Antioxidant potential of few selected medicinal plants of Mizoram and their anti-ulcer activity in induced albino rats

Thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Biotechnology

By

H. Lallawmawma

Ph.D. Registration No: MZU/Ph.D/597 of 31.10.2013

Under the Supervision of

Dr. N. Senthil Kumar
Professor
Department of Biotechnology

&

Joint- Supervision of

Dr. G. Gurusubramanian
Professor
Department of Zoology

Department of Biotechnology
School of Life Sciences
Mizoram University Aizawl, Mizoram

CERTIFICATE

I certify that the thesis entitled "**Antioxidant potential of few selected medicinal plants of Mizoram and investigation of their anti-ulcer activity in induced albino rats"** submitted to the Mizoram University for the award of a degree of Doctor of Philosophy in Biotechnology by H. LALLAWMAWMA is a record of research work carried out by him during the period from 2013 to 2016 under my guidance and supervision and this work has not formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles in this University or any other University or institution of higher learning.

Signature of the supervisor
(N. SENTHIL KUMAR)

Signature of the Co-Supervisor
(G. GURUSUBRAMANIAN)

Declaration of the Candidate

I, H. Lallawmawma Ph. D. scholar in Biotechnology Department, Mizoram University, Aizawl, do hereby solemnly declare that the subject matter of this thesis is the record of the work done by me. I have duly worked on my Ph. D. thesis under the supervision of Prof. N Senthil Kumar, Department of Biotechnology, Mizoram University and Prof. G. Gurusubramanian, Department of Zoology, Mizoram University. This is being submitted to Mizoram University for the degree of Doctor of Philosophy in Biotechnology and that I have not submitted this thesis to any other University/ Institute for any other degree.

(Prof. N. Senthil Kumar)
Head and Supervisor

(H.Lallawmawma)
Candidate

ACKNOWLEDGEMENTS

First and foremost, I bowed with humility in respect to Almighty God. I would like to thank Him for his guidance, protecting me from every corner of mental and physical conditions during my research work, for providing me an opportunity and abundant blessing.

My countless thanks goes to my Supervisors, Professor **N. Senthil Kumar**, Head of Department of Biotechnology, and Professor **G. Gurusubramanian**, Head of Department of Zoology, Mizoram University, Aizawl. I express great appreciation for their valuable time, guidance and effort, who constantly motivated me to do better, and assisted me during my difficult stages, as well as inspiring me a lot, sharing their precious treasure of knowledge such that I finally could complete this thesis.

I earnestly wish to express my sincere thanks to the faculties and non-teaching staffs from the Department of Biotechnology, Mizoram University for their innumerable help, providing much needed assistance and keeping my work running smoothly.

I sincerely give thanks to all my fellow research scholars from the Department of Biotechnology and Department of Zoology who have supported and provided a friendly and cooperative atmosphere at work and also many useful feedback and comments on my work.

And also, my heartfelt thanks goes to Dr. H Lalhlenmawia, Head of the Pharmacy Department, Regional Institute of Pharmaceutical and Nursing Science, Zemabawk, Aizawl and his colleagues for all their help, sacrifices and efforts that they have contributed on my work.

I am deeply grateful to my family for their love, support, prayers, understanding and for providing constant words of encouragement throughout the duration of my work. I express my sincere thanks to my wife for her patience and endurance during my research work for her valuable support and assistance. I also extend my gratitude to all my friends for they all have the most important hands in sparing their valuable time and gave me constant support and necessary help.

Last, but not the least, my thank goes to the State Biotech Hub and Bioinformatics Infrastructure Facility sponsored by Department of Biotechnology (DBT), Govt. of India, New Delhi for providing me the necessary facilities for my research. And I'm also thankful to MZU-UGC fellowship for supporting me for my research work.

(H. LALLAWMAWMA)

CONTENTS **Page no**

ACKNOWLEDGEMENTS **i-ii**

CONTENTS **iii-vii**

LIST OF FIGURES **viii-xiii**

LIST OF TABLES **xiv-xv**

LIST OF ACRONYMS **xvi-xix**

CHAPTER 1: INTRODUCTION AND REVIEW OF LITERATURE **1-21**

1.1 Antioxidants 1-2

1.2 Medicinal Plants 2-5

1.3 Reactive oxygen species 5

1.4 Gastric ulcers 5-7

1.5 History of gastric ulcers 7

1.6 Nanoparticles 7-9

1.7 High Performance Liquid Chromatography 9-11

1.8 Review of Literature 11-19

1.9 Peptic ulcer 19-20

1.10 Studies of antiulcer activities 20-21

CHAPTER 2: OBJECTIVE OF THE STUDY **22**

CHAPTER 3: STUDY AREA AND SURVEY **23-28**

3.1 Study area 23

3.2 Mampui village 24-26

3.3 Sialsuk village 26-28

CHAPTER 4: MATERIALS AND METHODOLOGY **29-46**

4.1 Plant materials 29-32

4.2 Omeprazole 32

4.3 Reagents 32

4.4 Method of Extraction (Soxhlet extraction) 32-33

4.5 Preliminary phytochemical screening 33-35

4.6 Evaluation of antioxidant activity of the plants by the following assays 35-39

4.6.1 Determination of total flavonoid content 35

4.6.2 Determination of β-carotene content 35

4.6.3 DPPH radical scavenging activity 36

4.6.4 Determination of reducing power (iron chelation) 36

4.6.5 High Performance Liquid Chromatography (HPLC) 37

4.7 Synthesis of silver and gold nanoparticles by using *Jasmimum nervosum* leaf extracts 37-38

4.8 Characterization of Gold Nanoparticle (AgNPs) and Silver Nanoparticle (AuNPs) 38-40

4.8.1 UV-Visible spectrophotometer 38-39

4.8.2 Fourier transforms infrared (FTIR) and X-ray diffraction (XRD) analysis 39

4.8.3 Transmission Electron Microscope (TEM) measurement 40

4.9 Synthesis and Characterization of silver and gold nanoparticles by using *Helicia robusta* bark extracts 40

4.10 Animal Stock 40

4.11 Acute Toxicity 40-42

4.11.1 Ethical committee 40-41

4.11.2 Assignment of animals 41

4.11.3 Housing and Diet 41

4.11.4 Mode of administration 41

4.11.5 Administration Dose 42

4.11.6 Observation period 42

4.11.7 Signs recorded during acute toxicity studies 42

4.12 Gastric Ulcer Induction by Ethanol 42-44

4.13 Ulcer index 44

4.14 Total acidity and free acidity 44-45

4.15 Measurement of Gastric Juice Acidity 45

4.16 Histological Examination of the Gastric Mucosa 45-46

4.16.1 Blocking 45-46

4.16.2 Staining 46

4.17 Statistical analysis 46

CHAPTER 5: RESULTS **47-81**

5.1 Phytochemical screening 47-48

5.2 Determination of β-carotene content 48-49

5.3 Determination of total flavonoid content 49-50

5.4 DPPH radical scavenging activity 50-52

5.5 Determination of Reducing Power 52-54

5.6 High Performance Liquid Chromatography 54-56

5.7 Silver and Gold nanoparticles characterization and their interactions 57-67

5.7.1 Synthesis of AgNPs and AuNPs - *Jasminum nervosum* leaf extract 57

5.7.2 Characterization of AgNPs and AuNPs - *Jasminum nervosum* leaf extract 57-61

5.7.2.1 UV-Visible spectrophotometer 57-58

5.7.2.2 Fourier transform infrared(FTIR) and X-ray Diffraction(XRD) Analysis 58-60

5.7.2.3 Transmission Electron Miscroscope (TEM) analysis 60-61

5.8 Synthesis of AgNPs and AuNPs - *Helicia robusta* bark extract 61-62

5.8.1 Characterization of AgNPs and AuNPs - *Helicia robusta* bark leaf extract 62-67

5.8.1.1 UV-Visible spectrophotometer 62-63

5.8.1.2 Fourier transform infrared (FTIR) and X-ray Diffraction (XRD) Analysis 63-65

5.8.1.3 Transmission Electron Microscope (TEM) analysis 65-67

5.9 Acute Toxicity Study 67-69

5.10 Gross Evaluation of Gastric Lesions 70-77

a) *Helicia robusta* methanolic bark extract 70-71

b) *Jasminum nervosum* methanolic leaf extract 72-73

c) Gold and Silver Nanoparticles of *Helicia robusta* methanolic bark extract 74-75

d) Gold and Silver Nanoparticles of *Jasminum nervosum* methanolic leaf extract 76-77

5.11 Histological Evaluation of Gastric Lesion 78-81

a) *Helicia robusta* methanolic bark extract 78

b) *Jasminum nervosum* methanolic leaf extract 79

c) Gold and Silver Nanoparticles of *Helicia robusta* methanolic bark extract 80

d) Gold and Silver Nanoparticles of *Jasminum nervosum* methanolic leaf extract 81

CHAPTER 6: DISCUSSION 82-97

CHAPTER 7: SUMMARY 98-104

CHA PTER 8: BIBILOGRAPHY 105-131

Published and communicated papers

LIST OF FIGURES

Figure 1. Google image of Mampui village.

Figure 2. Google image of Sialsuk village.

Figure 3. *Helicia robusta* tree and herbarium specimen of *H. robusta* showing leaves, fruits, flowers.

Figure 4. *Jasminum nervosum* (leaves and flower) and its herbarium specimen.

Figure 5. Standard curve of Quercetin.

Figure 6. DPPH free radical activity of Butylated hydroxyanisole (BHA) and methanolic extract of *Helicia robusta.*

Figure 7. DPPH free radical activity of Butylated hydroxyanisole (BHA) and methanolic extract of *Jasminum nervosum*

Figure 8. Reducing power of methanolic extract of *Jasmimum nervosum* in comparison with the standard, Ascorbic acid.

Figure 9. Reducing power of methanolic extract of *Helicia robusta* in comparison with the standard, Ascorbic acid.

Figure 10. HPLC chromatogram of phenolic compounds present in the methanolic extract of *Jasminum nervosum* (100 mg/ml *J.nervosum*).

Figure 11. HPLC chromatogram of phenolic compounds present in the methanolic extract of *Helicia robusta* (100 mg/ml *H.nervosum*).

Figure 12. a) Picture of leaves of *J.nervosum* b) Visible colour indicates the generation of nanoparticles of *J.nervosum* (1.leaf extract 2.AgNPs 3.AuNPs)

Figure 13. UV-Visbile spectroscopic analysis shows the intense absorbance spectra at (a) 520 nm and (b) 390 nm indicates the formation of AuNPs and AgNPs respectively. No SPR spectra were recorded for GERE and metal ion substrate ($AgNO_3$ and $HAuCl_4$).

Figure 14. Fourier transform infrared (FTIR) spectrum of aqueous and synthesized AgNPs and AuNPs using *J. nervosum* leaf extract showing the interaction of nanoparticles with water soluble phenolic biocompounds.

Figure 15. X-ray diffraction (XRD) pattern of synthesized: a) silver nanoparticles b) gold nanoparticles from aqueous leaf extract of *J. nervosum*.

Figure 16. High resolution transmission electron microscopic images of *J.nervosum* displays well dispersed

a) AgNPs in the size range between 4-22 nm with the average size of 10±2 nm
b)AuNPs in the size range between 2-20 nm with the average size of 9.4±4 nm

Selected area electron diffraction pattern of synthesized AuNPs confirms the face centre cubic crystalline structure and DLS analysis shows the particle size distribution and hydrodynamic diameter of synthesized AgNPs and AuNPs.

Figure 17. a) Visible colour indicates the generation of nanoparticles of *H.robusta* (1.bark extract 2.AgNPs 3.AuNPs). b) Picture of stem bark of *H.robusta*.

Figure 18. UV-Visbile spectroscopic analysis shows the intense absorbance spectra at (a) 550 nm and (b) 420 nm indicates the formation of AuNPs and AgNPs respectively. No SPR spectra were recorded for GERE and metal ion substrate ($AgNO_3$ and $HAucl_4$).

Figure 19. Fourier transform infrared spectroscopic analysis of synthesized AgNPs and AuNPs generates the transmittance corresponding to O-H, C=O and C-H functional group denotes the active role of CERE metabolites in reduction and stabilization.

Figure 20. a) X-ray Diffraction analysis of synthesized AgNPs shows the planes corresponds to both cubic and b) hexagonal crystalline structure whereas AuNPs possess the planes for face centred cubic (fcc) crystalline structure from aqueous leaf extract of *H.robusta.*

Figure 21a. a) High resolution transmission electron microscopic images displays dispersed AgNPs in the size range between 4.5-56.4 nm with the average size of 21±11 nm (b) SAED Pattern of synthesized AgNPs confirms the mixed phase of both cubic and hexagonal crystalline structure
(c) DLS analysis shows the particle size distribution and hydrodynamic diameter of synthesized AgNPs.

Figure 21b. (a)High resolution transmission electron microscopic images displays well dispersed AuNPs in the size range between 4.5-30.2 nm with the average size of 12.8±6 nm
(b) Selected area electron diffraction pattern of synthesized AuNPs confirms the face centered cubic crystalline structure
(c) Dynamic light scattering analysis shows the particle size distribution and hydrodynamic diameter of synthesized AuNPs.

Figure 22. The effect of *Helicia robusta* bark on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.
(G1) (Normal control group); (G2) (Ulcer control group); (G3) (Omeprazole); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Helicia robusta* extract.

Figure 23. The effect of *Jasminum nervosum* leaf on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.
(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control group); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Jasminum nervosum* extract.

Figure 24. The effect of gold and silver nanoparticles of *Helicia robusta* on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in albino rats.
(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control group); (G4) (.1Au mg/kg), (G5) (.5Au mg/kg), (G6) (.1Ag mg/mg), (G7) (.5Ag mg/kg) *Helicia robusta* extract.

