.917	.832	.587	.137	.772	.785
K			1	-.603	-.109	.994	-.419	-.990	-.977	-.762	-.153	-.174
Ca				1	-.727	-.687	.977	.708	.418	-.058	.881	.891
Mn					1	.001	-.857	-.031	.319	.727	-.966	-.960
Fe						1	-.516	-1.000*	-.947	-.686	-.261	-.281
Cu							1	.541	.215	-.270	.961	.967
Zn								1	.937	.664	.289	.310
Se									1	.883	-.062	-.041
Br										1	-.524	-.506
Rb											1	1.000*
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.23. Karl Pearson's correlation co-efficient of different elements in *Brassica oleracea*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.818	.949	.999*	-.840	-.002	-.498	-.471	.801	.599	-.197	.029
S		1	.594	.790	-.999*	-.577	-.906	-.893	1.000*	.029	-.725	-.552
K			1	.963	-.625	.314	-.198	-.168	.571	.822	.124	.343
Ca				1	-.813	.045	-.456	-.428	.772	.636	-.150	.076
Mn					1	.544	.889	.874	-.998*	-.069	.697	.518
Fe						1	.868	.883	-.600	.799	.981	1.000*
Cu							1	1.000*	-.918	.396	.948	.852
Zn								1	-.905	.424	.958	.868
Se									1	.001	-.744	-.575
Br										1	.667	.818
Rb											1	.974
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.24. Karl Pearson's correlation co-efficient of different elements in *Solanum nigrum linn*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.800	-.525	-.971	-.679	-.272	-.546	-.940	.194	.779	-.743	.444
S		1	.931	.919	.984	.795	.940	.547	.434	-.246	.192	-.893
K			1	.712	.981	.962	1.000*	.203	.733	.124	-.180	-.996
Ca				1	.834	.493	.729	.832	.044	-.608	.562	-.644
Mn					1	.891	.986	.387	.588	-.068	.012	-.959
Fe						1	.955	-.073	.891	.392	-.443	-.983
Cu							1	.227	.716	.100	-.156	-.993
Zn								1	-.517	-.946	.927	-.112
Se									1	.766	-.801	-.793
Br										1	-.998*	-.216
Rb											1	.270
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.25. Karl Pearson's correlation co-efficient of different elements in *Brassica oleracea*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.992	.614	-.806	-.059	.351	.891	.913	.692	.387	.915	1.000*
S		1	.510	-.874	.067	.465	.941	.957	.596	.268	.857	.989
K			1	-.027	-.824	-.524	.189	.238	.995	.965	.881	.630
Ca				1	-.544	-.837	-.987	-.977	-.130	.234	-.498	-.793
Mn					1	.914	.401	.354	-.761	-.943	-.457	-.080
Fe						1	.738	.703	-.433	-.728	-.058	.331
Cu							1	.999*	.289	-.074	.632	.881
Zn								1	.336	-.024	.670	.904
Se									1	.933	.925	.707
Br										1	.727	.406
Rb											1	.923
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.26. Karl Pearson's correlation co-efficient of different elements in *Solanum melongena*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.986	.932	.860	-.534	-.984	-.842	-.614	.923	.792	-.995	.653
S		1	.859	.764	-.665	-1.000**	-.919	-.736	.848	.681	-.966	.769
K			1	.987	-.190	-.852	-.588	-.286	1.000*	.960	-.963	.334
Ca				1	-.027	-.755	-.448	-.126	.990	.993	-.906	.175
Mn					1	.676	.906	.995	-.168	.093	.449	-.989
Fe						1	.924	.745	-.840	-.671	.962	-.778
Cu							1	.943	-.570	-.337	.785	-.959
Zn								1	-.264	-.006	.535	-.999*
Se									1	.966	-.956	.313
Br										1	-.848	.056
Rb											1	-.577
Sr												1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.27. Karl Pearson's correlation co-efficient of different elements in *Solanum incanum*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.870	.965	.993	.842	.025	.284	-.609	-.989	-.143	1.000*	.106
S		1	.709	.806	.466	.515	.721	-.138	-.932	-.613	.878	-.399
K			1	.989	.954	-.239	.022	-.796	-.916	.123	.960	.364
Ca				1	.900	-.092	.170	-.698	-.966	-.026	.991	.221
Mn					1	-.518	-.278	-.941	-.755	.413	.832	.625
Fe						1	.966	.778	-.170	-.993	.043	-.991
Cu							1	.588	-.420	-.990	.301	-.923
Zn								1	.487	-.698	-.595	-.853
Se									1	.285	-.992	.040
Br										1	-.160	.969
Rb											1	.088
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.28. Karl Pearson's correlation co-efficient of different elements in *Acmella oleracea*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.644	-.658	.958	1.000**	.975	-.085	.971	-.345	.734	-.703	.595
S		1	.152	.836	.634	.798	.708	.808	-.940	.992	.091	-.232
K			1	-.415	-.668	-.474	.806	-.460	-.480	.028	.998*	-.997
Ca				1	.954	.998*	.204	.999*	-.599	.898	-.470	.340
Mn					1	.972	-.098	.968	-.332	.725	-.713	.605
Fe						1	.139	1.000*	-.545	.867	-.527	.401
Cu							1	.155	-.906	.615	.768	-.851
Zn								1	-.559	.875	-.513	.386
Se									1	-.891	-.425	.549
Br										1	-.033	-.110
Rb											1	-.990
Sr												1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.5.29. Karl Pearson's correlation co-efficient of different elements in *Paciflora edulis L*

	P	S	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.650	.822	-.600	.375	-.034	-.126	-.044	-.356	-.417	-.460	.053
S		1	-.967	.998*	.461	-.737	-.672	-.730	-.479	.962	-.375	-.793
K			1	-.949	-.220	.541	.461	.532	.239	-.860	.127	.612
Ca				1	.517	-.779	-.719	-.773	-.534	.977	-.434	-.831
Mn					1	-.939	-.967	-.943	-1.000*	.687	-.996	-.906
Fe						1	.996	1.000**	.946	-.894	.903	.996
Cu							1	.997	.972	-.849	.939	.984
Zn								1	.949	-.890	.907	.995
Se									1	-.701	.993	.914
Br										1	-.615	-.930
Rb											1	.862
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.30. Karl Pearson's correlation co-efficient of different elements in *Gallus gallus domesticus*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.061	-.655	-.959	.988	.119	-.946	-.114	-.015	.018	-.408	-.838	.900
S		1	.794	.342	.092	.984	.382	-.985	-.997*	.997	-.886	.595	.381
Cl			1	.843	-.533	.672	.865	-.675	-.745	.743	-.422	.961	-.260
K				1	-.904	.168	.999*	-.173	-.270	.267	.132	.959	-.739
Ca					1	.269	-.885	-.264	-.167	.170	-.542	-.745	.956
Mn						1	.210	-1.000**	-.995	.995	-.955	.442	.540
Fe							1	-.215	-.311	.308	.089	.970	-.709
Cu								1	.995	-.995	.954	-.446	-.536
Zn									1	-1.000**	.919	-.533	-.450
Se										1	-.920	.530	.452
Br											1	-.156	-.766
Rb												1	-.516
Sr													1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.5.31. Karl Pearson's correlation co-efficient of different elements in *Labeo rohita*

	P	S	Cl	K	Ca	Mn	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	-.771	-.038	-.568	.929	-.129	-.974	-.247	-.980	-.123	.940	.048	.826
S		1	.665	.962	-.952	-.532	.895	-.426	.630	.726	-.509	.598	-.996
Cl			1	.844	-.405	-.986	.263	-.959	-.160	.996	.305	.996	-.595
K				1	-.832	-.743	.739	-.657	.394	.886	-.254	.794	-.933
Ca					1	.247	-.989	.129	-.838	-.481	.748	-.324	.976
Mn						1	-.098	.993	.323	-.968	-.459	-.997	.453
Fe							1	.021	.910	.344	-.839	.178	-.932
Cu								1	.433	-.931	-.562	-.980	.343
Zn									1	-.076	-.989	-.245	-.698
Se										1	.223	.985	-.661
Br											1	.386	.584
Rb												1	-.523
Sr													1

Table 3.5.32. Karl Pearson's correlation co-efficient of different elements in *Bos taurus*

	P	S	Cl	K	Ca	Fe	Cu	Zn	Se	Br	Rb	Sr
P	1	.991	.984	.964	.984	.953	-.957	.823	.951	.661	.673	.943
S		1	.999*	.991	.950	.985	-.909	.738	.899	.553	.767	.889
Cl			1	.996	.936	.992	-.890	.708	.880	.516	.794	.869
K				1	.900	.999*	-.845	.641	.833	.437	.846	.820
Ca					1	.882	-.994	.911	.991	.785	.529	.987
Fe						1	-.824	.611	.811	.401	.866	.797
Cu							1	-.952	-1.000*	-.850	-.430	-.999*
Zn								1	.959	.970	.133	.965
Se									1	.861	.410	1.000*
Br										1	-.111	.873
Rb											1	.388
Sr												1

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3.6.1. Comparison of mean ± SD of plasma malondialdehyde (MDA), plasma vitamin-A, plasma vitamin-E and plasma vitamin-C levels between control group-I and group-II patients with urolithiasis.

Parameters	**Group-I (Control, n=40) Mean** ± SD	**Group-II (Patients, n=50) Mean** ± SD	**P value**
Plasma MDA(nmol/ml)	1.94 ± 0.456	4.06 ± 0.693	<0.001
Erythrocyte SOD(Unit/mg Hb)	12.17 ± 1.21	7.25 ± 1.87	<0.001
Erythrocyte CAT(µmol/l)	71.4 ± 6.23	56.5 ± 4.98	<0.001
Vitamin -E(mg/dl)	1.66 ± 0.03	1.59 ± 0.04	>0.05
Vitamin- C(mg/dl)	0.47 ± 0.02	0.44 ± 0.04	>0.05

Table 3.6.2. Correlation coefficient between MDA, SOD, CAT, VitaminE and VitaminC

Plasma level	**MDA(r)**	**P value**
SOD	.180	>0.05
CAT	-.161	>0.05
Vitamin-E	-.046	>0.05
Vitamin-C	-.116	>0.05

4. DISCUSSION

Urolithiasis, like many other disease processes, is influenced by a number of genetic and environmental factors. Diet is a major environmental component; however, it frequently is ignored in the management of individuals with this disorder, with more emphasis placed on minimally invasive stone-removing procedures and preventive medical therapy**.** Epidemiological studies and metabolic investigations of the chemical composition of urine have suggested that a number of nutrients may influence the formation of stones in the upper urinary tract. The lithogenic potential of dietary factor is believed to be through different mechanisms. It results from a combination of a higher renal load of lithogenic substances, and a tendency towards their increased precipitation in the kidneys (Assimos and Holmes, 2000).