Figure 25. The effect of gold and silver nanoparticles of *Jasmimum nervosum* on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control); (G4) (.1Au mg/ml), (G5) (.5Au mg/ml), (G6) (.1Ag mg/ml), (G7) (.5Ag mg/ml) *Jasmimum nervosum* extract.

Figure 26. The effect of *H. robusta* on the histology of ethanol-induced gastric mucosal damage in rats.

(G1) (Normal control group); (G2) (Ulcer control group); (G3) (Omeprazole); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Helicia robusta* extract.

Figure 27. The effect of *J. nervosum* on the histology of ethanol-induced gastric mucosal damage in rats.

(G1) (Normal control group); (G2) (Ulcer control group); (G3) (Omeprazole); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Jasminum nervosum* extract.

Figure 28. The effect of gold and silver nanoparticle of *H. robusta* on the histology of ethanol-induced gastric mucosal damage in rats.

(G1) (Normal control group); (G2) (Positive control group); (G3) (Negative control group); (G4) (.1Au mg/ml), (G5) (.5Au mg/ml), (G6) (.1Ag mg/ml), (G7) (.5Ag mg/ml) *Helicia robusta* extract.

Figure 29. The effect of gold and silver nanoparticle of *J.nervosum* on the histology of ethanol-induced gastric mucosal damage in rats.

(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control); (G4) (.1Au mg/ml), (G5) (.5Au mg/ml), (G6) (.1Ag mg/ml), (G7) (.5Ag mg/ml) *Helicia robusta* extract.

LIST OF TABLES

Table 1. Information on Mampui Village, Mizoram.

Table 1a. Information on Sialsuk Village, Mizoram.

Table 2. Preliminary phytochemical screening of petroleum ether and methanolic extracts of *H. robusta.*

Table 3. Preliminary phytochemical screening of pet ether and methanolic extracts of *J. nervosum.*

Table 4. Determination of total β-carotene content in methanolic extracts of *H. robusta* and *J. nervosum* at absorbance 453nm, 505nm, 663nm.

Table 5. Total flavonoid content of the methanolic extracts *H. robusta* and *J. nervosum* at different concentrations at absorbance of 415 nm in comparison with the standard, Quercetin.

Table 6. DPPH free radical scavenging activity of methanolic extracts *H. robusta* and *J. nervosum* at absorbance of 517 nm. Standard Butylated hydroxyanisole (BHA) control absorbance = 0.168).

Table 7. Reducing power of methanolic extracts of *H. robusta* and *J. nervosum* at an absorbance of 700 nm in comparison with the standard, ascorbic acid.

Table 8. Identification and quantification of phenolic compounds present in the methanolic extracts of *J. nervosum*.

Table 9. Identification and quantification of phenolic compounds present in the methanolic extracts of *H. robusta.*

Table 10. Potential toxic effects of the crude extract of *H.robusta*, *J.nervosum*, Gold and Silver nanoparticles of *H.robusta*, Gold and Silver nanoparticles of *J.nervosum*

Table 11. General appearance and behavioral observations for control and treated groups.

Table 12. Effects of *H. robusta* bark methanolic extract on the gastric mucosa of rats.

Table 13. Effects of *J. nervosum* leaf methanolic extract on the gastric mucosa of rats.

Table 14. Effects of gold and silver nano particle of *H. robusta* bark methanolic extract on the gastric mucosa of albino rats.

Table 15. Effects of gold and silver nanoparticle of *J. nervosum* methanolic extract on the gastric mucosa of rats.

LIST OF ACRONYMS

JN	:	*Jasminum nervosum*
HR	:	*Helicia robusta*
ROS	:	Reactive Oxygen Species
WHO	:	World Health Organisation
NSAID	:	Non-steroidal anti-inflammatory agents
H. pylori	:	*Helicobacter pylori*
HPLC	:	High-performance liquid chromatography
e.g	:	Example
UNESCO	:	The United Nations Educational, Scientific and Cultural Organization
BHT	:	Butylated hydroxytoluene
BHA	:	Butylated hydroxyanisole
DNA	:	Deoxyribo Nucleic Acid
B.C	:	Before Christ
Etc.	:	etcetera
cm	:	centimetre
m	:	metres
MDA	:	Malondialdehyde
CAT	:	Chloramphenicol acetyltransferase
GSH	:	Glutathione
SOD	:	Superoxide dismutase

NO	:	Nitric oxide
PGE2	:	Prostaglandins E2
g	:	gram
Kg	:	kilogram
mm	:	milimetre
ºC	:	degree celsius
km	:	kilometre
ml	:	mililitre
E	:	East
N	:	North
CMC	:	Carboxy methyl cellulose
MUMC	:	Mizoram University Medical Centre
UK	:	United Kingdom
USA	:	United States of America
NaOH	:	Sodium Hydroxide
$NaNO_2$	:	Sodium Nitrite
$AlCl_3$	:	Aluminium Chloride
QE	:	Quercetin Equivalent
β	:	beta
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
%	:	percentage
pH	:	Negative logarithm of the hydrogen ion concentration/the power of hydrogen

rpm	:	revolutions per minute
UV-Vis	:	Ultraviolet visible
Min	:	Minute
$AgNO_3$	:	Silver Nitrate
$HAuCl_4$	:	Chloroauric acid
Ag	:	Gold
Au	:	Silver
AgNPs	:	Gold Nanoparticle
AuNPs	:	Silver Nanoparticle
mM	:	milimolar
FTIR	:	Fourier transforms infrared
XRD	:	X-ray diffraction
TEM	:	Transmission electron microscopy
kV	:	kilovolt
mA	:	milliampere-hour
Kev	:	Kiloelectron volt
OECD	:	Organization for Economic Co-operation and Development
hr	:	hour
mg	:	miligram
Hcl	:	Hydrochloric Acid
ANOVA	:	Analysis of variance
i.e	:	that is
µL	:	microlitre

Fig.	:	figure
SPR	:	Surface Plasmon Resonance
mEq	:	miliequivalents
ltr	:	litre
UI	:	Ulcer Index
N.C	:	Normal Control
CSE	:	Cystathionine gamma-lyase

CHAPTER 1
INTRODUCTION AND REVIEW OF LITERATURE

1.1 Antioxidants

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress (Ozsoy *et al.,* 2008). These antioxidants are capable of neutralizing the harmful effects of the ROS through the endogenous enzymatic defense system such as the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in human system. This is because antioxidants compounds can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, which therefore, appear to be very important in the prevention of many diseases (Halliwell *et al*., 1992). Therefore, the search for natural antioxidants to replace synthetic antioxidants is necessary.Within the antioxidant compounds flavonoids and phenolics, with a large distribution in nature, have been studied (Li *et al.*, 2009). Phenolics or polyphenols, including flavonoids, have received considerable attention because of their physiological functions such as antioxidant, antimutagenic and antitumor activities (Othman *et al*., 2007). Mohammad *et al*., (2010) also explicitly demonstrated that pharmacological effects may be attributed, at least in part, to the presence of phenols and flavonoids in the extracts.The compounds such as quercetin, rutin, narigin, catechins, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents because of their antioxidant activities (Paganga *et al.*, 1999), which are also used widely as standard substances today. Antioxidant research is an important topic in the medical field as well as in the food industry.

Recent research with important bioactive compounds in many plant and food materials have received much attention. Although the body possesses defense mechanisms, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS (Halliwell *et al*., 1995; Sies,

1993), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng *et al*., 1997). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares *et al*., 1997). In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects including antioxidant activity (Brown and Rice-Evans, 1998; Gil *et al*., 1999; Kähkönen *et al*., 1999; Vinson *et al*., 1995). Currently, the possible toxicity of synthetic antioxidants has been criticized. It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status (Halliwell, 1996). Thus interest in natural antioxidant, especially of plant origin has greatly increased in recent years (Jayaprakash and Rao, 2000).

1.2 Medicinal plants

The definition of Medicinal Plant has been formulated by WHO (World Health Organization) as follows- "A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Plants with medicinal properties "The gift of mother nature to mankind" are in use for centuries in the traditional system of medicine like Ayurveda, Unani, Siddha etc, in India and other countries for the treatment of diseases including ulcer. Plants maintain a complex system of multiple types of antioxidants. It has been estimated by WHO that approximately 80% of the world's population

from developing countries rely mainly on traditional medicinal plants for their primary health (Akarele, 1988). Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects. They are considered to be effective and non-toxic. Natural products from plants are a rich resource used for centuries to cure various ailments. The use of natural medicine in the treatment of various diseases like peptic ulcer is an absolute requirement of our time (Sasmal *et al*., 2007).

People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Maheshwari *et al*., 1986; Van Wyk *et al*., 2000). Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell *et al*., 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people. Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001). Plants are used medicinally in

different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996; Mahesh and Sathish, 2008). The plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty acids,carbohydrates etc.). Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and many pharmaceutical drugs. From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases (Wink *et al*., 2005).

Secondary metabolites (compounds) have no apparent function in a plant's primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro-organisms, insects and higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Karuppusamy, 2009; Sathishkumar and Paulsamy, 2009). In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites (e.g. steroids, quinines, alkaloids, terpenoids and flavonoids), which are used in drug

manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents.

1.3 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are major free radicals generated in many redox processes, which may induce some damage to the human body. Increased production of various forms of activated oxygen species, such as oxygen radicals and non- free radical species is considered to be the main contributor to oxidative stress, which has been linked to several diseases like atherosclerosis, Parkinson's disease, Alzheimer's disease, stroke, arthritis, chronic inflammatory diseases, cancers, and other degenerative diseases (Halliwell and Grootveld, 1987; McDermott, 2000). Free radicals have been shown to be harmful as they react with important cellular components such as proteins, DNA and cell membrane. The body on the other hand, requires free radicals for immune responses. Reactive oxygen species (ROS) collectively refer to both the oxygen free radicals (the O_2·–superoxide and the OH· hydroxyl radical) and some non-radical (hydrogen peroxide, H_2O_2) derivatives of oxygen (Shoji *et al*., 2008) are generated as products normal metabolism in the body (Zhang *et al*., 2009).

1.4 Gastric Ulcers

Gastric ulcer is a major health hazard in terms of both morbidity and mortality (Chaturvedi *et al*., 2007). Ulcer is the end result of an imbalance between digestive fluids in the stomach and duodenum. Most ulcers are caused by an infection with a type of bacteria called *Helicobacter pylori* . The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus

secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermic growth factors (Repetto and Llesuy, 2002). According to Malyshenko *et al.,* (2005) and Kim, (2008) some other factors, such as inadequate dietary habits, cigarette smoking, excessive ingestion of non-steroidal anti-inflammatory agents (NSAID), stress and hereditary predisposition may be responsible for the development of peptic ulcer. Peptic ulcers can occur also with prolonged NSAIDs use, hiatal hernia, vitamin deficiency such as folic acid and vitamin C can also cause an imbalance of gastric juices which can make the stomach susceptible to erosion. NSAIDs are known to induce ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway. Prostaglandins are found in many tissues including the stomach, where they play a vital protective role via stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair. Thus, the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and subsequently gastric ulceration. Untreated gastric ulcer is capable of inducing upper gastrointestinal bleeding (Tortora and Grabowski, 2003). An ulcer may or may not have symptoms. When symptoms occur, they may include gnawing or burning pain in the middle or upper stomach between meals or at night, bloating, heartburn, nausea or vomiting . In severe cases, symptoms can include dark or black stool (due to bleeding), vomiting blood (that can look like "coffee-grounds"), weight loss, severe pain in the mid to upper abdomen. Peptic ulcers are present in around 4% of the population .

Three stages of ulceration have been identified: **Erosion** is the first stage where a marked destruction of the mucosal lining approximately 1-2 cm across. Stage two, considered a **True Ulcer** can emerge if erosion is not care for and can be identified by the marked or scarred disruption of the smooth tissue in the stomach and duodenum. The final and most life threatening stage is a **Bleeding Ulcer**. This type of ulcer is a partial or complete hole in the stomach tissue

causing hemorrhage. This condition is a medical emergency and can cause significant GI complications.

1.5 History of Gastric Ulcers

Helicobacter pylori which caused most of the ulcers was identified in 1982 by two Australian scientists, Robin Warren and Barry J. Marshall. Warren and Marshall contended that most gastric ulcers and gastritis were caused by colonization with this bacterium, not by stress or spicy food as had been assumed before. In 1997, the Centers for Disease Control and Prevention, with other government agencies, academic institutions, and industry, launched a national education campaign to inform health care providers and consumers about the link between *H. pylori* and ulcers. This campaign reinforced the news that ulcers are a curable infection, and that health can be greatly improved and money saved by disseminating information about *H. pylori*.

In 2005, the Karolinska Institute in Stockholm awarded the Nobel Prize in Physiology or Medicine to Dr. Marshall and his long-time collaborator Dr. Warren "for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease.

1.6 Nanoparticles

According to Dr Ananya Mandal, 2014 nanoparticle is a small microscopic particle of matter that is measured on the nanoscale, usually one that measures less than 100 nanometers that behaves as a whole unit in terms of its transport and properties. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. For bulk materials larger than one micrometre the percentage of atoms at the surface is minuscule relative to the total number of atoms of the material.The interesting and sometimes unexpected properties of nanoparticles are not partly due to the aspects of the surface of the

material dominating the properties in lieu of the bulk properties. Nanoparticles exhibit a number of special properties relative to bulk material.Suspensions of nanoparticles are possible because the interaction of the particle surface with the solvent is strong enough to overcome differences in density, which usually result in a material either sinking or floating in a liquid. Nanoparticles often have unexpected visible properties because they are small enough to confine their electrons and produce quantum effects. Nanoparticles have a very high surface area to volume ratio, this provides a tremendous driving force for diffusion, especially at elevated temperatures. Nanoparticle research is currently the most studied branch of science with the number of uses of nanoparticles in various fields. The particles have wide variety of potential applications in biomedical, optical and electronic fields.