On the other hand, as one of the important indispensable constituent of human diet, vegetable foodstuffs serve as an indispensable constituent of the human diet supplying the body with essential elements in addition to vitamins, protein, certain hormone precursors and energy for well being and good health (Oyenuga *et al.,* 1975). The nutritional value of vegetable foodstuffs is an important aspect that should be considered especially with respect to essential element intake useful for maintenance of health and prevention and treatment of diseases. Moreover, Meat and meat products are important for human diet because they provide a great part of nutrients, including necessary elements. Reliable database on the nutrient composition of foods including elements content are important in many areas of endeavour including health assessment, formulation of appropriate institutional and therapeutic diets, nutrition education, food and nutrition training, epidemiological research on relationships between diet and diseases, plant breeding, nutrition labelling, food regulation and consumer protection as well as a variety of applications in agriculture, trade, research, development and assistance (Scrimshaw ., 1994). Thus, analysis plays an important role in

the assessment and maintenance of food quality and safety, both in industry and for enforcement authorities at the national and international levels (Kirk *et al.*, 1991). The used of direct and multi-elemental analysis methods of vegetation samples including XRF has increased over the last few years. Simplicity of sample preparation, minimum need for manipulation, speed and opportunity of analyzing several different elements have promoted XRF as a useful alternative to conventional spectroscopic techniques (Margui *et al.*, 2005). In addition to this, XRF can be directly applied in dried samples, avoiding sample digestion dissolution by toxic and corrosive acids (Noda et *al.*, 2006). In ED-XRF spectrometers, all of the elements in the sample are excited simultaneously, and an energy dispersive detector in combination with a multi-channel analyzer is used to simultaneously collect the fluorescence radiation emitted from the sample and then separate the different energies of the characteristic radiation from each of the different sample elements.

Mizoram is a State in the North Eastern Region of India and is a part of the Indo-Mayammar biodiversity hot spot of the world. The State harbour variety of edible plant species including number of medicinal plants used in the traditional practices (Lalramnghinglova., 2003). Most commonly consumed vegetables foodstuffs of the native of this region comprises of recipes of various leafy vegetables prepared in the traditional way and which are not normally consumed in other places of the country. Moreover, in the State of Mizoram, fish, chicken, beef and pork meat are a major source of protein to the population and are widely consumed. The study of the elemental contents of native vegetables and meat foodstuffs commonly consumed by living population are relevant because these vegetables are major nutrient suppliers including important essential elements acting on a metabolism of several functions in human body. Any long term excess and deficiency of these metals lead to the deterioration of the important metabolic pathways in human body which resulted serious diseases and disorders etc. Therefore ingestion of foods is an obvious means of exposure to

elements, not only because many metals are natural components of foodstuffs but also because of contamination during process (Ashraf, 2006). Obviously components of the foodstuffs at the point of consumption are necessary for the estimation of the actual amount of elements exposure to humans from the ingestions of these food items. The study of the metal composition of food is great significance because some of these metals are essential or toxic at higher level. The essential metals namely Mn, Cu, Zn, Se etc own their essentiality being constituents of enzymes and other important metabolic dysfunction causing diseases (Iwegbue, 2011).

In the present investigation, the thirteen (13) micro and macro elements in the twenty-three (23) selected commonly consumed vegetables foodstuffs and three (3) meat food stuffs were analyzed by using Energy Dispersive X-ray fluorescence (ED-XRF) for the first time and their possible role in urolithiasis the human body has been discussed as a result of the detrimental nature of these elements in the body.

The results of the present study revealed that K is observed generally higher in all the vegetables studied as compared to other macro elements recorded. The content of K, Ca, P, Cl and S in all the selected leafy vegetables ranged from 8701.1 ± 41.7 mg/Kg to 42883.6 ± 2215.4 mg/Kg, 2483.2 ± 59.1 mg/Kg to 20536.7 ± 312.3 mg/Kg, 2027.4 ± 19.1 mg/Kg to 13272.4 ± 82.3 mg/Kg, 579 ± 0 mg/Kg to 20434.5 ± 2373.6 mg/Kg and 1750.4 ± 121.6 mg/Kg to 31870.8± 631.8 mg/Kg respectively. The highest content of K was recorded in *Brassica oleracea* and Cl was recorded in the *Colocasis esculenta L* while the highest content of Ca was found in *Cucurbita maxima* and P was in the *Solanum incanum* with *Acacia gagaena* containing highest content of S. The study reveals that the leafy vegetables contained high and notable amount of K and Ca. While in case of the meat foodstuffs, the result of the present study revealed wide and appreciable variations in the major elements concentration in the different meat samples studied. K is observed generally higher in all the

meat samples studied as compared to other macro elements recorded like in the case of vegetables foodstuffs. The content of K, Ca, P, Cl and S in all the selected meat samples ranged from 2532.6 ± 58.2 mg/Kg to 3365.8 ± 89.6 mg/Kg, 413.1 ± 21.6 mg/Kg to 1993.4 ± 291.5 mg/Kg, 1493 ± 91.9 mg/Kg to 1894.9 ± 124.6 mg/Kg, 544.5 ± 3.5 mg/Kg to 594.7 ± 6.2 mg/Kg and 1648.8 ± 44.3 mg/Kg to 1676±93.7 mg/Kg respectively. The highest content of K and Cl were recorded in beef (3365.8 ± 89.6 mg/Kg of K and 594.7 ± 6.2 mg/Kg of Cl) while the highest content of Ca and P were found in fish sample (1993.4 ± 291.5 mg/Kg of Ca and 1894.9 ± 124.6 mg/Kg of P) with while chicken contained highest amount of S (1676 ± 93.7 mg/Kg) (Table 3.2.1). These observations indicated that the meat samples contained high and notable amount of K and Ca. Studies related to the contents of macro elements in the commonly consumed foodstuffs from other parts of the world is limited. These observations indicated that the meat samples contained high and notable amount of K and Ca. Studies related to the contents of macro elements in the commonly consumed foodstuffs from other parts of the world is limited. The content of elements recorded in our study is more than those reported by Stihi *et al.*, in the year 2008, Masson *et al.*, in the year 2010 and Vincevica Gaile and Klavins in the year 2011. As revealed from the study, the specified twenty three vegetable samples contain varied mean levels of elements which could be attributed to factors such as variation in genetic makeup of plant species and competitive interaction between metal ions within the lithosphere or variation in the amounts of the mineral in the soils where the vegetables grew (Anyawu *et al.,* 2004). High concentration of some metals recorded in some leafy vegetables in the present study may be due to high content of these metals in the soil. Moreover, a major pathway of soil contamination is through atmospheric deposition of heavy metals from point sources such as metalliferous mining, smelting and industrial activities and contamination of agricultural soils due to various inputs such as fertilizers, pesticides, sewage sludge, organic manure and compost (Singh, 2001). Additionally foliar

uptake of atmospheric heavy metals from emissions has also been identified as an important pathway of heavy metal contamination in vegetable crops (Salim *et al.*, 1992) and it has been reported that leafy vegetables accumulate higher metal contents than others (Al Jassir *et al.*, 2005). In case of the meat foodstuffs, the concentrations of major elements were less than but comparable to those reported by Rachida *et al.*, 2012. The main source of metals in these meat foodstuffs arises from processing, manipulation of feeds, contamination and drinking water sources. It has been reported that the recommended dietary allowances for Ca is set at 700mg, 1000mg and 1300mg for 1-3 years, 4-8 years and 9-8 years respectively while the upper tolerable intake of Ca is set 2500mg and 3000mg for children and adults respectively (Institute of Medicine, 2010; Daida *et al*., 2006). On the other hand the Recommended Nutrient Intake (RNI) for K and P is 4900 mg/day and 550-990 mg/day respectively for adults of both sexes (COMA, 1991). On the other hand, the permissible limit of S in the solid food is 3000 mg/kg (Australia-New Zealand, 2000). However, all the major elements concentration recorded in the vegetables foodstuffs in the present study were above the permissible limit as reported by these regulatory organizations and may posed threat to health on long term consumption. Contrarily, the concentrations of major elements mention *per se* recorded in the meat foodstuffs were within the permissible limit as revealed by present study.

Ca is an important element which plays a pivotal role in the physiology and biochemistry of the cell of human. It has an important role in signal transduction pathways, where it acts as a second messenger, in release of neurotransmitter from neurons, contraction of all muscle cell types, and fertilization. Many enzymes require Ca ions as a cofactor including those of the blood-clotting cascade. Extracellular Ca is also important for maintaining the potential difference across excitable cell membranes as well as proper bone formation. Like Ca, K is also one of the essential elements of human diet and

play important role in vital cellular mechanisms. As cofactors, it catalysis the conversion of ADP to ATP during the excitability of the nerve (Viarengo *et al.,* 1996) and K deficiency can create resistance in fat and muscle cells etc from insulin, increased serum triglycerides, may lower HDL and blood supply to the vital organs and increase the chances of stroke (Otten *et al.,* 2006). On the other hand, Cl is an element making up 0.14% of the concentration of elements in the human body and one of its important roles in the body is their electrolyte function. Another role of Cl in the body is to combine with hydrogen to form HCl, which breaks down proteins for absorption of other metallic minerals. By combining with Na and K, Cl works well to keep all of our biological systems running smoothly (Sevenhyusen, 1994). As an important major element, P is an element that makes up 1% of a person's total body weight and present in every cell of the body. The main function of P in the human body is in the formation of bones and teeth. It also plays an important role in how the body uses carbohydrates and fats and needed for the body to make protein for the growth, maintenance, and repair of cells and tissues. P also helps the body to make ATP, a molecule the body uses to store energy (Mason, 2001). As a macro element of the body, the role of S on the body is to acts as an integral part of many important compounds found in all body cells which are indispensable for life. The primary sulfur-containing compounds of interest in humans for the proper functioning of the cells and organs are methionine, cysteine, homocysteine, cystathione, S-adenosylmethionine (SAMe), taurine, α-keto-γ-CH3-thiobutyrate, methanethiol, thiamin, biotin, alpha-lipoic acid (ALA), coenzyme A, glutathione (GSH), chondroitin sulfate, glucosamine sulfate, fibrinogen, heparin, metallothionein, inorganic sulphate and vitamins etc. In addition to this, S is needed for a number of chemical reactions involved in the metabolism of drugs, steroids and xenobiotics (Baker, 2006).

Trace element plays an important role in human health because they participate in biological functions that contribute to growth and good health. Inadequate nutrition of these elements compromises immune function, causes impaired physical and mental growth, affects reproductive performance and reduces work productivity and consequently affects country's economy (Underwood, 1999). Analysis of data revealed that among the trace element studied, Fe concentration is generally found to be higher in all the leafy vegetables studied. The Fe content ranges between 54.0 ± 1.6 mg/Kg to 828.9 ± 26.1 mg/Kg in the vegetables studied with the highest Fe content found in *Passiflora edulis L*. While, in case of meat foodstuffs, the Fe content ranges between 47.9 ± 2.3 mg/Kg to 161.2 ± 6.2 mg/Kg with the highest Fe content found in beef (161.2 ± 6.2 mg/Kg). The concentration of Fe recorded in our study is less than those reported by Mohammed *et al.,* 2011and Ismail *et al.,* 2011. The high amount of Fe in the leafy vegetables may be due to the foliar absorption from the surroundings air (Kaplan, 1993). It has been reported that estimated average daily Fe requirements are 8.7 and 6.7 mg (COMA, 1991) while the permissible limit for Fe in food is in the range of 2.5-5.0 mg/kg depending on the foodstuff [Codex Alimentarius Commission (CAC). 2003]. And the minimum daily requirement of Fe is ranged from about 10 to 50 mg/day as per FAO/WHO, 2002. Therefore, the value recorded in our study is above the recommended dietary allowances of Fe (10-15 mg/day) as recommended by FAO/WHO, 2002. Fe is a necessary nutrient element and is core component of RBC. It is needed for healthy immune system and for energy production (Ullah *et al.,* 2012). Several studies have also shown the important role of Fe availability correlates with the bactericidal effect of lactoferrin and lysozyme, which can killed gram-negative bacteria (Ellison *et al.,* 1991).