Some of the uses of nanoparticles in biology and medicine include:

- Creating fluorescent biological labels for important biological markers and molecules in research and diagnosis of diseases
- Drug delivery systems
- Gene delivery systems in gene therapy
- For biological detection of disease causing organisms and diagnosis
- Detection of proteins
- Isolation and purification of biological molecules and cells in research
- Probing of DNA structure
- Genetic and tissue engineering
- Destruction of tumours with drugs or heat
- In MRI studies

- In pharmacokinetic studies.

Nanoparticles are being increasingly used in drug delivery systems. The advantages of using nanoparticles as a drug delivery system include:

- The size and surface characteristics of nanoparticles can be easily manipulated. This could be used for both passive and active drug targeting.
- Nanoparticles can be made to control and sustain release of the drug during the transportation as well as the location of the release. Since distribution and subsequent clearance of the drug from the body can be altered, an increase in drug therapeutic efficacy and reduction in side effects can be achieved.
- Choosing an appropriate matrix also helps in increasing the efficacy and reducing side effects
- Targeted drugs may be developed

1.7 High-performance liquid chromatography (HPLC)

High-performance liquid chromatography is a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds (Pauli *et al*., 2005). HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depends on the interactions between the stationary phase, the molecules being analyzed, and the solvent (s) used. (Liu *et al*., 2006) The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase. The amount of retardation depends on the nature of the analyte and composition of both stationary and mobile

phase. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time. Common solvents used include any miscible combinations of water or organic liquids (the most common are methanol and acetonitrile) (Abidi *et al*., 1991). Seperation has been done to vary the mobile phase composition during the analysis; this is known as gradient elution. The gradient separates the analyte mixtures as a function of the affinity of the analyte for the current mobile phase. The choice of solvents, additives and gradient depend on the nature of the stationary phase and the analyte. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column. HPLC has been used for manufacturing (e.g. during the production process of pharmaceutical and biological products), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and medical (e.g. detecting vitamin D levels in blood serum) purposes. The information that can be obtained using HPLC includes identification, quantification and resolution of a compound. Preparative HPLC refers to the process of isolation and purification of compounds. This differs from analytical HPLC, where the focus is to obtain information about the sample compound. Chemical separations is based on the fact that certain compounds have different migration rates given a particular column and mobile phase, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Purification is defined as the process of separating or extracting the target compound from a mixture of compounds or contaminants. Each compound showed a characteristic peak under certain chromatographic conditions. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any

other undesired compound. Generally assay of compounds are carried using HPLC. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. Other applications of HPLC includes Tablet dissolution study of armaceutical dosages form, Shelf-life determinations of parmaceutical products, Identification of active ingredients of dosage forms, Pharmaceutical quality control (Bergh, 1987; Stubbs 1990; MacNiel 1986; Bounine 1994) and Environmental applications (Lauback 1984; Wiklund 2005; Kwok 2006; Hongxia 2004) such as Detection of phenolic compounds in Drinking Water, Identification of diphenhydramine in sedimented samples, Bio-monitoring of pollute Forensics (Ayerton 1981; Bowden 1986; Haginaka 1984), Quantification of the drug in biological samples, Identification of anabolic steroids in serum, urine, sweat, and hair, Forensic analysis of textile dyes and determination of cocaine and metabolites in blood.

1.8 REVIEW OF LITERATURE

Available literatures on various studies of the antioxidant activities of medicinal plant and anti-ulcer studies appraisal have revealed yield informative features. Histological studies revealed that certain medicinal plants did not show any acute toxicity. Preliminary photochemical screening of medicinal plant identified the presence of important secondary metabolites like flavonoids and tannins. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of

the world's inhabitants relying mainly on traditional medicines for their primary health care.(Owolabi *et al*., 2007).

According to the World Health Organization (WHO, 1977) "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines (Anonymous, 2007a). Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products.

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo *et al*., 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine (Kala, 2005). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and

chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998). The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Cowman, 1999; Adesokan *et al*., 2008). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al*., 2000). Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important. The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges (Nautiyal *et al*., 2002), therefore they are more prone to extinction (Jablonski, 2004). Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socio-economic changes (Kala, 2000). Furthermore, the indigenous knowledge on the use of lesser-known medicinal plants is also rapidly declining. Continuous erosion in the traditional knowledge of many valuable plants for

medicine in the past and the renewal interest currently, the need existed to review the valuable knowledge with the expectation of developing the medicinal plants sector (Kala *et al*., 2006).

The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. All over the globe, especially in South American countries, the use of medicinal plants has significantly supported primary health care (Maciel *et al*., 2002). From 250 to 500 thousand plant species are estimated to exist on the planet, and only between 1 and 10% are used as food by humans and other animals. Such biodiversity is followed by a wide acceptance of the medicinal plant use (Carvalho *et al*., 2007). Most of the Brazilian population (80%) consumes only 37% of the commercially available drugs and depend almost exclusively on medicines of natural origin (Funari *et al*., 2005). Thus, phytotherapics entered the market promising a shorter and cheaper production, since basic requirements to use medicinal plants do not involve strict quality control regarding safety and efficacy compared to the other types of drugs (Niero *et al*., 2010). A variety of botanical products have been reported to possess antiulcer activity but the documented literature has centered primarily on pharmacological action in experimental animals. Except for a few phytogenic compounds (i.e. aloe, liquorice and chilly), limited clinical data are available to support the use of herbs as gastro-protective agents and thus, the data on efficacy and safety are limited. Despite this, there are several botanical products with potential therapeutic applications because of their high efficacy and low toxicity. Finally, it should be noted that substances such as flavonoids, aescin, aloe gel and many others, that possess antiulcer activity are of particular therapeutic importance as most of the anti inflammatory drugs used in modern medicine are ulcer genic. Active principles of antiulcer activity are flavonoids,

terpenoids and tannins. Many of these phytochemicals have significant antioxidant potentials that are associated with lower occurrence and lower mortality rates of several human diseases. In addition to their ability to act as an effective free radical scavengers, their natural origin represents an advantage to consumer in contrast to synthetic antioxidants which their use is restricted due to their carcinogenicity, ulceration etc. Alternative approach in recent days is the research of medicaments from ayurvedic or traditional medicinal system. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer. In this modern era also 75-80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Considerable evidence shows that more than 100 diseases affecting human beings are caused or aggravated by accumulation of free radicals or reactive oxygen species and the associated lipid per oxidation in the body (Chen and Yen, 2005). Some of these diseases include malaria, atherosclerosis, cancer, diabetes, acquired immunodeficiency syndrome (AIDS) and heart diseases (Luximon-Ramma *et al*., 2002; Li *et al.,* 2010). Experimental studies indicate a significant reduction in the risks for a variety of diseases upon feeding on polyphenol-rich foods, vegetables and beverages (Kris-Etherton *et al*., 2002). Polyphenols in plants are considered to be important ingredients in human diet. They are reported to exert a lot of biological effects such as antioxidant activity and inhibitory effects on carbohydrates hydrolyzing enzymes due to their ability to binds with protein (Griffiths and Moseley, 1980). The decrease in the cases of oxidative-stress associated diseases

like cancer, diabetes (Gerber *et al.,* 2002; Serafini *et al.*, 2002) and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (Di Matteo and Esposito, 2003) as well as inflammation and problems caused by cell and coetaneous aging (Ame, 1993) have been associated with the consumption of foods and vegetables that are rich in polyphenol antioxidant compounds.

Several studies reported the inhibitory effects of polyphenols on carbohydrates hydrolyzing enzymes. These include the green tea polyphenols that inhibit the activities of α-glycosidase and sucrose (Hara and Honda, 1992), berry polyphenols that inhibit the activity of α-glucosidase and α-amylase (McDougall and Stewart, 2005) and sweet potato polyphenols which inhibit the activity of α-glycosidase (Matsui *et al.,* 2001). Studies have shown a link between polyphenol content and lipo oxygenase (Aquila *et al*., 2009). Then, it is known that xanthine oxidase is an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues involved in many pathological processes such as inflammation, cancer, diabetes (Sweeney *et al*., 2001). Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in processed foods; these compounds have been reported to cause DNA damage induction and are carcinogenic (Sasaki *et al*., 2002; Ku and Mun, 2007). Therefore, the search for natural antioxidants that may be used in foods and synthetic antioxidants is necessary.India has several traditional medical systems, such as Ayurveda and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. The materia medica of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India. The ancient texts like Rig Veda (4500-1600 B.C) and Atharva Veda mention the use of several plants as medicine. The books on ayurvedic medicine such as Charaka Samhitaand Susruta Samhita refer to the use of more than

700 herbs (Jain, 1968). In India, the ayurvedic system has described a large number of such medicines based on plants or plant product and the determination of their morphological and pharmacological or pharmacognostical characters can provide a better understanding of their active principles and mode of action. However a large number of tropical plants have not been studied in detail for their chemical constituents, pharmacological properties of the extracts, and their pharmacognostical characterization including DNA sequencing etc.

The plant, *Helicia robusta* used in this study is asub- tropical plant which belongs to Proteaceae family. They grow naturally across the Malesia region with the major centre of species diversity of about fifty species in New Guinea. They grow naturally in the south west Pacific ocean region, and in north and eastern Australia. In some of the better known Australian species, the flowers and fruit are generally not prominent, and plants can be slow growing. They are generally propagated by seed, the viability of which drops rapidly with time. They grow naturally across southern and eastern Asia, including Indonesia, Malaysia and another centre of species diversity of about twenty species in southern China, extending to parts of the Indian subcontinent, the Philippines, Taiwan, and southern Japan. People living in all over India have been using plants from the same family as this species used for traditional medicine to treat various disorders. Therefore, *Helicia robusta* (Roxb.) Blum has been selected for this study in order to assess the gastroprotective potential of the methanolic extracts of leaves and stems in rats as an animal model.

Jasminum nervosum are straggling shrub with slender stems that scramble over the ground and climb into the surrounding vegetation. It belongs to Oleaceae family which contains about 150 species, mostly natives of the warmer regions of the old world. The stems are reported to be up to 20 metres long in Indo-China though only 1 - 5 metres long in China found in Guangdong,

Hainan and Guangxi provinces of China. The leaves and the stems are widely used for various applications in traditional medicine, exhibiting a remedy for diarrhea, malaria, sores and ulearations (Editorial committee of the National Chinese Medicine Administrative Bureau,. 2005). Some earlier works have been reported on the essential oils, phenylpropanoids, flavonoid and iridoid glycosides of various *Jasminum* species (Jin *et al*., 2006) which possess good bioactivities such as antioxidant activity, antiviral, antibiosis, antitumour, liver and gallbladder-protection, eliminating pain and relieving spasm, strengthening immunity as well as anti-diabetes and anti-hyperlipidenmia activity, playing significant roles in clinical medicine. It has cylindrical branches, leaves are opposite, pinnate and dark green, the leaflets are in three pairs, with an odd one and are pointed, the terminal one larger with a tapering point. The fragrant flowers bloom from June to October and as they are found chiefly on the young shoots, the plant should only be pruned in the autumn. It is mainly found in areas like Asia - China, northeast India, Nepal, Bangladesh, Myanmar, Thailand, Cambodia, Laos, and Vietnam. The habitat is rather humid, forested areas, slopes, thickets, mixed forests at elevations below 2,000 metres. A poultice of the crushed leaves theis used to treat ulcers and mastitis. In Cochin-China, decoction of the leaves and branches of *J.nervosum* is taken as a blood-purifier.The dried young shoots are used in the treatment of lymphadenopathy, metritis, galactophoritis, leucorrhoea, rheumatism, ostalgia, impetigo, dysmenorrhoea and haematometra. Other uses include wickerwork and binding and for making ropes and pot mats.

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue (Chan and Graham, 2004).Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcer such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital

ulcer. Of these peptic ulcer is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum (Debjit *et al*., 2010). The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer." The name refers to the site of ulceration. A person may have both gastric and duodenal ulcers at the same time. Gastric ulcers are located in the stomach, characterized by pain; ulcers are common in older age group. Eating may increase pain rather than relieve pain. Other symptoms may include nausea, vomiting, and weight loss. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur even in complete absence of acid (Vyawahare *et al*., 2009). Duodenal ulcers are found at the beginning of small intestine and are characterized by severe pain with burning sensation in upper abdomen that awakens patients from sleep. Generally, pain occurs when the stomach is empty and relieves after eating. A duodenal ulcer is more common in younger individuals and predominantly affects males. In the duodenum, ulcers may appear on both the anterior and posterior walls (Brooks *et al*., 1985).

1.9 Peptic ulcer

Peptic ulcer is one of the world's major gastrointestinal disorders and affecting 10% of the world population (Zapata-Colindres *et al*., 2006). About 19 out of 20 peptic ulcers are duodenal. An estimated 15000 deaths occur each year as a consequence of peptic ulcer. Annual incidence estimates of peptic ulcer hemorrhage and perforation were 19.4–57 and 3.8–14 per 100,000 individuals, respectively. The average 7-day recurrence of hemorrhage was 13.9% and the average long-term recurrence of perforation was 12.2% (Lau *et al*., 2011). In the Indian pharmaceutical industry, antacids and antiulcer drugs share 6.2 billion rupees and occupy 4.3% of the market share (Hoogerwerf and Pasricha, 2001).