Like Fe, Mn is one of the important essential elements required in carbohydrate metabolism as well as an antioxidant in SOD enzymes. Mn is also important in regulation of immune responses of the body by breakdown of amino acids, production of energy, by

regulating the metabolism of Vitamin B_1, C, and E and by activation of various enzymes important for proper digestion and utilization of foods (Chaturvedi et al, 2004). The content of the Mn recorded in the present study ranged between 10.9 ± 2.1 mg/L to 288.6 ± 6.3 mg/Kg and *Calamus sp* contained the highest amount. In case of meat foodstuffs, the Mn recorded in the present study ranged between 0 to 1.1±0.3 mg/Kg. The value of Mn obtained in our study in the vegetable foodstuffs is more than those reported by Singh and Taneja, 2010 and Gorbunov *et al*., 2004. The wide range of Mn values obtained in this study can be attributed to variations in the abundance of minerals in the lithosphere (EJ Underwood, 1977). As a contaminant however, no maximum permissible limit (MPL) has been fixed for Mn in vegetables. Upper tolerable limit of Mn for human is 2-11 mg/day.

In the present study, Zn concentration was recorded in the ranged between 31.8 ± 0.5 mg/Kg to 152.5 ± 8.9 mg/Kg and the highest Zn content was found in the *Acmella oleracea*. In case of meat foodstuffs, the Zn content ranges between 46.2±0.7 mg/Kg to 198.1±4.2 mg/Kg with the highest Zn content found in beef 198.1±4.2 mg/Kg. The concentration of Zn obtained in our study is in agreement with those reported by Singh and Taneja, 2010 but greater than the values recorded in the earlier studies from Egypt (AK Salama et al, 2005) and Latin America (M Olivares et al, 2004). This disparity between the results of the present study and those reported from other parts of the country could be explained by the indiscriminate used of excess Zn as micronutrients in Agricultural practices during the last decades (Benbi *et al*, 2006). However, Zn recorded in the present study except in the case of four of the twenty-three vegetables are within the permissible limit as set by USDA (2003). Moreover, during the last two decades in India, the exogenous treatment of growth hormone or its analogues couple with high concentration of Zn as zinc sulphate (ZnSO4) in commercial feeds is being exploited in rearing the livestock on large scale for higher yield of animal products. This may result an increase in bioavailability of Zn in the meat foodstuffs

investigated as observed in the case of beef in the present study. Zn is an important trace element involved in numerous aspects of cellular metabolism. It was estimated that about 10% of human proteins potentially bind Zn, in addition to hundreds which transport and traffic zinc. It is required for the catalytic activity of more than 200 enzymes (Stanstead, 1994). Zn also plays an important role in immune function (Solomon, 1988), wound healing (Mc Carthy *et al*, 1992), protein synthesis, DNA synthesis and cell division (Prasad, 1995).

Cu is known to play an important role in human metabolism, largely because it allows many critical enzymes to function properly (Harris, 2001). Cu is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissue throughout the body. Cu also plays a major role in the production of haemoglobin, myelin, and melanin and keeps thyroid gland functioning normally (Harris, 2001., Groff *et al*, 1995). As an antioxidant, Cu scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause (Bonham, 2002). As expected Cu was found to be presented in appreciable amount in the selected leafy vegetables foodstuffs studied between the ranged from the 4.3 ± 0.3 mg/Kg to 29.1 ± 2.1 mg/Kg and the highest level of the Cu was recorded from the *Solanum incanum*. In case of meat foodstuffs, the content of Cu in meat foodstuffs samples studied ranged from 3.4 ± 0.4 mg/Kg to 6.8 ± 1.4 mg/Kg and the highest level of the Cu was recorded from beef (6.8 ± 1.4 mg/Kg) (Table 3.2.2). The Cu content recorded in the present study is in agreement with Singh and Taneja, 2010 but less than that reported by Mohammed and N Sharif in the year 2011. The concentration of Cu in plants varied much with dependent nearby factors like proximity industries and use of fertilizers and Cu based fungicides. The value of the Cu obtained from this study is within the permissible limit of Cu in the vegetables set by Codex Alimentarous Commission (CAC), 1993.

In foods, Se is generally present as the amino acid derivatives selenomethionine and selenocysteine. The analysis of data from the present study revealed that Se contents in the

selected vegetables foodstuffs ranged between 0.12 ± 0.005mg/Kg to 13.9 ± 0.1mg/Kg and *Acmella oleracea* contained the highest. In case of meat foodstuffs, the content of Se recorded were between 0.17 mg/Kg to 1.8±0.1 mg/Kg. As contaminants no such limit for Se. The USRDA which is most widely quoted standard has been set similarly at 70 g/day for men and women on the basis of the optimization of plasma GPx activity (Thomas *et al*, 1993). Se is an element which behaves both as an antioxidant and anti-inflammatory agent. This is because Se in its antioxidant role in GPx, can reduce hydrogen peroxide, lipid and phospholipid hydroperoxides, thereby dampening the propagation of free radicals and reactive oxygen species. Moreover, Se reduce hydroperoxide intermediates in the cyclooxygenase and lipoxygenase pathways leading to inflammatory prostaglandins and leukotrienes (Sunde, 1997).

In the present study, Sr, Rb and Br were also detected in all the leafy vegetables studied with varying concentration in the ranged between 5.15 ± 1.01 mg/Kg to 122.50 ± 3.70 mg/Kg, 5.19 ± 0.43 mg/Kg to 155.25 ± 6.8 mg/Kg and 1.56 ± 0.6 mg/Kg to 110.4 ± 2.2 mg/Kg for Sr ,Rb and Br respectively. The highest content of the Sr, Rb and Br were recorded from the *Cucurbita maxima*, *Calamus sp* and *Acmella oleracea* respectively. In case of meat foodstuffs, Rb, Br Sr concentrations recorded in the range between 8.6 ±1.7 mg/Kg to 26.2 ±1.7 mg/Kg 3.4 ± 0.7 to 17.5 ± 0.8 mg/Kg and 11.4 ± 1.8 mg/Kg to 91.8 ± 5.9 mg/Kg respectively (Table 3.2.3). Generally Sr, Rb and Br have no known essential role in human or mammalian health. However, Rb ions are utilized by human body in a manner similar to potassium ions, being actively taken up by plants and by animal cells (O Julius, 1910). Besides this, Inorganic bromine and organobromine compounds do occur naturally, and some may be of use to higher organisms in dealing with parasites. For example, in the presence of H_2O_2 formed by the eosinophil, and either chloride or bromide ions, eosinophil

peroxidase provides a potent mechanism by which eosinophils kill multicellular parasites and also certain bacteria (Mayeno et al, 1989).

In the present study, the quantitative estimation of elements in the urinary stones collected from the urban area of Mizoram was also conducted. The results of the study reveal that Ca was found to be predominant constituent element as compared to other major elements. However, concentration varied over a large range viz. 0.06% to 38%. The concentrations recorded were between minimum of 939.4±30.1 mg/Kg to maximum of 118809.4±1968.7 mg/Kg of Ca, 61.9±10.5 mg/Kg to 35923.5±1580 mg/Kg of P, 725.96±0 mg/Kg to 2584.1±1394.1 mg/Kg of K, 30.5±3.3 mg/Kg to 1298.3±364.7 mg/Kg of S and 87.4±4.5 mg/Kg to 579±0 mg/Kg of Cl respectively. However, in some stones no Cl level were detected. In case of minor elements, the concentration of Mn, Fe, Cu, Zn and Se recorded were ranged between 9.4±0.6 mg/Kg to 80.3±13.2 mg/Kg, 27.7±2.1 mg/Kg to 110.7±3.7 mg/Kg, 0.19 1 mg/Kg to 364.1±18.7 mg/Kg, 1.4±1.1 mg/Kg to 107.8±2.3 mg/Kg and 0.12±0 mg/Kg to 1.0±0.6 mg/Kg respectively. Interestingly, earth elements namely Br and Sr were also detected in the urinary stones studied in the ranged between 1.1±0.2 mg/Kg to 6.4±2.1 mg/Kg and 0.1±0 mg/Kg to 88.3±1.3 mg/Kg respectively. The composition and clinical characteristics of urinary calculi can vary greatly from one part of the world to another and from one historic period to the next. This variation is related to climate, dietary, genetic, and socioeconomic factors as urolithiaisis can develop in patients as a result of metabolic disorders, anatomical malformations of the urinary tract, infection, and environmental and nutritional factors (Gearhart *et al*., 1991). The elements recorded in the present study may scattered in the urinary stones as impurities or as intergrowth constituents of the stone (Al-Kofahi and Hallak 1996).

It has been established that the formation of urinary stone is generally linked with excess of salts like ammomium acid urate, uric acid, calcium oxalate monohydrate etc.

However, insolubility alone is insufficient to explain the genesis of urinary stones. This may caused due to genetic, nutritional and environmental factors. There are three mechanisms which are importance in the urinary stone formation. These are nucleation, crystal growth and aggregation and nucleation refers to the birth of submicroscopic molecular species of critical size within the supersaturation solution (Nancollas, 1983; Vermeulen, 1966). It has been reported earlier that the descendants of African Negroes who migrated to USA formed renal stones as frequently as the rest of American population, establishing the role of dietary and environmental factors for formation of such stones. In the present study, in order to observe and establish, the etiologic role of major and minor elements recorded in the present study in the pathophysiology of urolithiasis, we carried out Karl's Pearson correlation analysis of the concentration of major and minor elements obtained in the meat and vegetables foodstuffs to that of their concentration recorded in the urinary stones. In addition to this, we also carried out Karl's Pearson correlation analysis within the elements for each vegetables and meat foodstuffs samples studied to see the interaction among elements. From the study of literature studies, it can be said that, among the major elements recorded in the vegetables and meat foodstuffs, major elements especially Ca, K, P and Cl were considered to play major role in the etiology of urolithiasis. In the present study, from the correlation analysis, it was revealed that, the positive correlation were observed in the concentration of Ca, K, P and Cl recorded in the vegetable foodstuffs namely *Clerodendron colebrookianum, Vigna unguiculata L., Zanthoxylum rhetsa L., Cucurbita pepo L., Gnetum gnemon L., Colocasis esculenta L., Musa paradisiacal, Solanum anguivi, Dysoxylum gobara merr, Calamus sp, Parkia timoriana, Brassica oleracea, Solanum nigrum linn, Passiflora edulis L.* to that of their concentration recorded in the urinary stones. Zn, Cu, Fe, Mn and Se obtained in the vegetable foodstuffs mentioned *per se* were also found to be positively correlated. In the case of meat foodstuffs, the concentration of Cl, K and P and Ca and K were found to be positively correlated in the

Gallus gallus domesticus and *Labeo rohita* respectively. All the major elements obtained in the *Bos Taurus* were positively correlated to that of their concentration recorded in the urinary stones. Only Se, Zn and Cu recorded in the meat foodstuffs studied in the present study were positively correlated to their concentration obtained in the urinary stones (Table No. 3.5.1).

In addition to this, the analysis of correlation coefficient among the elements in the vegetables foodstuffs samples studied in the present study reveals that in *Clerodendron colebrookianum, Vigna unguiculata L., Zanthoxylum rhetsa L., Cucurbita pepo L., Gnetum gnemon L., Colocasis esculenta L., Musa paradisiacal, Solanum anguivi, Dysoxylum gobara merr, Calamus sp, Parkia timoriana, Brassica oleracea, Solanum nigrum linn, Passiflora edulis L.,* Ca and Cl shows positive correlation with P, S, K, Se Mn, Fe, Cu, Zn, Br and Sr while K shows negative correlation with P, Zn, Mn, Rb, Cl, Ca, Cu and Sr. This mean that the concentration of Ca and Cl increased with the increased concentration of P, S, K, Se Mn, Fe, Cu, Zn, Br and Sr while the concentration of K decreases with increased concentration of P, Zn, Mn, Rb, Cl, Ca, Cu and Sr. In case of meat foodstuffs, Ca and Cl recorded in the *Bos Taurus* show positive correlation with all other remaining major and minor elements while K shows negative correlation with P, Ca, Cu, Zn and Sr. The positive correlation between Ca and Cl with other elements shows that the concentration of Ca and Cl increases with increase concentration of other elements while the level of K decrease with increase concentration of P, Ca, Cu, Zn and Sr in the case of *Gallus gallus domesticus.* Thus, the two ways of correlation coefficient analysis i.e. concentration of elements recorded in the vegetables and meat foodstuffs to that of concentration in the urinary stones and correlation coefficient within the elements concentration recorded in each vegetables and meat foodstuffs samples in this study revealed that major elements specially Ca, Cl, P and K recorded in the above mentioned 14 vegetables foodstuffs and two meat foodstuffs may have a role in the

etiology and prevalence of urolithiasis in the natives of this region. Since these foodstuffs were commonly and frequently consumed by the natives of this region, the intake excess amount of Ca, Cl and P more than the permissible limit may have hazard effect in the body and may be associated to high prevalence of urolithiasis in urban population of this region. Moreover, low intake of K by the natives as reveal from the correlation study of the elements in the present study may also be acted etiologic factors for the high prevalence of urolithiasis in this region.

It has been reported that Ca is one major component of the most common type of human kidney stones. Some studies suggested that people who take calcium as a dietary supplement have a higher risk of developing kidney stones. In the United States, these findings were used as the basis for setting the Reference Daily Intake for calcium in adults (Farmer *et al.,* 1984). Due to higher levels of calcium content in the food, it may causes renal insufficiency, vascular and soft tissue calcification, hypercalciuria (high levels of calcium in the urine) and kidney stones. One of the possible roles of Ca in the urolithiasis is that Ca may bind to dietary oxalate in the intestine, thereby reducing oxalate absorption and the subsequent concentration of urinary oxalate. Even a small increase in urinary oxalate excretion increases the risk for calcium oxalate stones (Straub M Hautmann 2005). As the natives of this region have been consuming excess Ca from the vegetables and meat based diets for a long period of time, perhaps a greater proportion of dietary calcium remains in the intestine available to bind oxalate and excrete more lithogenic promoters in the urine. Moreover, after most of the oxalate is bound, additional dietary calcium would simply result in more calcium absorption and would not protect against calcium stones. Our results also confirmed the importance of individual dietary factors in the development of symptomatic kidney stones. However, some epidemiological studies have shown an inverse relationship between dietary calcium and urinary stone formation recurrence rates, possibly caused by

calcium inhibiting the intestinal absorption of oxalate. This idea is supported by a recent study showing that subjects on a diet of high calcium and restricted oxalate, protein and salt had half as many stone recurrences as those on a low calcium diet (Borghi *et al*. 2002). In contrast, the results of a large randomized trial suggested an increased risk of kidney stones associated with calcium supplements, possibly because the calcium was not ingested with food or the supplements were taken by those who exceeded the upper level of 2500 mg/day as recorded in the present study. Moreover, Ca is thought to have an inhibitory effect on Fe absorption from the diet, even at low levels. High Ca in the diets has also been shown to reduce net Zn and Cu absorption and balance (Bonithon-Kopp, 2000). This changed in the Zn and Fe status may also reflects probability of immune response inhibition (Solomon, 1988), wound healing (Mc Carthy *et al.,* 1992), protein synthesis, DNA synthesis and cell division (Prasad, 1995) which might lead to progression of the disease concerned(Leroux *et al.,* 1985) even though all the foodstuffs studied have high and appreciable amount of Fe and Zn. Moreover, alterations in the Cu concentrations are indicative of alteration in the vascular functions, a condition which might be relevant in bladder stone formation (Kok *et al.,* 1988). In addition to this, Ca levels in the body are under control of genetic and hormonal factors. Therefore an excessive accumulation of calcium in blood or tissue solely through excessive Ca consumption should not occur in the absence of diseases such as bone cancer, hyperthyroidism, and hyperparathyroidism or in the absence of excessive vitamin D intake. Adverse effects which have been reported due to high Ca intakes include the formation of urinary stones in persons with a propensity for nephrolithiasis, hypercalciuria and for hyperabsorption of calcium, and interference with the absorption of other minerals (Whiting and Wood, 1997).

In addition to this, from the present study, it can be predicted that the living population of this region have been consuming excess P level from the vegetables and meat

foodstuffs as revealed from the by ED-XRF analysis of foodstuffs and correlation coefficient analysis. One of the major roles of P in the formation of urinary stones is revealed from the clinical studies which reported that high P intakes influenced the parathyroid-vitamin D axis, which maintains calcium balance in the body. The P loading in humans operates through mechanisms of nutritional or secondary hyperparathyroidism similar to those observed in animals fed excess P. High phosphate in the diets causes a reduction in the urinary excretion of Ca and may be contributed in the formation of urinary stones (Brixen, 1992). On the other hand, high Cl concentration recorded in the vegetables and meat foodstuffs and a positive correlation established in Cl concentration recorded in these foodstuffs to that of their concentration in the stone and with the concentration of other elements may have contributed in the formation of urinary stones. Cl in combination with a metal such as Na, it becomes chloride like NaCl which is essential for life and small amounts of chlorides are required for normal cell functions in plant and animal life.

However, high NaCl intake due to high availability of Cl in the diet is associated with increased excretion of urinary Ca, a risk factor for certain types of stones. Moreover, a high NaCl intake also decreases urinary citrate concentration, another risk factor for stone formation. Even for individuals with no prior history of stone formation, a high dietary NaCl intake through increase Cl in the diet increases risk for urinary stones formation (Munson, 2001). Therefore, it is important to continue the investigations on the cause of the increased levels of chlorate in food. The use of chlorine disinfectants in drinking, washing and irrigation water and the presence of chlorate in fertilizers have already been identified as possible sources, but it is important to have a full overview on all the causes and their relative importance as regards the findings of the increased levels of chlorate in fruits and vegetables and other foods.

On the contrary, it has been reported that dietary K restriction can increase urinary calcium excretion. Hypokalemia stimulates tubular citrate reabsorption, thereby decreasing the urinary excretion of citrate, an important inhibitor of calcium oxalate stone formation. Even though, the K level in the vegetables and meat foodstuffs were generally higher as compared to other major elements in this study and even more than the permissible limit, the negative correlation of K with other elements recorded in the present study might have reduced K bioavailability in the body and may link to the increased prevalence of urolithiasis in the urban population of this region. It has been reported that K in food accompanied organic anions such as citrate that are metabolized to bicarbonate. Thus, the consumption of K-containing foods such as fruits and vegetables represents an alkali load that increases the urinary excretion of citrate (Lemann et al 1991; and Curhan, 1997).

Beside these, trace elements like Zn, Cu, Mn and Se are important elements and cofactors of the many antioxidant enzymes namely SOD, CAT, glutathione peroxidase(GPx**)** (Failla *et al.,* 1985) . However, high levels of elements like Ca, P and Fe have antagonistic interaction in the intestinal absorption level with these minor elements. As a result of this, the natives of this region might have low bioavailability of these minor elements in the body even though these elements were recorded in the appreciable amount in the commonly consumed vegetables and meat foodstuffs by this population. Thus the low bioavailability of these trace elements in the body may link to decrease in the activities of antioxidant enzymes mentioned *per se.* This may ultimately lead to increased oxidative stress especially in the disease conditions like urolithiasis and the antioxidant enzymes activities might not function properly to protect the onslaught of high oxidative stress. In corroborate to this, the patients with urolithiasis from this region were associated with high oxidative stress level as revealed by the present study on the estimation of oxidative stress status in the patients with urolithiasis of this region.

In addition to these, even though Sr and Br have no known essential role in human, their positive correlation recorded to that of their concentration in urinary stones and also with other elements indicated these earth elements detected in the vegetables and meat foodstuffs have a significant contribution and acts as etiologic chemical factors in the pathophysiology of urolithiasis. It has been reported that organic material referred to as matrix and composed of serum and urinary proteins (albumin, globulin, mucoproteins) may acts as ground substance and framework for these impurities and elements in the process of stone formation (Jenkins, 1991). It has been said that the etiology and history of stone formation varies in different populations and it is represented by anatomical abnormalities, metabolic studies, dietary habits and stone composition. However, hereditary or induced by external causes, our present observations strongly support the view that elements also play major role in the formation of kidney stones. Thus, from the above discussion, it may be inferred that individuals as well as synergistic interactions between elements from dietary and environmental exposures play an important role in the formation of urinary stones.

The evaluation of fluoride in the water samples collected from the various water sources of Aizawl reveals that the highest content of fluoride was recorded in water sample collected from Edenthar with a fluoride concentration of 1.362 mg/L. An elevated level observed in the fluoride concentration in this study may be attributed to the location of source of water of this area to the vicinity of main highway. This may result contamination of fluoride from vehicles and other man-made activities. However, the fluoride concentration recorded is within the maximum permissible limit of 1.5 ppm in drinking water as reported by WHO 2004. As expected, the finding of the present study also reveals that this region in the North eastern part of India is not a fluoride endemic state like other states of India namely Andhra Pradesh, Karnataka, Tamil Nadu, Punjab, Haryana, Maharashtra, Gujarat, Rajasthan, Uttar Pradesh, Kerala, Jammu and Kashmir, and Delhi. Even though, epidemiological

studies reported that flouride as one of the chemical factor for the prevalence of urolithiasis, the finding of fluoride estimation in the various potable water samples of the Aizawl reveals that fluoride found in the drinking water may have not any role in the prevalence of urolithiasis in the living population of this region. It has been reported that fluoride in vivo may behave as a mild promoter of urinary stone formation by excretion of insoluble calcium fluoride, increasing oxalate excretion and mildly increasing the oxidative burden (Singh *et al.*,2001). Moreover, it has been reported that fluoride concentrations above 1.5 ppm in drinking water cause dental fluorosis and much higher concentration skeletal fluorosis. Low concentration (approximately 0.5 ppm) provides protection against dental caries. India is among the 23 nations around the globe where health problems occur due to the consumption of fluoride contamination water and the extent of fluoride contamination in water varies from 1.0 to 400 mg/l. In India, 20 million people are severely affected by fluorosis and 40 million people are exposed to risk of endemic fluorosis (WHO 2004).

The study of the oxidative stress and antioxidant vitamins status in the patients with urolithiasis from the urban region of Mizoram reveals that group-II patients with urolithiasis has a state of increase oxidative stress and weak antioxidant defence system as demonstrated by a highly significant value ($p<0.001$) in the level of mean plasma MDA (4.06 ± 0.693 nmol/ml) as compared to control group-I (1.94 ± 0.456nmol/ml).