1.10 Studies on antiulcer activities

(Kavitha *et al*., 2014) has performed antiulcer activity of *Pterolobium hexapetalum* (roth.) leaf and fruit extracts on pyloric ligated rats. Result of the study indicated that the methanol and aqueous extracts of *P. hexapetalum* leaf and fruit significantly protected the gastric mucosa injury at 100mg/kg b.wt. with 81.33% and 80.38%, respectively. (Elham Rouhollahi *et al*., 2014) worked on the acute toxicity and gastroprotective activity of *curcuma purpurascens* rhizome against ethanol-induced gastric mucosal injury in rats. Immunohistochemistry analysis of gastric homogenate elicited the critical role of Bax down-regulation and Hsp70 up-regulation.

(John-Africa *et al.,* 2014) has investigated that the Anti-ulcer and wound healing activities of *sida corymbosa* in rats. This study has shown that *Sida corymbosa* has constituents with the ability to reduce the severity of haemorrhagic gastric lesions reduce inflammation and increase the rate of wound healing in rodents.(Moghadamtousi *et al*., 2014) has performed the gastroprotective activity of *A. muricata* leaves against ethanol-induced gastric injury in rats via. Hsp70/Bax involvement. The results obtained in this study showed the safety of ethyl acetate extract of *A. muricata* in rats even at the highest dose of 2 g/kg. In addition, Hsp70 upregulation and Bax downregulation were found to be involved in the suppression of gastric injuries.The results suggest that the *P. speciosa* extract may act by enhancing the gastric mucosal defense and/or by inhibiting leukotriene synthesis. The plant extract protected the gastric tissues through the upregulation of the HSP70 and down regulation of the BAX protein. In addition, it also increased the GSH and SOD activities, and decreased the level of lipid peroxidation (MDA) in the *P. speciosa*-pretreated groups.

The objectives of this study were to evaluate the bark of *Helicia robusta* and the leaves of *Jasminum nervosum* Lour. as a source of natural antioxidants using different fractions to

determine their antioxidant capacities by different scavenging methods and also investigation of the anti-ulcer activity of the crude extracts of both plants using Wistar Albino rats. In this study, both the plants are taken under the same investigation as they are known to be used traditionally by the local people for the remedy of gastric problems where our aim was to find out whether these two plants have a good antioxidant potential as well as anti-ulcer activity.

CHAPTER 2
OBJECTIVES

- Screening of phytochemicals of Petroleum Ether and Methanol extracts of *Helicia robusta* bark and *Jasminum nervosum* leaf.

- Evaluation of the antioxidant activity the bark extracts of *Helicia robusta* and leaf extract of *Jasminum nervosum.*

- Investigation of anti-ulcer activity of of *Helicia robusta* bark extract and *Jasminum nervosum* leaf extract using ulcer induced male Wistar strain Albino rats

CHAPTER 3
STUDY AREA AND SURVEY

3.1 Study Area

The main area for this study falls under the Tropical wet evergreen forests and Sub-tropical Hill forest. The tropical wet evergreen forest is the most important forest type in Mizoram.The natural forests are least disturbed by exogenous factors. The hill ranges also runs from north-south direction and is criss-crossed by numerous small hillocks, streams and valleys. The catchment ares of the rivers are scrubby and devoid of good forests except the riverine reserved forests. Varid types os vegetation are distinguished and dense forests consists of trees, climbers, liane and ptetidophytes. This area has a tropical humid climate being enroute south-west monsoon rains in low depression. The temperature varies from 6ºC - 35ºC with annual precipitation of 2000 to 3500 mm. The area is dry and cold during December to February; warm and moist in March and May; very wet and warm from June to September; and moderate in October and November. The period from June to August is not suitable for field work due to heavy rainfall .The best season for field collection starts from October upto the month of May.The soil of the study site is acidic in nature owing to the leaching out of organic matter in soil. The acidity gradually decreases in the area occupied by the secondary forests as well as the valleys and embankment of rivers. The dense forests has a humus, dark-brown to blackish and sandyto loamy soil with good aeration. The soil under bamboos, scrubby vegetation and teak plantations is generally brown, compact and clayey-loam, whereas the valleys and river banks accumulate deposits of sands and alluvial soils. Other areas are covered with mixed vegetation are generally reddish-brown, coarse sandy and friable.

3.2 Mampui village

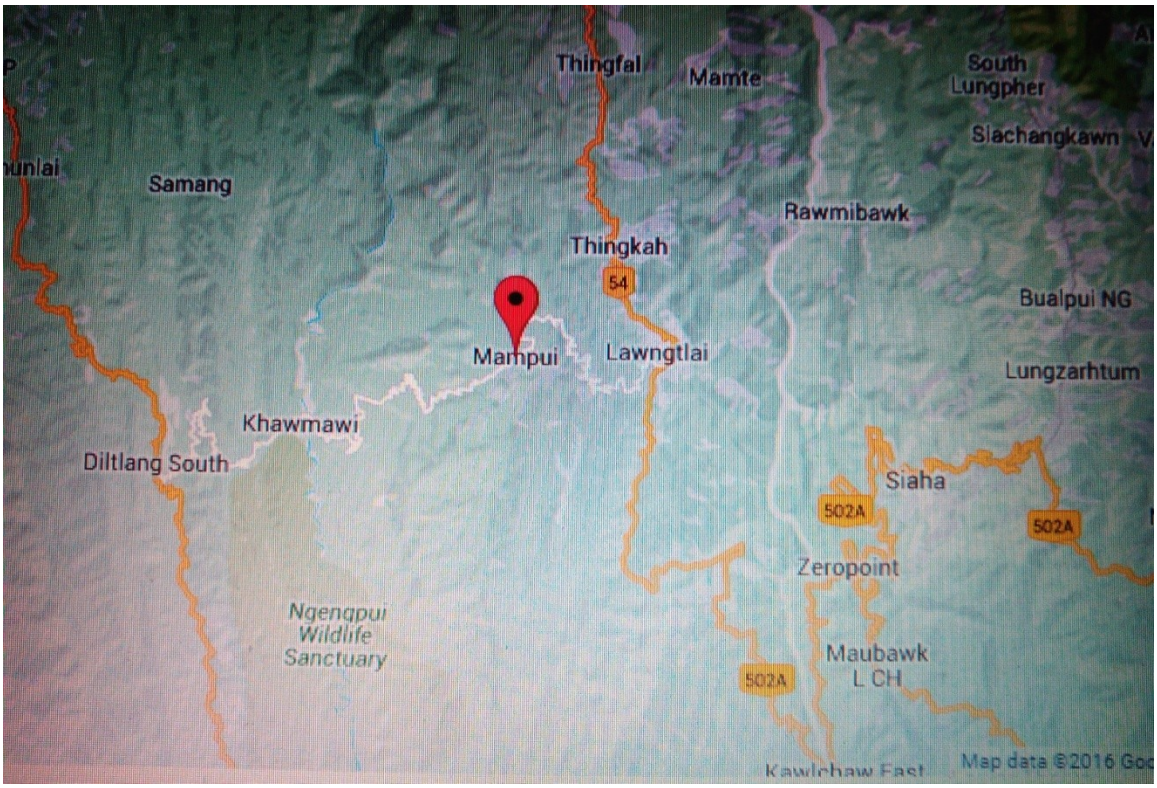


Figure 1: Google image of Mampui village

Mampui is a medium size village located in Lawngtlai district of Mizoram State, India. It is located 18 km towards South from District head quarters Lawngtlai and 314 km from State capital Aizawl. The village was established sometime in the year 1905. Mampui is surrounded by Saiha block towards East, Bungtlang South block towards South, Tuipang block towards South, Sangu block towards North. A deep surveyance was carried out and based on their traditional knowledge and practices, the local people have been using *J. nervosum* (Hruikha) leaves as an important medicine in the case of treatment against stomache-ache via ulcers. Based on their practices, the leaves were taken in a bowl, ground and filled with water (infusion) and is taken internally at a tablespoonful (10 mL) thrice daily and these practices were reported to be of great significance.

Table 1 : Information on Mampui Village

Location	Latitude: 22°31'-22°35'N Longitude: 92°46' –92°52'E
Temperature	6-24°C in winter 20-32°C in summer
Population	220 families/1200 approx members
Literacy	93.00 %
Altitude	918 metres

Mampui forest falls under tropical and mixed deciduous forest to sub-tropical nature. The climatic condition is moist-humid sub-tropical nature with high rainfall ranging between 2000 -3000 mm annually. The forest is stratified into ground flora, middle layer and top canopy. Ground flora exhibits the presence of *Lindernea ruelloids, lepidaeathus incuria, Ageratum conizoide etc. the lower middle layer consists of Chromolena aderata, Lantana camera, Dendrokcnide sinuate, Jasminum nervosum, Bridelia monoica*. The upper middle layer consists *Ficus semicordata, Macaranga denticulatus, Aporosa octandra, Albizia procera* etc. and the top canopy is covered by *Dipterocarpus turbinatus, Michellia champaca, Dillenia indica, Terminalia myriocarpa,*

Aphanamixis wallichi, Haldina cordiflora, Mitragyna rotundifolia, Lagerstroemia speciosa, Chukrassia tabularis, Bischofia javanica, Duabanga grandiflora, Toona ciliata, Calophyllum polyanthum, Podocarpus neriifolia, Stereospermum colais, Knema linifolia, Gynocardia ordorata, Hydnocarpus kurzii, Helicia robusta etc. Of cane species *Calmus , Zalaca baccarii, Plectocarpia khasiana* are common. Of palm species *Borassus flabellifera, Licula peltata* and *Typha elephantine* are very common. *Melocanna baccifera* is predominant over the species of *Dendrocalamus longispathus, Bambus tulda* etc. A moderately sloped gradients of secondary forests are being utilized for large scale plantations of the most valuable timber spcies *Tectonia grandis*. Also epiphytes and climbers are abundant.

3.3 Sialsuk village

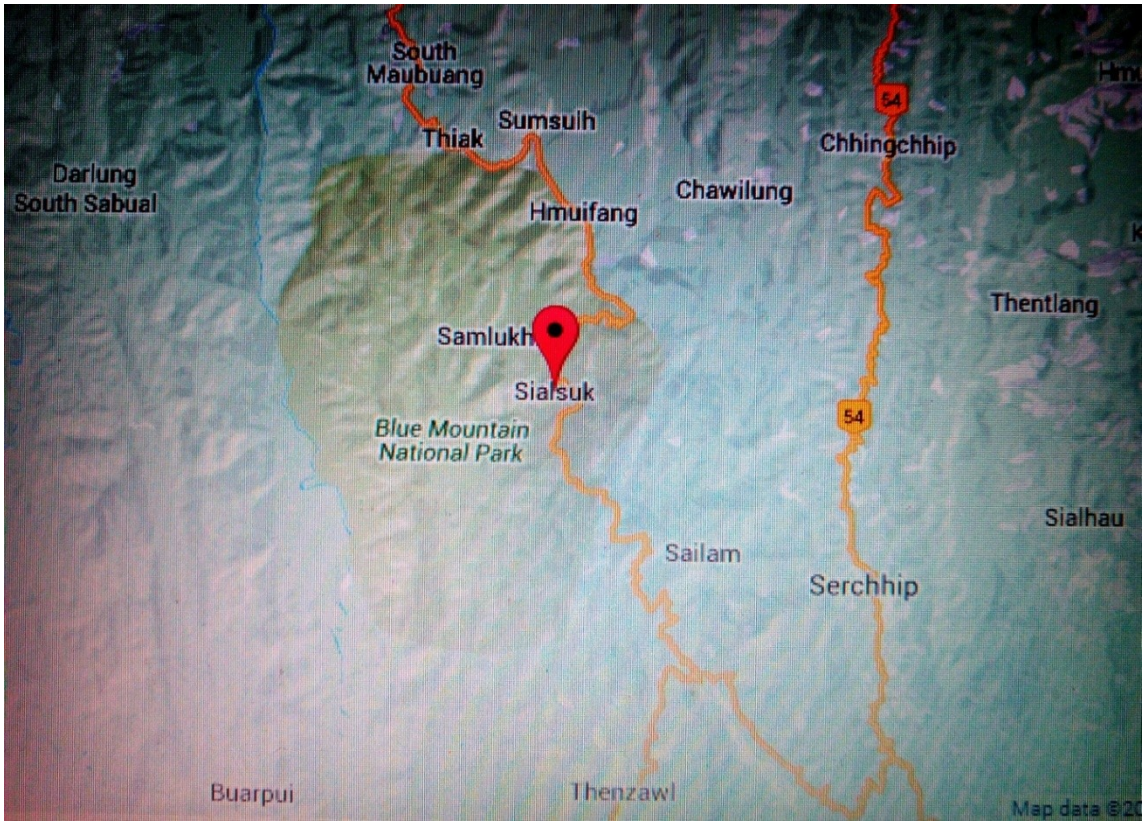


Figure 2: Google image of Sialsuk village

Sialsuk is a medium size village located in Aibawk, Aizawl district, Mizoram. It is located 45 km towards South from District head quarters Aizawl and 20 km from Aibawk, 43 km from State capital Aizawl. Sialsuk is surrounded by Aibawk block towards North, Tlangnuam block towards North, Thingsulthliah block towards North, Reiek block towards North. A deep surveyance was also carried out and based on their traditional knowledge and practices, the local people have been using *H. robusta* (Pasaltakaza) bark as an important medicine for treatment against colic, stomache-ache and for strengthening the functions of uterus. The bark of the tree is boiled with water (decoction) and is taken internally, these practices were reported to be of great significance.The bark of the trees were collected and stored in a polythene for further purposes.