Since the group-II patient have increased lipid peroxidation, as expected the activities of SOD (7.25 ± 1.87 Unit/mg Hb) and CAT (56.5 ± 4.98 µmol/l) were significantly decreased ($p<0.001$) in them than those of group-I control (12.17 ± 1.21 Unit/mg Hb of SOD and 71.4 ± 6.23 µmol/l of CAT) indicating that the patients group rats are under the oxidative stress. (Table 3.6.1). A positive correlation was recorded between MDA and SOD activity ($r= .180$, $p>0.05$) while a negative correlation between MDA and CAT activity ($r = -.161$, $p>0.05$), however, the coorelations are not significant (Tables 3.6.2). Along with antioxidant

enzymes, the nutritional antioxidants such as vitamin-E and vitamin-C also play a pivotal role in scavenging free radicals. A non significant decreased ($p>0.05$) in the Vitamin-E(1.59 ± 0.04mg/dl and Vitamin-C (0.44 ± 0.04 mg/dl) were observed in the group- II patients as compared to the control groups (1.66 ± 0.03 mg/dl of vitamin- E and 0.47 ± 0.02 mg/dl of vitamin-C) (Tables 3.6.1). A negative correlation was observed between MDA and vitamin-E ($r= -0.46$, $p>0.05$) and MDA and also MDA and Vitamin-C ($r = -0.116$, $p>0.05$), however, the correlation is not significant (Tables 3.6.2).

Crystal aggregation and retention are critical events in the formation of stones in the urolithiasis. There is close association between crystal development and free radical activity in vivo. In this study, the relation between urolithiasis and oxidative stress could be corroborated in the group-II patients studied, which shows elevated levels of LPO with significant decrease in the activity of the antioxidant enzymes. The activity of the inbuilt antioxidant enzymes SOD and CAT were significantly lowered in the group-II patients with urolithiasis as compared to control group-I and this could mean an exaggerated oxidative stress due to formation of stones in the kidney/or ureter. These results are coherent with previous workers (Carraco- Valiente *et al*., 2012. Vasavidevi 2006; Mahmoud *et al.,* 2009). It has been reported that oxalate stone formation induced lipid peroxidation in urolithiasis. This may be the reason for the elevation of LPO products in the group-II patients as it have been revealed by in vivo and in vitro studies that oxalate can induced LPO through inhibition of antioxidant enzymes (Kato et *al.,* 2007; Muthukumar and Selvam 1997; Lenin *et al.,* 2002) Upon oxalate binding, the condition of peroxidation enhances and increase along with the depletion of thiol content which may in turn promotes nucleation and aggregation property of stone matrix protein fractions. This type of activity is also associated with peroxidized mitochondria and nuclei, suggesting that the peroxidation can be a causative factor for the initial stage of stone formation (Govindraj and Selvam, 2001). In addition to this, reduction in

either nutritional antioxidants or enxymatic antioxidants, shall also enhances to the progression of lipid peroxidation (Bharathi *et al.,* 2013; Selvam and Bijikurien 1992).

The production of lipid peroxidation products depends on activities of antioxidant enzymes such as SOD and CAT which registered a significant reduction in erythrocyte of group-II patient's subjects. A positive correlation was recorded between MDA and SOD activity while a negative correlation between MDA and CAT activity, however, the correlations are not significant. There is conflicting data on the status of the antioxidant enzymes in the subjects with urolithiais with some observe higher (Carrasco *et al.,* 2012) while other report significant decreased (Mahmoud *et al.,* 2009) as compare to healthy control. The positive correlation between MDA and SOD in this study may be due to increase activity of SOD in response to increased lipid peroxidation product. Our result is in agreement with those reported by Singh PP and Barjatia, 2002 who hypothesized that SOD must be over stretching itself to dismutate $O^{\cdot 2}$ to H_2O_2. SOD is the only antioxidant enzyme which effectively dismutates $O^{\cdot 2}$ to H_2O_2 and which in turn covert to water by CAT to retards the impact of free radical damage (Selvam and Bijikurien, 1992; Mahmoud *et al.,* 2009). Therefore, reduction in the plasma level of SOD and CAT here can be explained due to consumption of antioxidant enzymes by increasing lipid peroxidation.

Along with antioxidant enzymes, α- tocopherol play a pivotal role in scavenging free radicals. In corroboration with above concepts a decrease in the level of α-tocopherol was observed in group-II patients with urinary stones ($p>0.05$) and a negative correlation was observed between MDA and vitamin-E which is in agreement with other results (Kato J *et al.,* 2007; Singh PP and Barjatia MK, 2002 and Mehta *et al,* 1994 Santosh and Selvan, 2003). α-tocopherol is a major lipid soluble chain-breaking antioxidant. In addition, it has also demonstrated positive effect not only in restoring antioxidant status but also in preventing crystal deposition during oxalate challenge (Thanmilselvan *et al.,* 2003; Lenin *et al.,* 2002).

The regeneration of α-tocopherol from tocopherol radical involves synergistic reaction between α- tocopherol and ascorbate. This recycling reaction leads to the formation of dehydro-ascorbate which is further reduced to ascorbate by a non-enzymatic reaction with reduced glutathione. However, a no significant decrease ($p>0.05$) in plasma ascorbate level was observed in the present study which is in accordance with other authors (Mehta *et al*., 1994).

In this study vitamin-C level in plasma is not significantly different in group-II patients with urolithiasis as compared to control group-I($p>0.05$, Table- 3.6.1). A negative correlation is also observed between MDA and Vitamin-C, however, the correlation is not significant. It is possible that ascorbic acid is endogenously converted to oxalate and appears to increase the absorption of dietary oxalate which in turn induces free radical generation thereby causing renal stones (Massey *et al.,* 2005). It is not clear whether the negative corelation between MDA and antioxidant enzymes like SOD and CAT and antioxidant vitamins like vitamin-E and vitamin-C are result or the cause of this disease. The result of the present study suggests that oxidative stress is an evident and bearings in pathogenesis of urolithiasis with depletion in antioxidant status.

In summary, the chemical characterization of the commonly consumed vegetables and meat foodstuffs reveals the presence of variable amount of different type of major, minor and earth elements in these foodstuffs. The positive correlation observed in the concentration of some of the elements namely Ca, P, Cl recorded in vegetables and meat foodstuffs samples with their concentration recorded in the urinary stones, positive correlation of Ca and Cl with other elements and negative correlations of K with other elements as revealed from the correlation analysis and low bioavailability of important trace elements namely Cu, Zn, Fe, Se, Mn due to high and long term consumption of element like Ca indicated that the some of the

selected vegetables and meat foodstuffs have etiologic chemical factors which may contributed in the pathophysiology of urolithiasis in this living population of urban area of Mizoram. However, the fluoride concentartion recorded in the drinking water doesn't have any role in the prevalence of urolithiasis in the urban native of this region of the north eastern part of India. On the other hand, the study of oxidative stress status in the patients with urolithiais reveals that significant increased in the oxidative stress parameters and low levels of vitamin-E and vitamin-C between group-I control and group-II patients with urolithiasis in this population suggests that oxidative stress may also be the major contributing factor leading to crystal aggregation and adherence on the surface of renal epithelial cells thereby leading to genesis of urolithiasis. Further evaluation need to be done in other tribe population to find out the level oxidative stress indicators and vitamin-E and Vitamin-C which can contribute to the genesis of the urolithiasis.

5. CONCLUSION

The present study deals with evaluation of the etiologic chemical factors and oxidative stress status related to high prevalence of urolithiasis in the urban natives of Mizoram taking into account both the physical and biological parameters and their correlation with urolithiasis. The following points can be concluded from the present study:

a) The chemical characterization of the commonly consumed vegetables and meat foodstuffs by ED-XRF reveals the presence of variable amount of different type of major elements (Ca, P, Cl, K and S) minor elements (Zn, Fe, Cu, Mn and Se) and earth elements (Sr, Rb and Br) in these foodstuffs.

b) The results of the present study also reveals that the concentration of all the major elements recorded in the vegetables and meat foodstuffs were more than the permissible limit as reported by regulatory organizations. Moreover, all the vegetables and meat foodstuffs studied were found to contain high and notable amount of Fe, Mn, Zn and appreciable amount of Cu, Se, Br, Rb and Sr. This means that the living population of this region have been consuming excess amount of these major element specially Ca, Cl, P and S from these indispensible dietary sources.

c) The chemical analysis of urinary stones collected from the patients of the urban area of Mizoram reveals Ca as a predominant chemical constituent of the urinary stone followed by other elements like P, Cl, K, S, Zn, Fe, Cu, Mn, Se, Sr and Br. This finding indicated that these elements were scattered as impurities or as intergrowth inside the constituent of the urinary stones. These reflect the conditions of metabolic disorders, anatomical malformations of the urinary tract, environmental and dietary habits which may vary greatly from one part of the country to another.

d) Karl's Pearson Correlation analysis revealed the positive correlation observed in the concentration of the elements namely Ca, P, Cl recorded in fourteen vegetables foodstuffs samples out of twenty three namely *Clerodendron colebrookianum, Vigna unguiculata L., Zanthoxylum rhetsa L., Cucurbita pepo L., Gnetum gnemon L., Colocasis esculenta L., Musa paradisiacal, Solanum anguivi, Dysoxylum gobara merr, Calamus sp, Parkia timoriana, Brassica oleracea, Solanum nigrum linn, Passiflora edulis L.* and two meat foodstuffs i.e *Bos Taurus* and *Gallus gallus domesticus* with their concentration recorded in the urinary stones, positive correlation of Ca and Cl with other elements studied and negative correlations of K with other elements recorded. These findings indicated that these elements recorded in the above mentioned vegetables and meat foodstuffs play an important role in the pathophysiology of urolithiasis in this living population of urban area of Mizoram.

e) Further, excess and long term consumption of Ca and Fe from these vegetables and meat foodstuffs studied may be resulting in the low bioavailability of important trace elements namely Cu, Zn, Se, Mn in the body due their antagonistic interactions and may lead to decrease in the activities of antioxidant enzymes like SOD and CAT causing high oxidative stress associated with disease conditions and also observed in the present investigations of activities of antioxidant enzymes mentioned *per se*.

f) In the present study, the fluoride concentration recorded in the drinking water samples from the various water sources of the urban area of Mizoram reveals fluoride concentrations were within the permissible limit and as such, fluoride present in the drinking water of this region may not have any role in the prevalence of urolithiasis in the natives of this region.

g) The study of oxidative stress status in the patients with urolithiasis reveals a significant increased in the oxidative stress parameters and low levels of vitamin-E and vitamin-C in the group-II patients with urolithiasis as compared to group-I healthy control. The result of the present study suggests that oxidative stress is evident and has bearings in pathogenesis of urolithiasis with depletion in antioxidant status.

As the causes of urolithiasis is multifatorial and maltilateral and moreover, limited studies have been done in this particular health problem from this region of India, the results of the present study provides valuable information to the general population of this region to understand the root causes of the urolithiasis, dietary habits that have to be restricted and other remedies and precautions need to be exercised to prevent the formation and recurrence of stone diseases. Nevertheless, the results of the present study also provide supporting information on the root causes of variations of stone prevalence on worldwide epidemiology of Urolithiasis. Since the natives of urban area of this region of the North Eastern part of India were ethnically different and have different dietary habits from other part of India, the parameters from the present study may also be of great importance both as a guide for the clinical management and also for better understanding of physicochemical principles underlying the formation of calculi that may be helpful to give advice, generate awareness and provide suggestive measures for the people to carry out preventive measures in reducing the risk of prevalence and recurrence of urolithiasis in this region. Finally, the findings of the present study provides a significant insight about this disease to the researcher of other fields of applied sciences like agricultural sciences, food and nutrition sciences, health sciences, biochemistry and molecular biology to initiate collaborative strategies to contain this health disorder.

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9. Educational Qualifications:

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2	HSSLC	Mizoram Board of School Education, Mizoram	2005	II	50.00
3	B.SC	Mizoram University, Mizoram	2009	II	53.60
4	M.SC	Mizoram University, Mizoram	2011	I	65.39

LIST OF PUBLICATIONS:

- **R. Lawmzuali**, N. Mohondas Singh and Ksh. Birla Singh (2014). Spectroscopic Determination of Trace Elements Present in Selected Vegetable Foodstuffs Commonly Consumed by the Urban Population of Aizawl. *Proceedings: International Conference cum Exhibition on Drugs Discovery & Development from Natural Resources*. 10-14p. ISBN 978-81-923224-1-4.

- **R. Lawmzuali**, K. Birla Singh, M. Sudarshan and N. Mohondas Singh (2015). Determination of elements present in meat foodstuffs in Aizawl with ED-XRF. *Science and Technology Journal*. 3(2):169-172. ISSN:2321-3388.

- R. Lawmzuali, N Mohondas Singh, ST. Lalruatfela, K. Birla Singh (2016). Oxidative Stress and Antioxidant Vitamins Status in Patients with Urolithiasis from North Eastern Part of India. *Asian Journal of Biological and Life Sciences*. 5(1). In press.

- R. Lawmzuali, K. Birla Singh, M. Sudarshan, S. S. Ram and N. M. Singh (2016). Determination of Elements Content in the Selected Leafy Vegetables Commonly Consumed by Natives of Mizoram, India using ED-XRF. *Indian Journal of Nutrition*. (Accepted on 23rd May, 2016).

- R. Lawmzuali, K. Birla Singh and N. Mohondas Singh (2016). Elemental Determination of Two Medicinal Plants of Mizoram Using EDXRF. *Proceedings: International Symposium on Sustainable Horticulture.*

- R. Lawmzuali, K. Birla Singh and N. Mohondas Singh (2016). Analysis of Fluoride Content in Drinking Water of Aizawl Using SPADNS Method. *Proceedings: Solid Waste Management and Safe Drinking Water in Context of Mizoram and Other States in India.*

- R. Lawmzuali, K. B. Singh, M. Sudarshan, S. S. Ram and N. M. Singh (2016). Determination of Elements in the Selected Vegetables Commonly Consumed by Natives of Mizoram using ED-XRF. *Proceedings: Solid Waste Management and Safe Drinking Water in Context of Mizoram and Other States in India*

LIST OF PAPERS PRESENTED IN INTERNATIONAL/NATIONAL CONFERENCES:

- Presented a paper entitled *Trace element analysis of some selected vegetable foodstuffs commonly consumed by the urban population of Aizawl using ED-XRF* in the UGC-Sponsored National Level "Interaction Programme for Ph.D Scholars" organized by the UGC-Academic Staff College, Mizoram University on September 23- October 12, 2013.

- Presented a paper entitled *Spectroscopic Characterization of Tuibur and its Interaction with Copper(II) Ions* in the International Conference on Harnessing Natural Resources for Sustainable Development: Global Trends organized by Cotton College, Guwahati on January 29-31, 2014.

- Presented a paper entitled *Spectroscopic Determination of Trace Elements present in selected Vegetable Foodstuffs Commonly Consumed by the Urban Population of Aizawl* in the International Conference cum Exhibition on Drugs Discovery and Development from natural Resources organized by Department of Pharmacy, RIPANS on February 5-6, 2014.

- Presented a paper entitled *Sprectroscopic Analysis of Gall Stones in Mizoram Using ED-XRF* in the Orientation Workshop on radiation – Its applications in Chemical, Physical and Life Sciences organized by Department of Chemistry, Mizoram University, Aizawl and UGC-DAE Consortium for Scientific Research, Kolkata Centre on October 29-31, 2014.

- Presented a paper entitled *Analysis of Eight Commonly Consumed vegetables of Mizoram Using ED-XRF* in the REACH-2015: An International Symposium on Recent Advances in Chemistry organized by UGC-Centre for Advanced Studies in Chemistry, Department of Chemistry, North Eastern Hill University, Shillong on March 3-5, 2015.

- Presented a paper entitled *Determination of Elements in the Selected Vegetables Commonly Consumed by Natives of Mizoram using ED-XRF* in the National Seminar on Solid Waste Management and Safe Drinking Water in Context of Mizoram and

Other States in India organized by Mizoram Polytechnic, Lunglei on March 8-9, 2016.

PARTICIPATION IN INTERNATIONAL/NATIONAL CONFERENCES:

- Participated in the *National Seminar on Green Chemistry for Greener Environment* organized by Department of Chemistry, Pachhunga University College, Aizawl on November 26-27, 2012.
- Participated in the *National Seminar on Recent Advances in Natural Product Research* organized by Department of Zoology, Pachhunga University College and Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Aizawl on November 29- December 1, 2012.
- Participated in the *National workshop on Mathematical Analysis* organized by Department of Mathematics and Computer Scence, Mizoram University on March 7-8, 2013.
- Participated in the *Thematic Orientation workshop on Trace Element Analysis and Radiological Sciences* organized by Manipur University, Imphal and UGC-DAE Consortium for Scientific Research, Kolkata Centre on March 12-14, 2013.
- Participated in the *One Week Workshop on Applied Statistics* organized by Academic Staff College, Mizoram University on September 23-28, 2013.
- Participated in the *UGC-Sponsored National Level "Interaction Programme for Ph.D Scholars"* organized by the UGC-Academic Staff College, Mizoram University on September 23- October 12, 2013.
- Participated in the *One Day State Level Acquaintance Programme* organized by the Department of Physics, Mizoram University on March 28, 2014.
- Participated in the *Science Academies Lecture Workshop on recent Advances in Chemistry* organized by the Department of Chemistry, Mizoram University on June 2-4, 2015.
- Participated in the *Short Term Course on Instrumentation* organized by the UGC-Academic Staff College, Mizoram University on September July 22-28, 2014.
- Participated in the *Research Monitoring and sensitization workshop 2015* organized by the Project Committee, Pachhunga University College on November 9, 2015.

RIPANS/OP - 02

Spectroscopic Determination of Trace Elements Present in Selected Vegetable Foodstuffs Commonly Consumed by the Urban Population of Aizawl.

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The main aim of the present study is to identify essential elements present in the vegetable foodstuffs commonly consumed by the population of Aizawl, Mizoram, a North Eastern State of India due to their principal role as detrimental elements in human nutrition based on their absorbance label in the spectroscopy measurement. The absorbance label detected in the samples of vegetable foodstuffs ranged from 4.987 to 5.533 for mustard leaves, 5.121 to 6.046 for colocasia leaves, 5.028 to 6.54 for Chingit, 4.672 to 5.15 for East India glory blower, 4.818 to 6.042 for Cowpea leaves and 4.838 to 5.434 for pumpkin leaves. This spectrometric measurement revealed the presence of trace elements like calcium (Ca), Zinc (Zn), Iron (Fe) and Magnesium (Mg) in the commonly consumed vegetables foodstuffs by the natives of this region.

Keywords: Trace Elements, Spectroscopic measurement, Mizoram State

INTRODUCTION:

Vegetables serve as an indispensable constituent of the human diet supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy (Oyenuga and Fetuga, 1975). Reliable data on the nutrient composition of foods are important in many areas of endeavour including health assessment, formulation of appropriate institutional and therapeutic diets, nutrition education, food and nutrition training, epidemiological research on relationships between diet and disease, plant breeding, nutrition labelling, food regulation and consumer protection as well as a variety of applications in agriculture, trade, research, development and assistance (Scrimshaw, 1994). Food composition tables and databases are available in most countries, yet the data they contain are invariably criticised as being too inaccurate for many purposes(Sevenhyusen, 1994); one of the reasons is that different work-

ers have used different techniques of analysis and that the samples so analysed had undergone different sample preparations leading to varying degrees of losses without ignoring the inherent variable factors that affect food composition namely growing conditions, stage of ripeness or product formulation that are commonly not specified. Analysis plays an important role in the assessment and maintenance of food quality and safety, both in industry and for enforcement authorities at the national and international levels (Kirk & Sawyer, 1991).

The natives of Mizoram, a north eastern state of India are different culturally and socially including the food habits from those living in the mainland India. Mizo food comprises traditional recipes of various leafy vegetables which are not normally consumed in other places. Among them are recipes prepared from Antam (Mustard Leaves), Dawl(Elephant ear), Chingit, Phuihnam(East India Glory Blower), Behlawi (Cowpea leaves) and Maian (Pumpkin leaves). Hence, the need to determine the contents of essential minerals in these commonly consumed vegetables becomes imperative due to their principal role as detrimental elements in the body. However, the survey of the literature indicates that studies related to determination elements status in different food stuffs available in India are scarce, particularly in North- Eastern States of India. In view of the fact that there have been non-existent of data on contents of these minerals in the foodstuffs commonly consumed by the living urban population of Mizoram, study was undertaken to determine their level using standard techniques of spectroscopic measurement. The results of the study are reported in this communication.

2. MATERIALS AND METHODS

2.1 Sample Preparation.

The vegetables were collected from the local markets in Aizawl. The leaves were destalked, washed with distilled water and ovendried at 60^{o}C for about 2-3 days depending upon the vegetable. After drying, the leaves were ground into fine powder using mortar and pestle and then sieved, and stored in a well labelled air tight container for analysis.1 gram of the sample was weighed and mixed with 50ml of distilled water. This mixture was heated in an oven at 80^{o}C for 90 minutes and cooled. After cooling, the mixture was filtered and the filtrate was stored in a container for analysis.

2.2 Analysis using Evolution 220 UV-Visible Spectrometer.

In a UV-Visible spectroscopy, a sample is probed with photons of wavelength in the range of ultraviolet-visible light and absorbance is measured relative to an appropriate control. UV-Vis has immense analytical applications because analytes in mixtures that exclusively absorb photon radiation at particular wavelengths can be quantified independently of other components and an unknown material can be characterized by comparing with known materials. Solutions of transition metal ions, organic compounds with a high degree of conjugation, or those with alternating single and multiple bonds, and charge transfer complexes can be analyzed by UV-Vis spectroscopy. In its simplest form, a sample is placed between a light source and a photodetector, and the intensity of a beam of light is measured before and after passing through the sample. These measurements are compared at each wavelength to quantify the sample's wavelength dependent extinction spectrum. The data is typically plotted as extinction as a function of wavelength. Each spectrum is background corrected using a "blank" – a cuvette filled with only the dispersing medium – to guarantee that spectral features from the solvent are not included in the sample extinction spectrum. Evolution 220 is an excellent choice to perform quantitative and qualitative analyses of a wide range of sample types by fixed wavelength, scan, quant and kinetic measurements with the help of comprehensive quantitative analysis solutions.

3. RESULTS AND DISCUSSION

The absorbance label of the spectroscopic measurement of vegetables food stuffs analyzed are presented in Table 1 to 6.