Table 1a : Information on Sialsuk Village

Location	Latitude: 23°19’ - 23° 24’N Longitude: 92°40- 92° 45’E
Temperature	6°C- 21°C in winter 20°C- 34°C in summer
Population	396 families/1881 approx members
Literacy	97.99 %
Altitude	1519 metres

Sialsuk forest falls under the major group- Montane Sub-tropical forests or sub-tropical broad-leaved hill forest extending from 1500-2158 m and the peak is about 1500 metres. It is a rocky mountainous hill range in north-south direction. It is very windy and cold during the winter season. The forests are characterized by *Rhodedendron arboretum, Myrica esculenta, Eugelhardtia, Pinus kesiya, Lithocarpus dealbata, Quercus serrata*. The common trees are *Ulmus laevigata, Mosena ferrae, Picrasma javanica. Helicia robusta, Schima wallichii, baukinia variegetta, Syzygium spp., Castanopsis tribuloids, Eurya accuminata, Erythrine stricta, Vitex pediumcularis* etc. *Arundinaria callosa, Chimonobambusa khasiana, Dendrocalamus sikkimensis and D.giganteus* are the characteristic bamboo species. *Melocalamus compactiflora* are also present where as distribution of *Melocanna bacciflora* is restricted to the forests, *Trachycarpus martiana* and few Cynas are also present. Among shrubs *Artesemia indica, Clerodendrum viscosium, Cordia dichotoma, Flemingia macrophylla, Laea asiatica* are frequent and *Plantago major, Cassia tora, Lepidagathin rigida, Elsholtia blanda* and grasses are a few examples of herbaceous plants. This forest type is the natural abode of epiphytic orchids like *Renanthera inschootiana, Vanda coerulae Griff ex Linda, Mantisia saltoria and M.wengirii Fisher*

CHAPTER 4
MATERIALS AND METHODOLOGY

4.1 Plant materials: Eight important medicinal plants were selected for this study in the beginning, but depending on their availability, location, literature and its medical uses only two plants were selected namely *Helicia robusta and Jasminum nervosum*.

Plant Taxonomy

Scientific name: *Helicia robusta (Roxb.) Blume*

Kingdom : Plantae

Unranked : Angiosperms

Family : Proteaceae

Phylum : Tracheophyta

Class : Magnoliopsida

Genus : *Helicia*

Species : *H. robusta*

Figure 3: *Helicia robusta* tree and herbarium specimen of *H. robusta* showing leaves, fruits, flowers.

H. robusta (Roxb.)R.Br.ex.Blume belongs to the family Protoceae. Locally, it is named Pasaltakaza/Sialhhma. It is a small to medium-sized evergreen tree of sub-tropical forests and found to be present in Ngopa, Sialsuk, Biate, N.Vanlaiphai forest etc in Mizoram at an altitude of 700-1400 m, it is also found in Bangladesh, Myanmar, Malaysia, Meghalaya and Andamans. The bark is grayish-white and the inner bark is pungent and irritating to taste. The leaves are oblong or oblanceolate (3.5-10 x 5-15 cm), acuminate coarsely serrate, nerves 7-10 pairs, anastomosing, base cuneate, narrowed into the petiole (1-2.5 cm) long. Flowers are borne on the trunk and branches and look like a bottle-brush, yellowish-brown, by pairs, on rusty-villous peduncle. Flowers are seen between March-April; November- February. Fruits are ellipsoid or obliquely ovoid. Decoction of the bark is used for colic/stomach ulcer at the rate of half a cup twice a day and for the strengthening the function of uterus (Lalramnghinglova, 1998). It is further narrated that the sap of the root is more active than that of the bark.

Scientific name: *Jasminum nervosum Lour.*

Kingdom : Plantae

Family : Oleaceae

Phylum : Magnaliophyta

Class : Magnoliopsida

Order : Lamiales

Genus : Jasminum

Species : *J.nervosum*

Figure 4: *Jasminum nervosum* (leaves and flower) and its herbarium specimen

J. nervosum Lour. belonging to the Oleaceae family, locally named as Hruikha. It is a natural scandent shrub of 1-5 m height, found in the tropical dense ever green forest of Mampui village, Lawngtlai District, Mizoram at an altitude of 250 -1000 m. It is also found in Bhutan, Bangladesh and Myanmar. The stem is green and smooth. Leaves are ovate-lanceolate, acuminate, chartaceous, glossy 3-nerved (2-4 x 3.5-12 cm) a pair of basal nerves extending up to the apex and anastmosing with secondary nerves, the base are rounded or sub-cordate. Flowers are white, axillary 2-5 flowered cymes, lobes 7-10. Fruits are ellipsoid and small. Flowers are between January- march ; March-May. The leaves and the stems are widely used for variousapplications in

traditional medicine, exhibiting a remedy for diarrhea, malaria, sores and ulearations. Some earlier works have been reported on the essential oils, phenylpropanoids, flavonoid and iridoid glycosides of various *Jasminum* species which possess good bioactivities such as antioxidant activity, antiviral, antibiosis, antitumour, liver and gallbladder-protection, eliminating pain and relieving spasm, strengthening immunity as well as anti-diabetes and anti-hyperlipidenmia activity, playing significant roles in clinical medicine (Jin *et. al.*, 2006).

4.2 Omeprazole

Omeprazole as a common reference antiulcer drug (Abdulla *et al*., 2009; Rashdi *et al*., 2012; Golbabapour *et al*., 2013) and was used as a positive control in this study. This drug was obtained from Mizoram University Medical Centre (MUMC). The drug was prepared by dissolving it in CMC (Carboxy methyl cellulose) and given orally to the animals at a dose of 20 mg/kg body weight (5 mL/kg).

4.3 Reagents

Omeprazole was dissolved in 0.5% (w/v) Carboxy methyl cellulose (CMC) and orally administered to the rats at a dosage of 20 mg/kg body weight (5 ml/kg) (Mahmood *et al*., 2010). Deionized water, gallic acid (Fluka, UK), 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich Co., USA). Ascorbic acid, Methanol, Petroleum ether, Chloroform and all other chemicals were of analytical grade (BDH, UK).

4.4 Method of Extraction (Soxhlet Extraction)

Soxhlet extraction is a common conventional method used for extracting heat-stable compounds. The soxhlet extractor consists of a distillation flask, an extractor, and a condenser. The solvent in the distillation flask is heated and the resulting vapor is condensed in the

condenser. The condensed solvent from the condenser fills into the thimble-holder containing the sample that needs to be extracted. When the solution in the extractor reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying dissolved solute into the bulk liquid. The solute is left in the distillation flask while the solvent is evaporated, condensed, and passed back into the sample solid bed. This process is repeated 3 to 5 times or until a complete extraction are achieved (Tandon and Rane, 2008). 250g of *H. robusta* powder (from raw 1.2 kg) was used for extraction using soxhlet apparatus. The method of extraction was done successively by using petroleum ether at 60°C for 6 hours and with methanol at 50°C for 8 hours.

280g powder of *Jasmimum nervosum* (from raw 900g) was used for extraction using Soxhlet apparatus. The extraction was also done successively using petroleum ether at 60°C for 12 hours followed by chloroform extraction at 60°C for 18 hours and then lastly with methanolic extraction at 50°C for 22 hours.

4.5 Preliminary phytochemical screening

About 1.5 ml of petroleum ether and methanolic extracts of *Helicia robusta* and *Jasmimum nervosum* were both used to determine the presence of secondary metabolites using the standard protocols of Harborne, 1998 as follows:

(i) Test for Flavonoids

A portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicates the presence of flavonoids.

(ii) Test for amino acids (Ninhydrin Reaction):

2-3 ml of sample solution was taken in a test tube and 3-4 drops of ninhydrin solution was added and heated for 2-3 min. Appearance of purple or violet colour indicates the presence of protein.

(iii) Test for steroids

About 100 mg of the extract was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface is indicative of the presence of steroidal ring.

(iv) Test for Tannins

0.5 g of extract was boiled in 10 ml of distilled water in a test tube and then filtered. 0.1 % $FeCl_3$ was added to the filtered samples and observed for brownish green or blue-black colorations which indicates the presence of tannins.

(v) Test for Alkaloids

Saturated solution of picric acid was prepared by taking 1 g of picric acid in 100 ml of distilled water and dissolved properly. One to two drops of extract was added to one or two drops of reagent and dark yellow precipitate indicates the presence of alkaloids.

(vi) Test for Glycosides

0.5 g of extract diluted with 5 ml of water was added with 2 ml of acetic acid containing one drop of ferric chloride solution and mixed with 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of a deoxysugar. A violet ring may appear below the brown ring while in the acetic layer a greenish ring may form just above the brown ring.

(vii) Test for Carbohydrates

0.5 mL of powdered sample of extract, 5 mL of Benedict's reagent was added and boiled for 5 min. Formation of bluish green colour showed the presence of carbohydrate solution was boiled for few minutes. In the presence of flavonoids, reddish pink or dirty brown colour was produced.

4.6 Evaluation of antioxidant activity of the plants by the following assays

4.6.1 Determination of total flavonoid content

The total flavonoid content of methanolic extract of *Helicia robusta* and *Jasmimum nervosum* were determined by Anoosh *et al*., 2010. 1 ml of the extract was mixed with 2 ml of distilled water. After 5 minutes, 3 ml of 5 % sodium nitrite ($NaNO_2$) and 0.3 ml of 10% aluminium chloride ($AlCl_3$) were added. After 6 minutes, 2 ml of NaOH (1M) was added and the volume was made upto 10 ml with distilled water. After 1 hour, absorbance reading was taken at 510 nm. A standard curve was prepared with Quercetin at different concentrations (10, 20, 40, 60, 80, 100 μg/ml). From the calibration curve of reference standard, the total flavonoid content was determined and expressed as milligrams of quercetin equivalents (QE/g) of dried extract.

4.6.2 Determination of β-carotene content

β-carotene content of methanolic extract of *Helicia robusta* and *Jasmimum nervosum* extract were determined according to the method of Nagata and Yamashita (1992). The dried methanolic and petroleum ether extract (100 mg) was vigorously shaken with 10 ml of acetone, hexane mixture (4:6 ml) for 1 min and filtered. The absorbance of the filtrate was measured at 453, 505, and 663 nm. The total β-carotene content was calculated according to the equation

$$\beta\text{ –carotene (mg/100ml)} = 0.216(A_{663}).\ 0.304(A_{505}) + 0.452(A_{453})$$

4.6.3 DPPH radical scavenging activity

DPPH free radical scavenging activity methanolic extracts of *Helicia robusta* and *Jasmimum nervosum* were estimated according to the method described by Blois (1958) with minor modifications. Butylated hydroxyanisole (BHA) was used as reference standard. 0.5 ml of DPPH solution in methanol (0.1mM) was mixed with 3 ml of the extract and 3 ml of standard prepared in various concentrations (10, 20, 40, 60, 80, 100 µg/ml). The extract and standards were incubated for 30 minutes at 37°C. Absorbance was measured at 517 nm using UV-Vis Spectrophotometer. Control reading was also taken.

The % inhibition observed was calculated using the formula:

$$\% \text{ inhibition} = (Abs_{cont.} - Abs_{ext} / Abs_{cont.}) \times 100$$

Where Abs_{cont} is absorbance of control and Abs_{ext} is absorbance of extract.

IC_{50} was then calculated for the sample and standard from the graph by plotting the % Inhibition in Y-axis and concentration in X-axis.

4.6.4 Determination of reducing power (iron chelation)

The reducing power of methanolic extract of *Helicia robusta* and *Jasmimum nervosum* was determined by the method of Oyaizu *et al*.,1986 using Ascorbic acid as standard. 1 ml of the extract and 1 ml of the standard with various concentrations (10,20,40,60,80,100µg/ml) were mixed with 2.5ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 30 minutes. The reaction was stopped by adding 2.5 ml of 10% trichloroacetic acid and the mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride solution and the absorbance was taken at 700 nm using UV-Vis Spectrophotometer.

4.6.5 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) was performed according to Martin *et al*., 2005 at the Department of Pharmacy, Regional Institute of Pharmaceutical and Nursing Science, Zemabawk, Aizawl, Mizoram using 12 standards such as Gallic acid, Arjunic acid, Lutein, Catechin, Chlorogenic acid, Ferulic acid, Kaempferol, Ursolic acid, Boswellic acid, Ellagic acid, Chebulagic acid, Stigmasterol bought from Natural Remedies Private Liited, Bangalore. The instrument was set up at a flow of 0.3 ml/ min and UV at 254 nm where methanol was used as a mobile phase (solvent) using isocratic standards.

4.7 Synthesis of silver and gold nanoparticles by using *Jasmimum nervosum* leaf extracts

Fresh and healthy leaves of *Jasminum nervosum* were collected from Mampui Village, Lawngtlai, Mizoram, Northeast India ($22^{o}52'$ North latitude, $92^{o}89'$ East longitude). The taxonomic identification of the collected plant was authenticated by Prof. Lalramnghinglova, Department of Environmental Science, Mizoram University, Mizoram, India and assigned a voucher specimen number (MZULM 22133). The collected plant parts were thoroughly washed under running tap water, dried in shade and triturated into fine powders by using an electric grinder. The powder was stored in air sealed brown bottles at ambient temperature. 50 g of dried leaf powder was mixed with 500 mL of boiled and cooled distilled water with continuous stirring on a magnetic stirrer (Minjas and Sarda 1986). The suspension was left for 3 h, filtered through Whatman no. 1 filter paper and the filtrate was stored in amber colored air tight bottle at 10°C.