Table 1: Showing data for the Spectroscopic measurement of Mustard leaves

Sl. No.	Wavelength(nm)	Absorbance
1	362.984	5.533
2	276.954	5.224
3	339.075	5.185
4	230.920	5.176
5	315.069	5.103
6	318.412	5.084
7	242.050	5.071
8	385.018	5.039
9	337.000	5.004
10	221.292	4.987

Table 2: Showing data for the Spectroscopic measurement Colocasia Leaves

Sl. No.	Wavelength(nm)	Absorbance
1	202.040	6.046
2	389.989	5.744
3	286.038	5.630
4	316.032	5.572
5	301.984	5.542
6	380.161	5.528
7	248.938	5.460
8	265.980	5.354
9	278.012	5.191
10	220.194	5.121

Table 3. Showing data for the Spectroscopic measurement Chingit

Sl. No.	Wavelength(nm)	Absorbance
1	359.091	6.543
2	336.965	5.947
3	304.910	5.566
4	201.156	5.397
5	307.097	5.246
6	372.025	5.112
7	192.080	5.067
8	258.993	5.052
9	381.065	5.028
10	228.147	5.028

Table 4: Showing data for the Spectroscopic measurement for East India Glory Blower

Sl. No.	Wavelength(nm)	Absorbance
1	346.014	5.159
2	365.037	5.100
3	260.187	4.860
4	247.612	4.724
5	262.877	4.707
6	241.053	4.703
7	228.981	4.675
8	341.879	4.674
9	287.913	4.672
10	212.142	4.667

Table 5: Showing data for the Spectroscopic measurement for Cowpea leaves

Sl. No.	Wavelength(nm)	Absorbance
1	312.997	6.042
2	359.002	5.442
3	376.917	5.412
4	332.173	5.235
5	228.135	5.019
6	382.979	4.990
7	281.018	4.940
8	242.251	4.910
9	238.006	4.856
10	273.068	4.818

Table 6: Showing data for the Spectroscopic measurement for Pumpkin Leaves

Sl. No.	Wavelength(nm)	Absorbance
1	338.940	5.434
2	269.016	5.282
3	211.012	5.082
4	225.148	5.038
5	353.000	4.929
6	313.979	4.887
7	244.070	4.868
8	248.837	4.856
9	343.094	4.855
10	228.606	4.838

On evaluation the spectroscopic data and comparison with the known literature, it can be concluded that samples vegetables mention *per se* contain Calcium, Zinc, Iron and Magnesium although their exact quantities cannot be given.

4. CONCLUSION

The results of this study supply valuable information about the presence of elements in vegetables foodstuffs commonly consumed by urban population of Aizawl.

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Science and Technology Journal, Vol. 3 Issue: II ISSN: 2321-3388

Determination of Elements Present in Meat Foodstuffs in Aizawl with ED-XRF

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Abstract—Trace elements are required by human body and have various roles in biochemical functions mostly as catalyst for enzymatic activity in human body. Some trace elements are essential since they are inadequately or not at all synthesized by human body. The deficiency or excess of those essential trace elements may cause diseases and be deleterious to health. Since food is the primary source of essential elements for humans and it is an important source of exposure to toxic elements either, the levels of trace elements in consumed meat foodstuffs must be determined. The determination of trace elements content in meat foodstuffs widely consumed in Aizawl city was conducted. Meat samples were collected from markets in the city and analyzed using Energy Dispersive X-ray Fluorescence (ED-XRF). A large variability of essential trace elements concentration was observed (Ca, K, Cl, P, S, Fe, Mn, Zn, Cu, Se Sr, Rb and Br). These results could provide information of nutritional status of the society and can be used as a reference for government and related institution to effectively making policies and solution for public health improvement.

Keywords: ED-XRF, Trace Elements, Biochemical, Meat Foodstuffs

INTRODUCTION

Trace elements play an important role in human health because they participate in biological functions that contribute to growth and good health. The trend roles of metal ions in health and disease range from the requirement for intake of essential trace elements to toxicity associated with metal overload. One of the major sources of trace elements is the diet consumed. Therefore, it is important to ensure that the consumed diet contains the essential minerals in adequate quantities. Inadequate nutrition of these elements compromises immune function, causes impaired physical and mental growth in children, affects reproductive performance, and reduces work productivity and consequently affects a country's economy [1-3]. Some trace elements, such as iron, zinc, manganese, chromium, cobalt and selenium, are essential since they are either inadequately synthesized or not synthesized in the body [4-6]. Most of the people affected by trace elements deficiency do not show any clinical symptoms nor are they aware of the deficiency; this is a phenomenon referred to as hidden hunger. However, those essential trace elements become toxic when their concentration becomes excessive. The high concentrations, as also depletion in the concentration of those essential trace elements, may cause various metabolic instabilities due to enzyme dysfunction [7-9].

Meat as the flesh of animals used for food is a relevant dietary source of proteins, essential amino acids, chemical elements (e.g. iron, zinc) and vitamins (e.g. B12, D). Yet, the healthy image of meat is tarnished by its negative association with non-nutritional issues like the presence of various toxic contaminants. Taking consumer behavior into consideration, there was primarily the demand for sufficient food, afterwards the desire for more and more quality in the food area and nowadays everybody asks for safe and healthy food with high quality. With increasing global distribution of feed, food and ingredients the different countries in our world have never been before more dependent on each other with respect to their food supply. Chemical composition of meat depends on kinds and degrees of animal feeding. The demand for mineral compounds depends on age, physiological state, and feed intake as well as on living conditions. In order to promote animal growth and prevent diseases, all kinds of metals are added into animal feed excessively [10].

Considering that meat is an important source of toxic metal exposure to humans, but also a valuable source of some essential elements (mainly Cu, Fe, Zn and Se) [11], it is important to explore heavy metal and trace element (Ca, K, Cl, P, S, Fe, Mn, Zn, Cu, Se Sr, Rb and Br) concentrations in various meat foodstuffs consumed in Aizawl market. Moreover, the data were compared with the permissible limits and estimates of dietary exposures.

MATERIALS AND METHODS

Sample Preparation

Three different types of commonly consumed meat (Chicken, Beef and Fish) were selected for analysis. The meat foodstuffs were purchased from the Central Markets of Aizawl, Mizoram, a North Eastern State of India. They were thoroughly washed with triple distilled water to eliminate contamination due to dust and environmental pollution, air-dried and then oven dried at 60^0C and stored in plastic bags until needed. Drying takes about 3-4 days depending upon the meat foodstuffs. After drying, they were grounded into fine powder using mortar and pestle, and stored in a well labelled air tight container for analysis. The powdered samples were then formed into pellets (150mg each) by using a KBr press pelletizer. The die used was 13mm.

Analysis using ED-XRF Spectrometer

The elemental analysis of meat samples was carried out using a Xenematrix Ex-3600 Energy dispersive X-ray fluorescence ED-XRF spectrometer which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV). The measurements were carried out in vacuum using different filters (between the source and sample) for optimum detection of elements. For example, for P, S, Cl, K and Ca, no filter was used, and a voltage of 6 kV and current of 200 mA were used and samples were run for 200 sec. A 0.05-mm-thick Ti filter was used in front of the source for Mn, Fe, Cu and Zn, with an applied voltage of 14 kV and a current 900 mA and samples were run for 400 sec. For higher *Z* elements such as Se, Br, Rb and Sr Fe filter of 0.05mm thickness was used at a voltage of 23 kV and 200 mA current and samples were run for 600 sec. The X-rays were detected using a liquid-nitrogen-cooled 12.5 mm^2 Si (Li) semiconductor detector (resolution 150 eV at 5.9KeV). The X-ray fluorescence spectra were quantitatively analyzed by the software next integrated with the system. This software uses the Fundamental Parameter Method approach, which combines a theoretical basis of X-ray emission and absorption with experimental measurements for unknown sample analyses. Here all matrix corrections etc. are taken into account. The experimental results were subject to statistical analysis using Excel 2007 and SPSS package v.17.0.

RESULTS AND DISCUSSIONS

In this study, the total thirteen elements were determined in the powdered pellet of three different meat foodstuffs by using ED-XRF (Fig.). The mean concentration of the various elements in the meat samples collected from the central markets of Aizawl, Mizoram, India were shown in the Tables 1, 2 & 3. These Tables revealed wide and appreciable variations in the elemental concentration in the different meat samples studied.

The results of the present study revealed that K is observed generally higher in all the meat samples studied as compared to other macro elements recorded. The content of K, Ca, P, Cl and S in all the selected meat samples ranged from 2532.6±58.2 mgL^{-1} to 3365.8 ± 89.6 mgL^{-1}, 413.1 ± 21.6 mgL^{-1} to 1993.4±291.5 mgL^{-1}, 1493±91.9 mgL^{-1} to 1894.9±124.6 mgL^{-1}, 544.5±3.5 mgL^{-1} to 594.7±6.2 mgL^{-1} and 1648.8±44.3 mgL^{-1} to 1676±93.7 mgL^{-1} respectively. The highest content of K and Cl was recorded in beef (3365.8 ± 89.6 mgL^{-1} of K and 594.7±6.2 mgL^{-1} of Cl) while the highest content of Ca and P was in fish (1993.4±291.5 mg/l of Ca and 1894.9±124.6 mg/l of P) with chicken containing highest amount of S (1676±93.7 mgL^{-1}) (Table 1). The study reveals that the meat samples contained high and notable amount of K and Ca. However the content of elements recorded in our study is in agreement with those reported by Stihi et al in the year 2008[12], Masson et al in the year 2010 [13] and Vincevica Gaile and Klavins in the year 2011[14]. However, all the macro elements were within the maximum permissible limit in vegetables as reported by Institute of Medicine[15], Stihi et al [12], Masson et al [13] and Vincevica Gaile and Klavins [14].

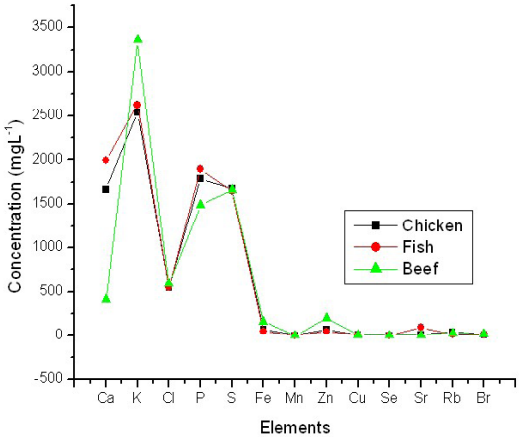

Fig. 1: ED-XRF Spectra for Determination of Concentration of Different Elements

Analysis of data revealed that among the trace element studied, Fe and Zn concentration is generally found to be higher in all the meat samples studied. The Fe and Zn content ranges between 47.9±2.3 mgL^{-1} to 161.2±6.2 mgL^{-1} and 46.2±0.7 mgL^{-1} to 198.1±4.2 mgL^{-1}, with the highest Fe and Zn content found in beef (161.2±6.2 mgL^{-1} and198.1±4.2 mgL^{-1}) (Table 2). The value recorded in our study is within the recommended dietary allowances of Fe (10-15 $mgday^{-1}$) as per FAO/WHO [16]. Fe is a necessary nutrient element and is core component of RBC. It is needed for healthy immune system and for energy production. Several studies have also shown the important role of Fe availability correlates with the bactericidal effect of lactoferrin and lysozyme, which can killed gram-negative bacteria [17]. The concentration of Zn obtained in our study is in agreement with those reported by Singh and Taneja [18]. Zn also plays an important role in immune function, wound healing, protein synthesis, DNA synthesis and cell division.

The content of the Mn recorded in the present study ranged between 0 to 1.1±0.3 mgL^{-1} and can be neglected. As a contaminant however, no maximum permissible limit (MPL) has been fixed for Mn in foodstuffs. Upper tolerable limit of Mn for human is 2-11 $mgday^{-1}$.

Cu is known to play an important role in human metabolism, largely because it allows many critical enzymes to function properly. As an antioxidant, Cu scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause. As expected Cu was found to be present in appreciable amount in the selected meat samples studied from 3.4±0.4 mgL^{-1} to 6.8±1.4 mgL^{-1} and the highest level of the Cu was recorded from beef (6.8±1.4 mgL^{-1}) (Table 2). The Cu content recorded in the present study is in agreement with Singh and Taneja [19]. The concentration of Cu in plants varied much with dependent nearby factors like proximity industries and use of fertilizers and Cu based fungicides.