The broth solution of *J. nervosum* leaves was prepared by taking 20 g of dried powder in a 500-mL Erlenmeyer flask along with 200 mL Milli-Q water and boiled for 10 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1, stored at −4 °C, and used

within one week. The filtrate was treated separately with aqueous 1 mM $AgNO_3$ and $HAuCl_4$ (21.2 mg of $AgNO_3$ and 49.2mg of $HAuCl_4$ powder in 125 mL Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. The reaction mixture was checked for the development of colour change and absorbance spectra was monitored using UV-visible spectroscopy. To enhance the efficiency, monodispersity and yield of AgNPs and AuNPs, different parameters such as pH (3-10), temperature (37°C -100°C), reaction time (0-60 min), substrate concentrations (0.25 mM, 0.5 mM, 1 mM, 2 mM and 3 mM) and volume of *J. nervosum* leaf extract (5, 10, 15, 20, and 25 ml) were fixed. The reaction volume was quantified by measuring the absorbance of mixtures using UV−visible spectrophotometer in the ranges of 300–700 nm. Ninety five-millilitre aqueous solution of 1 mM of AgNOs and $HAuCl_4$ was reduced using 5 mL of *J. nervosum* leaves extract at room temperature for 10 min resulting in a brown-yellow and purple colouration of the solutions indicating the formation of AgNPs and AuNPs. It was found that aqueous silver and gold ions can be reduced by aqueous extract of *J. nervosum* to generate extremely stable AgNPs and AuNPs in water (Parashar et al. 2009; Veerakumar et al. 2013). Silver nitrate ($AgNO_3$) and Chloroauric acid ($HAuCl_4$) were purchased from Sigma–Aldrich Mumbai, India.

4.8 Characterization of AgNPs and AuNPs

4.8.1 UV-Visible spectrophotometer

Synthesis of AgNPs and AuNPs solution with *J. nervosum* leaves extract was observed by UV–Vis spectroscopy. Generation of AgNP and AuNP exhibits unique and tunable optical properties on account of their surface plasmon resonance (SPR), dependent on shape, size and size distribution of the NPs (Ingle *et a*l., 2009). Excitation of surface plasmonic vibrations due to

the reduction of Ag^+ and Au^+ ions were measured spectrophotometrically at different time intervals. Briefly, a small aliquot of the sample was diluted with distilled water and an absorption maximum was scanned by UV– visible spectrophotometer at 300–700 nm using Perkin-Elmer Lambda 2 UV198 visible Spectroscopy.

4.8.2 Fourier transforms infrared (FTIR) and X-ray diffraction (XRD) analysis

FTIR is most useful to determine the composition of polymer materials and to identify possible biomolecules responsible for the stabilization of the synthesised AgNPs and AuNPs (Zhang *et al*., 2011). The reaction mixture was centrifuged at 60,000×g for 40 min and the pellet was dissolved in deionized water, filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing silver and gold nanoparticles was used for FTIR, XRD and transmission electron microscopy (TEM) analysis. FTIR spectroscopic studies were carried out to find possible bio-reducing agent present in the *J. nervosum* leaf extract. The wavelength spectrum of the extracts before and after the addition of $AgNO_3$ and $HAuCl_4$ were recorded, by mixing the samples with KBr powder and pelletizing after drying, by measuring the spectra in the diffuse reflectance mode using Perkin Elmer make model spectrum RX1 (Wavelength range between 4000 cm^{-1} to 400 cm^{-1}). X-ray diffraction was performed to determine the dimension of biologically synthesized AgNPs and AuNPs with h, k, l values. The XRD diffractogram of synthesized AgNPs and AuNPs were carried out on films of the respective solutions drop-coated onto glass substrates on a Phillips PW 1830 instrument operating with conditions at a voltage of 40 kV and a current of 30 mA in Cu, Kα1 radiation. Particles size (L) of the Ag and Au was calculated using PAN analytical X pert PRO Model instrument following Debye-Scherrrer's equation: $L = 0.9\lambda/\beta \cos h\ \theta$ where, λ is the wavelength of the X-ray, β is full width and half maximum and θ is the Bragg's angle.

4.8.3 Transition Electron Microscopy (TEM) measurement

The morphology, size and diffraction of the AgNPs and AuNPs were measured at different magnification at 100Kev JEOL 3010 transmission electron microscope. Ag and Au colloidal solution were purified by repeated centrifugation and allowed for sonication; a drop of this solution was used to make a thin layer on the copper coated grid and allowed to dry.

4.9 Synthesis of silver and gold nanoparticles by using *Helicia robusta* bark extracts

Synthesis of silver and gold nanoparticles by using *Helicia robusta* bark extracts was performed by following the methods mentioned in the above section by using *J. nervosum* in relation to characterization of AgNPs and AuNPs by UV-Visible spectrophotometer, Fourier transforms infrared (FTIR) and X-ray diffraction (XRD) analysis and TEM measurements.

4.10 Animal Stock

Healthy adult male Albino Wister rats (4 - 6 weeks old), each weighing between 85 - 150 g, were obtained from the animal house at the Department of Zoology, Mizoram University (Ethical Permit No. MZUIAEC15-16-06/ MAA/R). The rats were randomly divided into 7 groups of 6 rats each and fed with standard pellet diet and tap water *ad libitum*. The rats were individually placed in separate cages with wide mesh wire bottoms to prevent coprophagy during the experiment.

4.11 Acute Toxicity

4.11.1 Ethical committee

The ethics committee for animal experimentation of the Department of Zoology, Mizoram University, Mizoram, Aizawl, India, approved the experiment. Throughout the experiments, all

animals were treated humanely according to the criteria outlined in the ‘‘Guide for the Care and Use of Laboratory Animals’’. Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423. Experiments were performed using healthy young adult male Wistar albino rats, weighing 80-150 g.

4.11.2 Assignment of animals

The animals were randomly divided into six groups each containing six rats. They were identified by the markings using a yellow stain. One rat was unmarked and the others were marked on head, body, tail, head and body, body and tail, to ease the observation.

4.11.3 Housing and Diet

The animals were housed in polypropylene cages (55 x 32.7 x 19 cm), with sawdust litter in a temperature controlled environment (23 ± 2ºC). Lighting was controlled to supply 12 hr of light and 12 hr of dark for each 24-hr period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The animals were fed with standard laboratory animal food pellets with water ad libitum.

4.11.4 Mode of administration

The test substance (*J. nervosum* leaf extract, *H. robusta* bark extract, silver and gold nanpaticle from *J. nervosum* leaf extract and *H. robusta* bark extract) was administered in a single dose by gavage using specially designed rat oral needle. Animals were fasted 3 hr prior to dosing (only food was withheld for 3 hr but not water).

4.11.5 Administration Dose

Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 50, 100, 200 and 400 mg/kg. After the administration of test substance, food for the rat was withheld for 2 hr.

4.11.6 Observation period

Animals were observed individually after atleast once during the first 30 min, periodically during the first 24 hr, with special attention given during the first 4 hr, and daily thereafter, for a total of 14 days. All the rats were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes.

4.11.7 Signs recorded during acute toxicity studies

Direct observation parameters include tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern are the other parameters observed. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 hr. The number of survivors was noted after 24 hr and then these were maintained for a further 14 days with a daily observation. OECD. *OECD Guidelines for Acute Toxicity of Chemicals*; Organisation for Economic Co-operation and Development: Paris, France, 2001; No. 420.

4.12 Gastric Ulcer Induction by Ethanol

Ethanol is considered a risk factor for developing gastric ulcers. It readily penetrates the gastric mucosa due to its ability to solubilize the protective mucous and expose the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin (Oates *et al*., 1988), causing

damage to the membrane (Sener *et al*., 2004). Moreover, alcohol stimulates acid secretion and reduces blood flow leading to microvascular injuries, through disruption of the vascular endothelium and facilitating vascular permeability; it also increases activity of xanthine oxidase. Ethanol also triggers imbalances in cellular antioxidant processes. For example, it causes the release of superoxide anion and hydroperoxy-free radicals, and hence increased oxidative stress in the tissues, evidenced by increased levels of malondialdehyde, a marker of increased lipid peroxidation (Glavin 1992; Repetto 2002; Marotta 1999). The harmful effects of ethanol thus manifest either through direct generation of reactive metabolites,including free radical species that react with most of the cell components, therefore changing their structures and functions, or by contributing to other mechanisms that finally support oxidative damage (Kato 1990; Nordmann 1994). Ethanol also produces necrotic lesions in the gastric mucosa of animals by a direct toxic effect thereby reducing the secretion of bicarbonates and depleting gastric mucus production in animals (Marhuenda *et al*., 1993).

Furthermore, ethanol-induced membrane damage is associated with increased permeability of the plasma membrane by sodium and water. It also produces massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Massignani *et al*., 2009). Also, ethanol-induced ulceration is linked to reduced mucosa microcirculation and to increased apoptosis (R. Hernandez-Munoz *et al*., 2000).The damaging effects of ethanol have been exploited in developing the ethanol model of peptic ulcers. The model is independent of gastric acid secretion and resembles acute peptic ulcers in humans (Brzozowski *et al*., 1998). As a model, ethanol-induced ulcer may not be appropriate or useful for the assessment of the usefulness of antisecretory drugs or testing materials due to the absence of gastric acid secretion where acid

secretion underlies the development of the ulcer. Instead, the ethanol-induced ulcer model is useful for studying the efficacy of potential drugs or testing agents that have cytoprotective and/or antioxidant activities.

The animals were fasted for 24 hr prior to the experiment (Mahmood *et al*., 2010). Groups 1 and 2 received vehicle (0.5% CMC) orally. Group 3 received an oral dose of 20 mg/kg omeprazole in 0.5% CMC (5 ml/kg), and groups 4–7 received methanolic bark extract of *Helicia robusta* at doses of 50, 100, 200 or 400 mg/kg as a pretreatment. At 1 hr after pretreatment, the vehicle or absolute ethanol was orally administered to groups 2–7 (Abdulla *et al*., 2010). One hour later, the rats were euthanized, and their stomachs were dissected.

The same experimental design was followed for *Jasminum nervosum* antiulcer activity with seven groups. One hour later, the rats were euthanized, and their stomachs were dissected.

4.13 Ulcer index

The following arbitrary scoring system was used to grade the incidence and severity of lesion, Kulkarni (2002). 0 = Normal, 1 = Red coloration, 2 = Spot ulcers, 3 = Haemorrhagic streaks, 4 = Ulcers > 3 but < 5 and 5 = Ulcers > 5. Mean ulcer score for each animal is expressed as Ulcer Index. The percentage of ulcer protection was determined by

$$\% \text{ Protection} = \frac{(\text{Control mean ulcer index} - \text{Test mean ulcer index})}{\text{Control mean ulcer index}} \times 100$$

4.14 Total acidity and free acidity

It is based on simple acid-base titration. Gastric juice (1mL) was pipette into a 100 mL conical flask and diluted with 9 mL distilled water. Two or three drops of Topfer's reagent was

then added and titrated with 0.01 N sodium hydroxide until all traces of red color disappeared and the colour of the solution was yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein were then added and the titration was continued until a definite red ting appeared; the volume of alkali added was noted. The volume corresponds to total acidity, Acidity was expressed in terms of mEq/L.

4.15 Measurement of Gastric Juice Acidity

The animals were sacrificed, and their stomachs were removed. The stomach contents were collected, measured, centrifuged, and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7 (Shay,1945).

$$\text{Acidity} = \frac{\text{Vol. of NaOH x 100 (mEq/L)}}{0.1}$$

4.16 Histological Examination of the Gastric Mucosa

The gastric wall specimens were fixed in 70% of Bouins fluid for 24 hr prior to paraffin tissue processing . The stomach tissues were sectioned at a thickness of 5 mm and stained with Hematoxylin and eosin to evaluate histological degeneration (Behmer 1976).

4.16.1 Blocking:

1. 90% alcohol two changes (each 1 hour)
2. Absolute alcohol (100%) two changes (each 1 hour)
3. Absolute xylene (5-10 minutes)
4. wax + xylene (1:1 ratio) (45 minutes)
5. wax-1 (45 minutes)

6. wax-2 (45 minutes)
7. wax-3 (45 minutes)
8. Block with paraffin wax

4.16.2 Staining:

1. Absolute xylene two changes (each 10 minutes)
2. Absolute alcohol (100%) two changes (each 10 minutes)
3. 90% alcohol two changes (each 10 minutes)
4. 70% alcohol two changes (each 10 minutes)
5. Wash with distilled water one change (10 minutes)
6. Stained with heamatoxylin one change (10 minutes)
7. Wash with running water (5 minutes)
8. Wash with Acid (Hcl) 1 drop or dip only
9. Wash with running water (5 minutes)
10. 50% alcohol one change (10 minutes)
11. 70% alcohol one change (10 minutes)
11. Stained with Eosin (1 minute)
12. 90% alcohol two changes (each 10 minutes)
13. Absolute alcohol two changes (each 10 minutes)
14. Absolute xylene two changes (each 10 minutes)
15. DPX Mounting with coverslips.

4.17 Statistical analysis

All the data are expressed as mean ± SEM. The values obtained for the above parameters with the extracts were compared with control group using one way ANOVA followed by Dunnett's test. The values of $p<0.0001$ and $p<0.0001$ were considered to indicate a significant difference between the groups

CHAPTER 5
RESULTS

5.1 Phytochemical screening

The preliminary phytochemical screening of petroleum ether extract and methanol extract in *Helicia robusta* showed the presence of different secondary metabolites such as flavonoids, alkaloids, amino acids, tannins, steroids, carbohydrates (Table 2) where methanolic extract tends to be much better and showed more presence of phytochemicals than petroleum ether extract similarly in *Jasmimum nervosum*, the methanol extract tends to show more presence of phytochemicals than pet ether extract which showed the presence of flavanoids, alkaloids, amino acids, tannins and carbohydrates (Table 3).

Table 2: Preliminary phytochemical screening of petroleum ether and methanolic extracts of *H. robusta*

Phytochemicals	Petroleum ether extract	Methanolic extract
Alkaloids	+	+
Flavonoids	+	+
Amino acids	+	+
Steroids	+	+
Tannins	-	+
Carbohydrates	-	+
Glycosides	-	-

Table 3: Preliminary phytochemical screening of pet ether and methanolic extracts of *J. nervosum*

Phytochemicals	Petroleum ether extract	Methanolic extract
Alkaloids	-	+
Flavanoids	+	+
Amino acids	+	+
Steroids	-	-
Tannins	+	+
Carbohydrates	-	+
Glycosides	-	-

5.2 D**etermination of β-carotene**

The total β-carotene content of the methanolic extracts of *H. robusta* and *J. nervosum* were determined. Absorbance were taken at 453, 505, 663 where at each respective absorbance. *H. robusta* indicated higher activity of total β-carotene content than that of *J. nervosum* (Table 4).

Table 4: Determination of total β-carotene content in methanolic extracts of *H. robusta* and *J. nervosum* at absorbance 453nm, 505nm, 663nm.

Absorbance (nm)	Total β-carotene content	
	Helicia robusta	*Jasmimum nervosum*
453	4.520	4.214
505	3.640	2.968
663	3.806	3.128

Total β-carotent content of *H. robusta* = 0.216 (3.806) x 0.304 (3.640) x 0.432 (4.520)
= 2.86 mg/100 mL

Total β-carotent content of *J. nervosum* = 0.216 (3.128) x 0.304 (2.968) x 0.432 (4.214)
= 1.11 mg/100 mL

From the analysis, it was observed that *H. robusta* have higher β-carotene content than that of *J. nervosum* i.e. (2.86 > 1.11) mg/100 mL.

5.3 Determination of total flavonoid content

Phenolic compounds like flavonoids are known to possess a broad range of antioxidant activity. Absorbance was taken using a spectrophotometer at 415 nm having different concentrations expressed in µL/mL. The result shows that at each concentration taken there was a difference in the antioxidant activity between the methanolic extracts of *H. robusta* and *J. nervosum* as shown in Table 5. The total flavonoid content in the methanolic extract of *H. robusta* and *J. nervosum* was determined from a regression equation for the calibration curve ($y=0.0026x+0.0293$, $R^2 = 0.9929$) (Fig. 5).

Table 5: Total flavonoid content of the methanolic extracts *H. robusta* and *J. nervosum* at different concentrations at absorbance of 415 nm in comparison with the standard, Quercetin.

Concentration (µg/ml)	Absorbance at 415 nm		
	Quercetin	*H. robusta*	*J. nervosum*
10	0.048	0.181	0.163
20	0.086	0.204	0.195
40	0.132	0.232	0.244
60	0.202	0.276	0.316
80	0.226	0.281	0.361
100	0.288	0.310	0.422

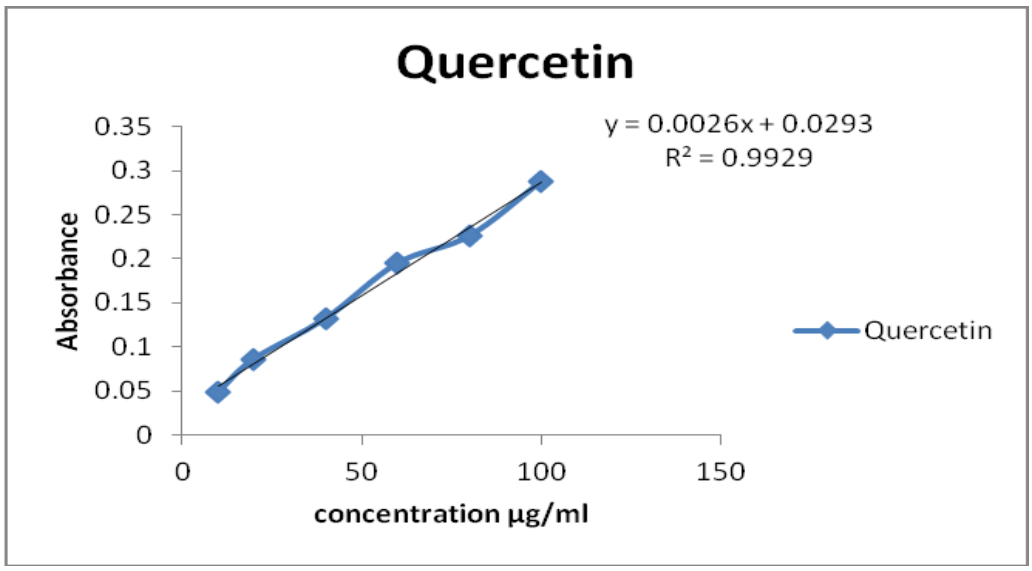


Figure 5: Standard curve of Quercetin.

From the above calibration curve of the reference standard, Quercetin (QE) the total flavonoid content for *Helicia robusta* was determined to be 83.46 mg (QE/g, dry extract) and 68 mg (QE/g, dry extract) for *Jasmimum nervosum* inferring *Helicia robusta* had higher flavonoid content than *Jasmimum nervosum.*

5.4 DPPH radical scavenging activity:

DPPH is a free radical which is stable at room temperature and this method is often employed to determine the antioxidant activity of many plant extracts. The concentration in g/mL of the extract to scavange 50% of the DPPH radical is called IC50 and lower IC50 values indicates higher anti-radical activity. Absorbance for both the plants was taken at 517 nm at different concentrations and expressed in µg/mL (Table 6). Then IC_{50} was calculated from the graph by plotting the % inhibition in Y-axis and concentration in X-axis for methanolic extracts *H. robusta* and *J. nervosum* against a standard, Butylated hydroxyanisole (BHA).

Table 6. DPPH free radical scavenging activity of methanolic extracts *H. robusta* and *J. nervosum* at absorbance of 517 nm. Standard Butylated hydroxyanisole (BHA) control absorbance = 0.168)

Concentration (µg/ml)	Absorbance at 517 nm	
	Helicia robusta	*Jasmimum nervosum*
10	0.130	0.118
20	0.113	0.104
40	0.079	0.098
60	0.057	0.064
80	0.051	0.049
100µg/ml	0.039	0.027

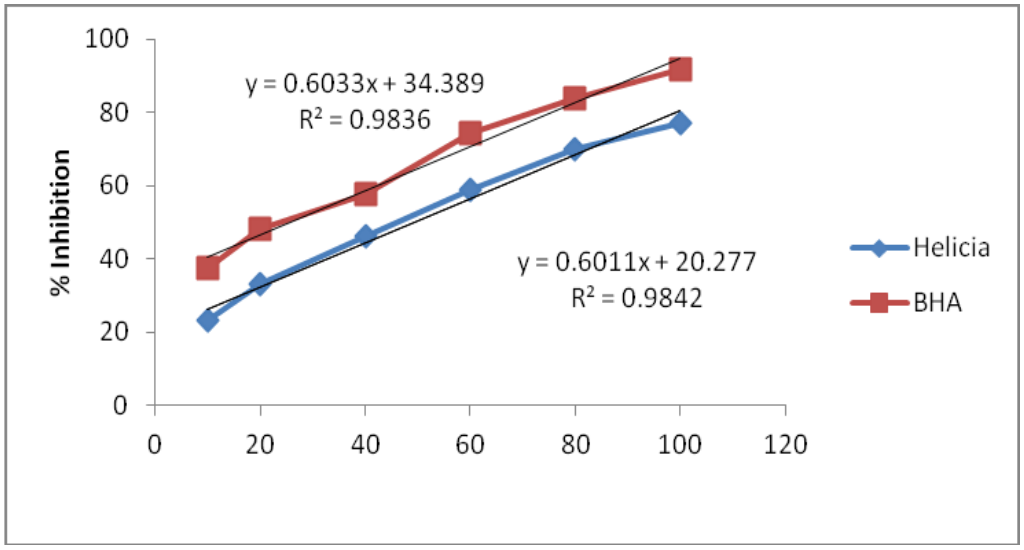


Figure 6: DPPH free radical activity of Butylated hydroxyanisole (BHA) and methanolic extract of *Helicia robusta*

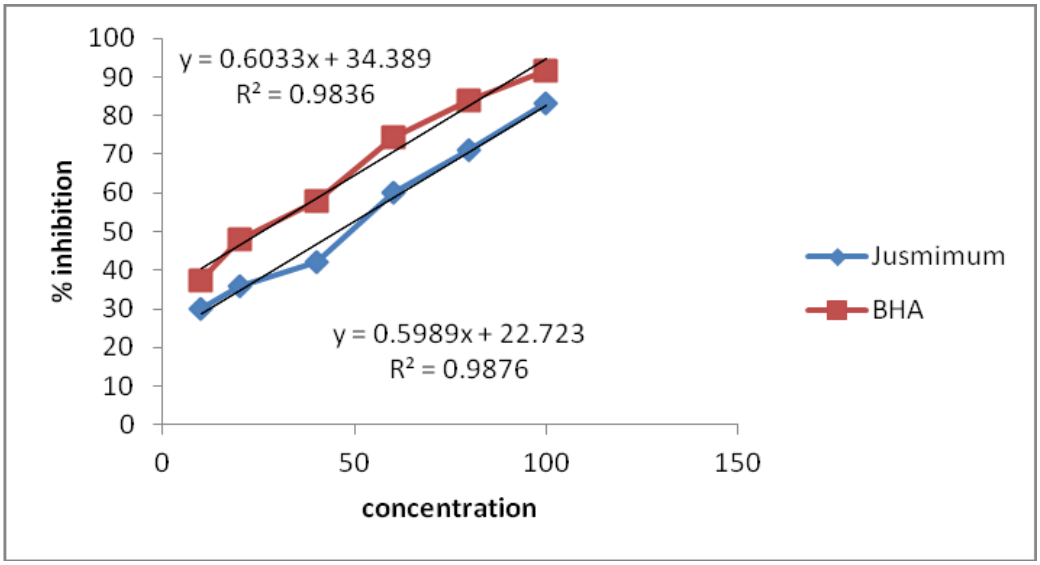


Figure 7: DPPH free radical activity of Butylated hydroxyanisole (BHA). and methanolic extract of *Jasminum nervosum*.

The result clearly showed that methanolic extracts of *H. robusta* and *J. nervosum* exhibited a good anti-radical scavenging activity. However, in comparison of the two plants , we found that *Helicia robusta* had lower IC50 value than *J. nervosum* which showed that *H. robusta* had higher anti-radical activity than that of *J. nervosum* i.e (0.494< 0.059).

5.5 Determination of Reducing Power:

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. In this assay, the presence of antioxidant in the extract resulted into reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). Higher is the absorbance of the reaction mixture, higher would be the reducing power. The reducing power of the extract increases as the concentration increases suggesting that some compound in the extract may be able to terminate the radical chain reaction. Table 7 represents the different concentrations of

methanolic extracts of *H. robusta* and *J. nervosum* (µg/mL) at an absorbance of 700 nm against a standard, Ascorbic acid (Fig.7 and Fig.8), respectively.

Table 7. Reducing power of methanolic extracts of *H. robusta* and *J. nervosum* at an absorbance of 700 nm in comparison with the standard, ascorbic acid.

Concentration (µg/ml)	Adsorbance at 700 nm		
	Ascorbic acid	*Helicia robusta*	*Jasmimum nervosum*
10	0.293	0.127	0.120
20	0.392	0.147	0.135
40	0.568	0.162	0.148
60	0.721	0.178	0.160
80	0.841	0.189	0.184
100	0.983	0.241	0.197

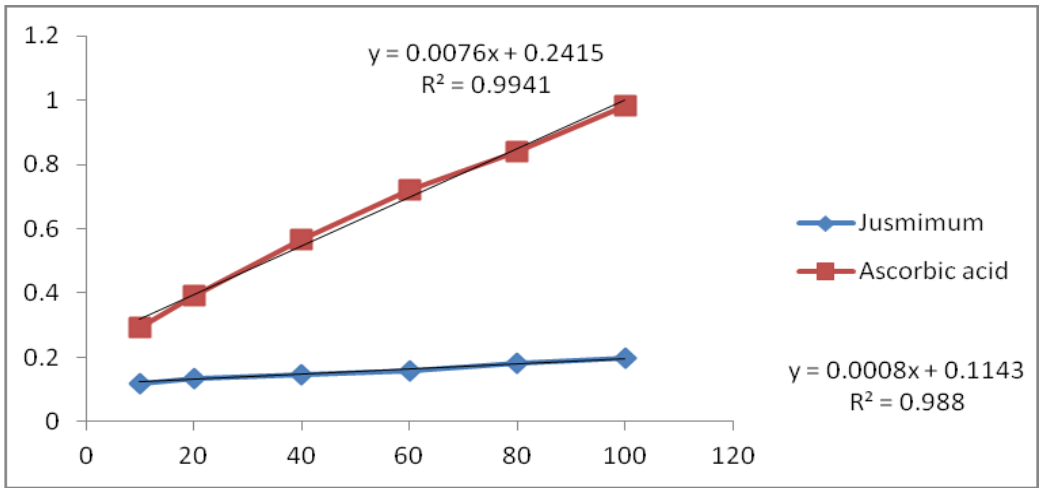


Figure 8: Reducing power of methanolic extract of *Jasmimum nervosum* in comparison with the standard, Ascorbic acid.

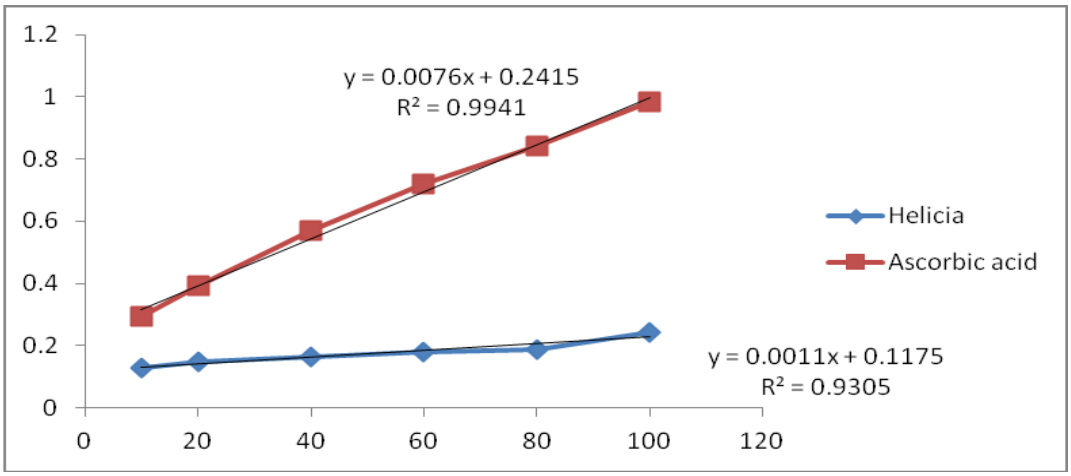


Figure 9: Reducing power of methanolic extract of *Helicia robusta* in comparison with the standard, Ascorbic acid.