In the present study, Rb and Br were also detected in all the meat samples studied with varying concentrations in the range between 8.6±1.7 mgL^{-1} to 26.2±1.7 mgL^{-1} and 3.4±0.7 to 17.5±0.8 mgL^{-1} respectively (Table 3). Generally Rb and Br have no known essential role in human or mammalian health. However, Rb ions are utilized by human body in a manner similar to potassium ions, being actively taken up by plants and by animal cells. Se (0.17 mgL^{-1} to 1.8±0.1 mgL^{-1}) is an element which behaves both as an antioxidant and anti-inflammatory agent.

Sr is considered as an essential element involved in Ca and P management for animals. Nevertheless, the possibility that Sr is essential has not been confirmed. Its mean concentration in chicken and fish samples examined were 11.4±1.8 mgL^{-1} and 91.8±5.9 mgL^{-1} respectively. These values were extremely greater than that reported by Jarzyńska and Falandysz [20] for muscle (0.13 $mgkg^{-1}$ dry weight), liver (0.11 $mgkg^{-1}$ dry weight) and kidney (0.18 $mgkg^{-1}$ dry weight).

Table 1: Ca, K, Cl, P and S Concentration (mgL^{-1}) of the Meat Samples of Aizawl. Values are Mean ±SE, 3 Observations Each

Name of the Meat Sample	Calcium(Ca)	Potassium (K)	Chlorine (Cl)	Phosphorus (P)	Sulphur (S)
Chicken	1667.6±197.5	2532.6 ±58.2	544.5 ±3.5	1788.5 ±71.4	1676 ±93.7
Fish	1993.4 ±291.5	2623.3 ±41.4	549.4 ±2	1894.9 ±124.6	1648.8 ±44.3
Beef	413.1 ±21.6	3365.8 ±89.6	594.7 ±6.2	1493.1 ±91.9	1666.2 ±139.2

Table 2: Fe, Mn, Zn and Cu Concentration (mgL^{-1}) of the Meat Samples of Aizawl. Values are Mean ±SE, 3 Observations Each

Name of the Meat Sample	Iron (Fe)	Manganese (Mn)	Zinc (Zn)	Copper (Cu)
Chicken	72.9 ±5.1	0.3 ±0.1	64.9 ±7.3	3.4 ±0.4
Fish	47.9 ±2.3	1.1 ±0.3	46.2 ±0.7	4.8 ±0.6
Beef	161.2 ±6.2	0	198.1 ±4.2	6.8 ±1.4

Table 3: Se Sr, Rb and Br Concentration (mgL^{-1}) of the Meat Samples of Aizawl. Values are Mean ±SE, 3 Observations Each

Name of the Meat Sample	Selenium (Se)	Strontium (Sr)	Rubidium (Rb)	Bromine (Br)
Chicken	1.8 ±0.1	11.4 ±1.8	43.2 ±3.2	3.4 ±0.7
Fish	0.12	91.8 ±5.9	8.6 ±1.7	10.2 ±0.9
Beef	0.17	0	26.2 ±1.7	17.5 ±0.8

CONCLUSION

Food consumption is the major pathway for human exposure to heavy metals and so threatens the health of the population. The level of heavy metals and trace elements in different meat samples in Aizawl were determined and assessed by comparing the results with the permissible limits. The results indicated that concentrations were mostly under the permissible limits and as such poses no threat to the health of the populace.

ACKNOWLEDGEMENTS

The authors are grateful to Director and Staff of UGC-DAE CSR, Kolkata Center, for providing the necessary Laboratory facilities and DBT, New Delhi for financial support under the DBT-NE-Twinning Project.

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Figure 2(a). Photographs of Vegetable foodstuffs

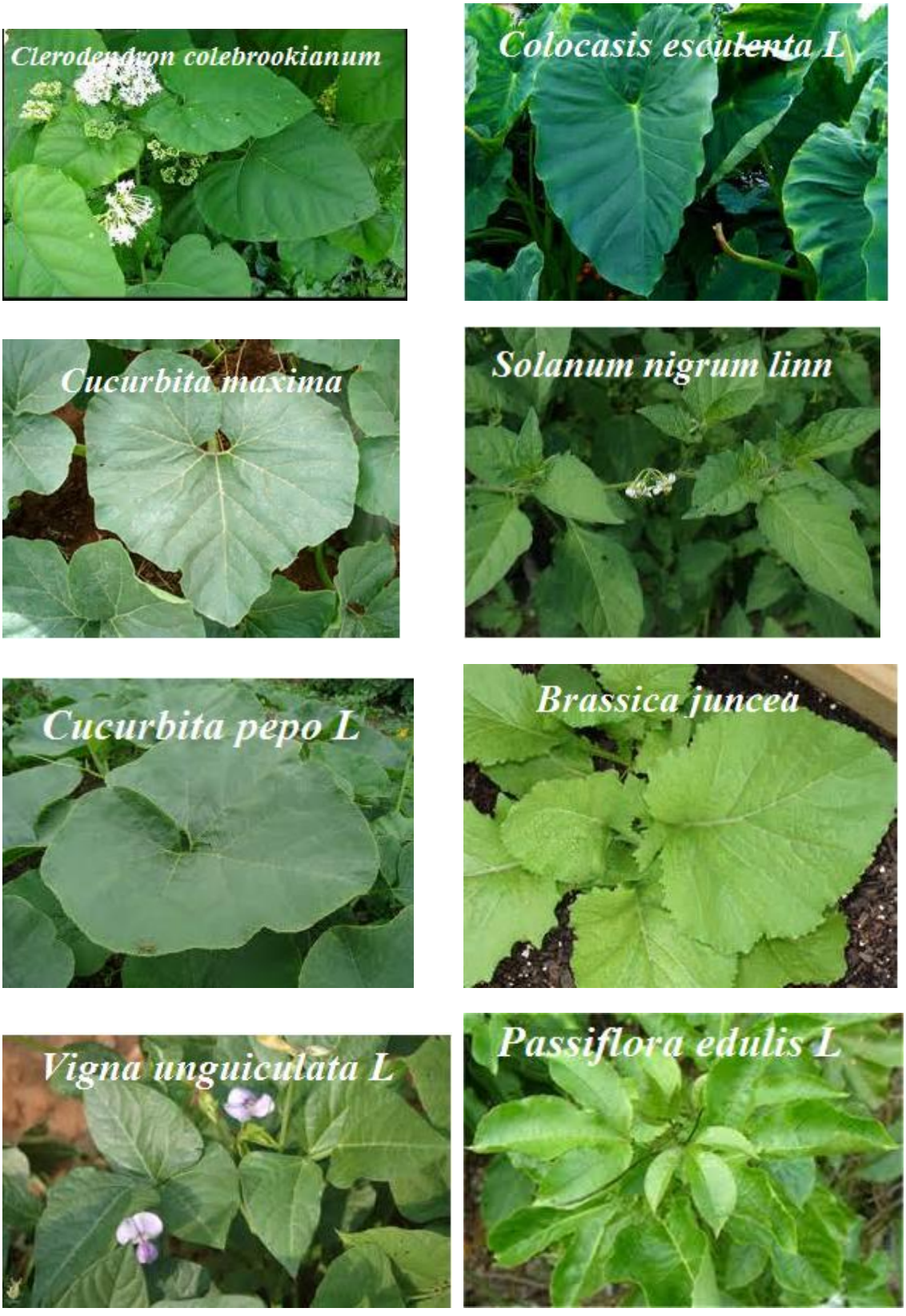

Figure 2(b). Photographs of Vegetable foodstuffs

Figure 2(c). Photographs of Vegetable foodstuffs

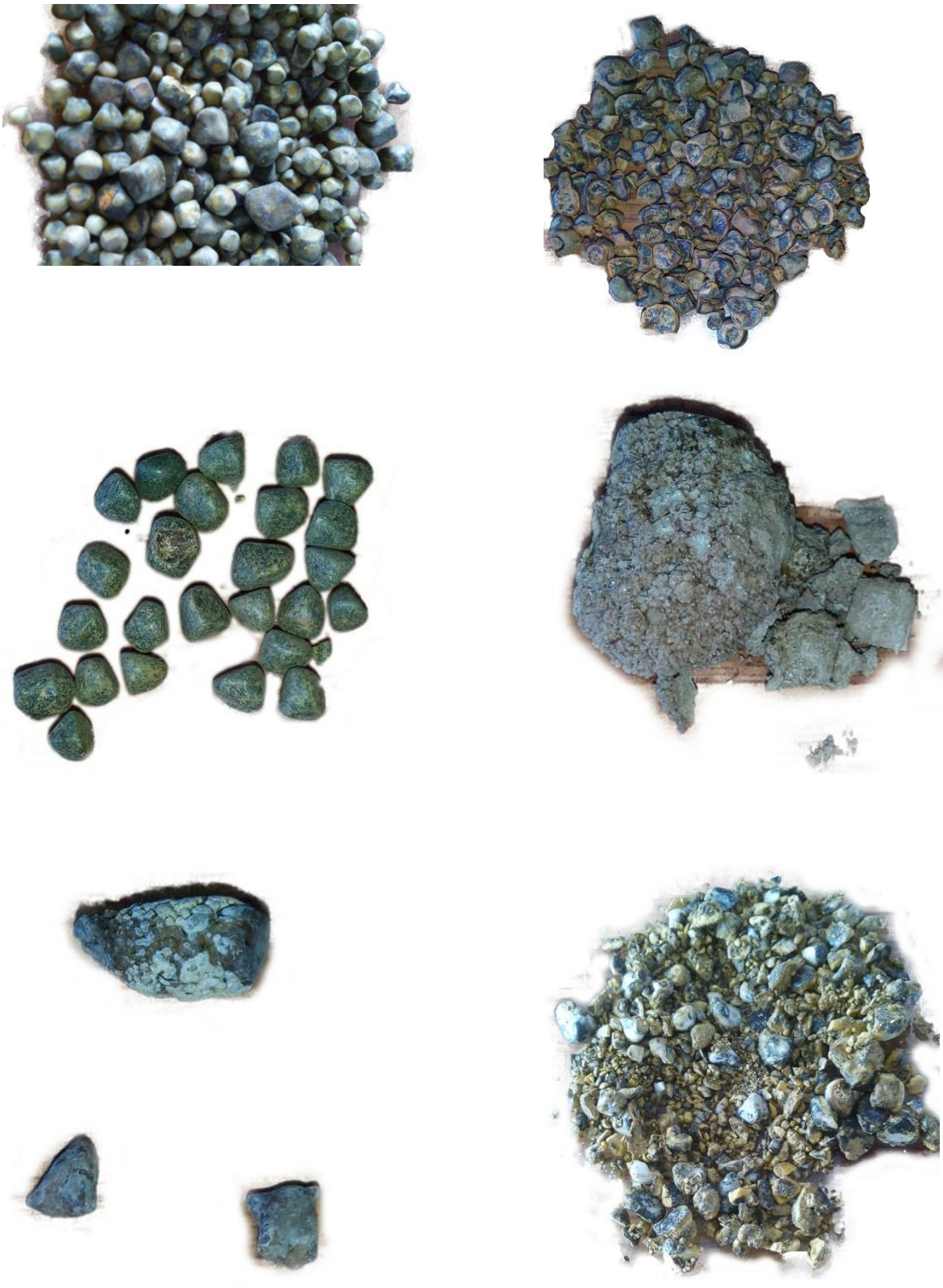

Figure 3. Photographs of Urolithiatic stones