The reducing power of the methanolic extracts of *J. nervosum* (0.2415 > 0.1143) and *H. robusta* (0.2415 > 0.1175) was lower than the standard ascorbic acid. The reducing power of *Helicia robusta* is higher than *Jasmimum nervosum* i.e (0.1175 > 0.1143). However, the antioxidant present in the extract cause the reduction of Fe^{3+} to Fe^{2+} and thus proved its reducing power (Fig. 8 and Fig. 9) as well as the antioxidant activity.

5.6 High Performance Liquid Chromatography

The HPLC was set up at a flow of 0.3 ml/ min and UV at 254 nm where methanol was used as a mobile phase (solvent) using isocratic condition. All the 12 standards such as gallic acid, arjunic acid, lutein, catechin, chlorogenic acid, ferulic acid, kaempferol, ursolic acid, boswellic acid, ellagic acid, chebulagic acid, and stigmasterol were tested and only five phenolic compounds (ferulic acid, kaemferol, lutein, gallic acid and chlorogenic acid) were detected by HPLC and showed good peaks for the standard phenolic compounds. Hence, these five phenolic compounds were taken into consideration for comparing their presence in the methanolic extracts of *J.nervosum* (Table 8, Fig. 10) and *H.robusta* (Table 9, Fig. 11).

Table 8 : Identification and quantification of phenolic compounds present in the methanolic extracts of *Jasminum nervosum*. (100 mg/ml)

S.No	Phenolic compound	Retention time	Amount (mg/ml)
1	Chlorogenic acid	9.204	-
2	Ferulic acid	8.191	-
3	Gallic acid	8.000	-
4	Kaemferol	8.355	-
5	Lutein	7.592	28.3

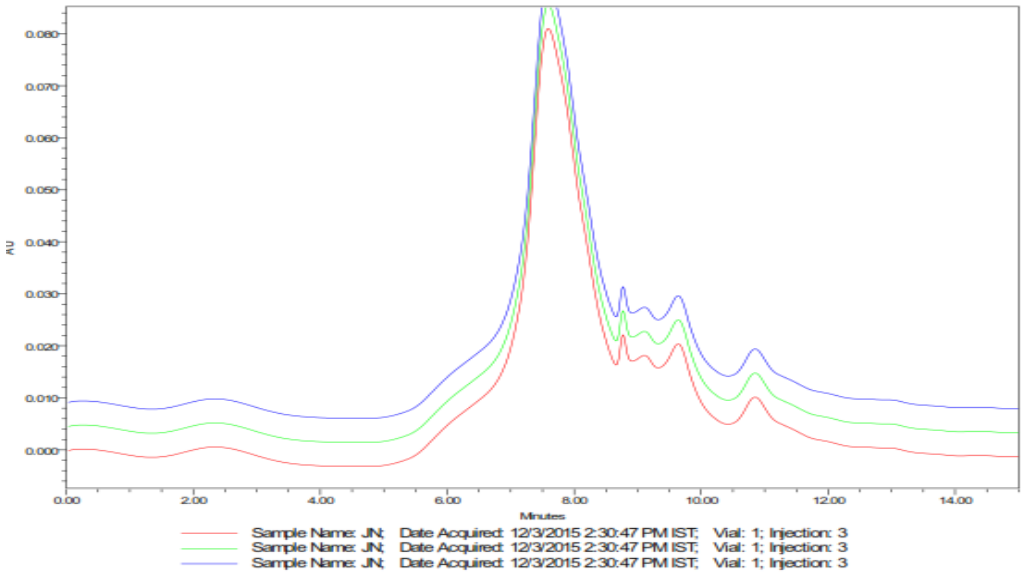


Figure 10: HPLC chromatogram of phenolic compounds present in the methanolic extract of *Jasminum nervosum* (100 mg/ml *J.nervosum*).

Table 9: Identification and quantification of phenolic compounds present in the methanolic extracts of *Helicia robusta.* (100 mg/ml)

S.No	Phenolic compound	Retention time	Amount (mg/ml)
1	Chlorogenic acid	9.204	-
2	Ferulic acid	8.191	4.9
3	Gallic acid	8.000	9.2
4	Kaemferol	8.355	-
5	Lutein	7.592	20.1

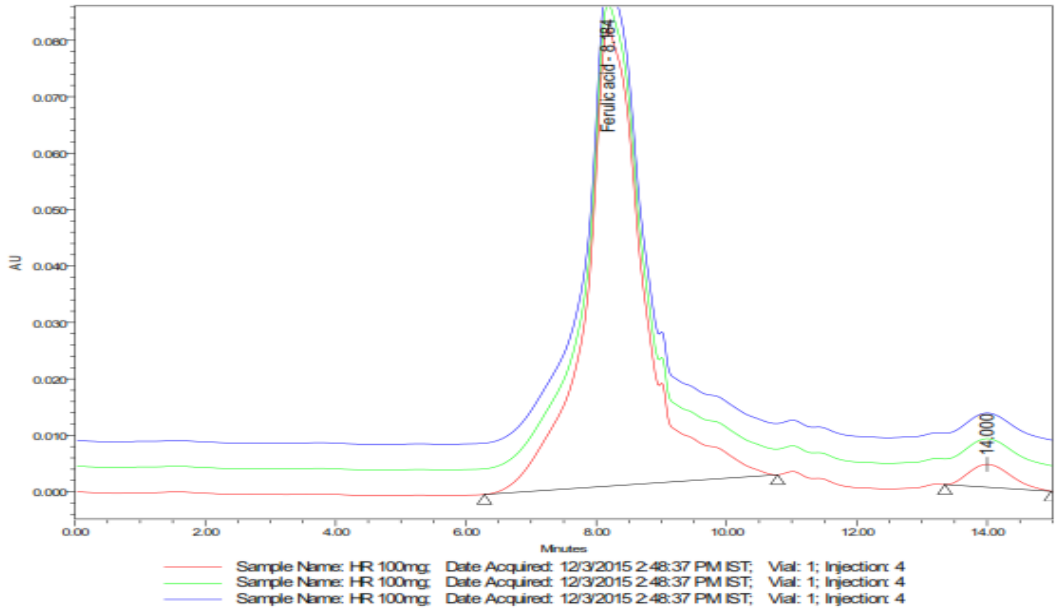


Figure 11: HPLC chromatogram of phenolic compounds present in the methanolic extract of *Helicia robusta* (100mg/ml *H.robusta*).

5.7 Silver and Gold nanoparticles characterization and their interactions

5.7.1 Synthesis of AgNPs and AuNPs - *Jasminum nervosum* leaf extract

AgNPs and AuNPs were synthesized using aqueous leaf extracts of *J. nervosum*. The $AgNO_3$ and $HAuCl_4$ solutions turned to yellowish brown colour and pinkish violet colour with the addition of leaf extract, respectively (Fig. 12). Interestingly, both the nanoparticles were synthesized rapidly within 1 hr of incubation period.

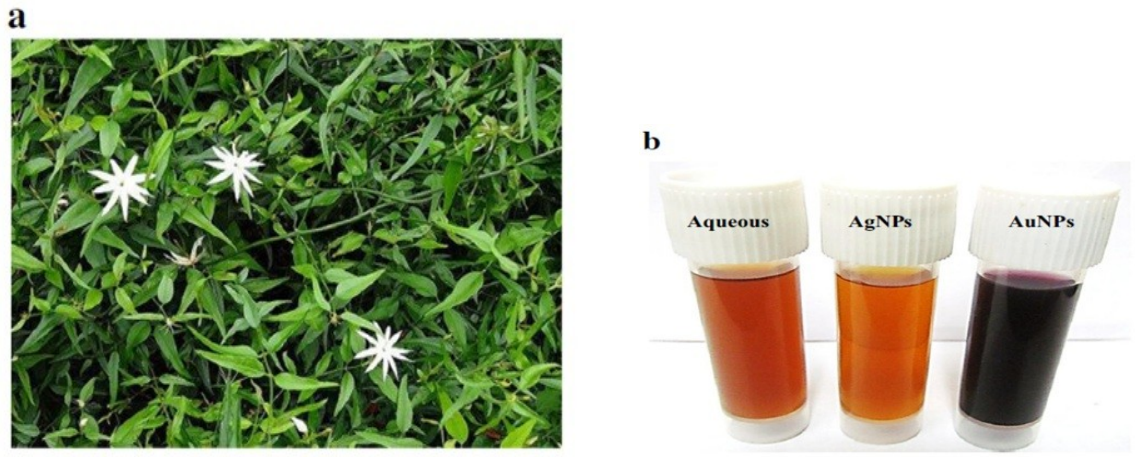


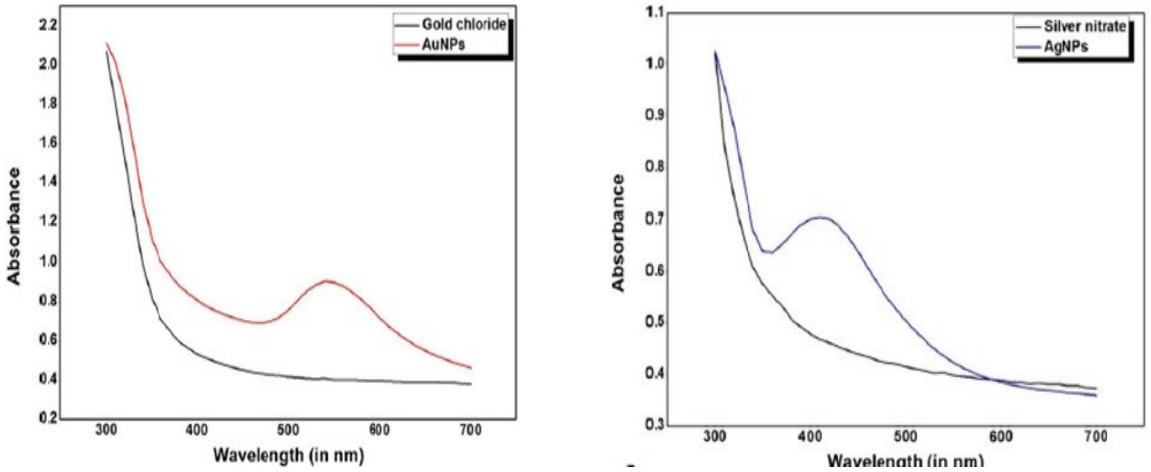
Figure 12: a) Picture of leaves of *J.nervosum* b) Visible colour indicates the generation of nanoparticles of *J.nervosum* (1.leaf extract 2.AgNPs 3.AuNPs)

5.7.2 Characterization of AgNPs and AuNPs - *Jasminum nervosum* leaf extract

5.7.2.1 UV-Visible spectrophotometer

Absorbance spectrum of the reaction mixture at different wavelengths ranging from 300 to 700 nm revealed a peak at 520 nm for AuNPs and at 390 nm for AgNPs. The optimal condition to synthesis narrow size range nanoparticles with high stability was fixed at 70^0C temperature, pH = 7, 1mM concentration of metal ion, stoichiometric ratio of reaction mixture =

95 mL of $AgNO_3$ and HAuCl4 with 5 mL of plant extract and 1 h incubation time. Intensity of yellowish brown and pinkish violet colour increased in direct proportion to the incubation period. It was due to the excitation of surface plasmon resonance (SPR) effect and reduction of metal ions into nanoscale particles. The silver and gold surface plasmon resonance were observed at 520 nm and 390 nm, respectively, which steadily increased in intensity as a function of time of reaction (ranging from 10 min to 6 h) without showing any shift of the wavelength maximum.



a b

Figure 13: UV-Visbile spectroscopic analysis shows the intense absorbance spectra at (a) 520 nm and (b) 390 nm indicates the formation of AuNPs and AgNPs respectively. No SPR spectra were recorded for GERE and metal ion substrate ($AgNO_3$ and $HAucl_4$).

5.7.2.2 Fourier transform infrared (FTIR) and X-ray Diffraction (XRD) analysis

FTIR spectroscopy analysis was performed to ascertain the involvement of possible plant biocompound responsible for reduction of Ag^+ and Au^+ ions and capping and stabilization of bioreduced AgNPs and AuNPs synthesized by using plant extract.(Fig. 14) shows the aqueous and synthesized AgNPs and AuNPs using *J. nervosum* leaf extract where the absorption spectrum manifests prominent transmittance located at 3433, 2922, 1644, 1632.45, 1383, 1069 and 1042

cm^{-1} in the region 500 – 4000 $cm^{-1.}$ The strong bands at 1644 cm^{-1} corresponded to the –C=C– stretches (flavanones) and broad peaks at 3433 and 2922 cm^{-1} indicating the –N-H– stretches (amide group) and cyclic CH2 stretches (aliphatic group). The prominent band at 1383 may be attributed to –C–O stretching mode showing the presence of carbonyl groups. The band at 1069 cm^{-1} corresponded to the presence of fatty acids (Fig. 15). A number of Bragg reflections with 2θ values of 38.13◦, 43.92◦ and 64.51◦ sets of lattice planes are observed which may be indexed to the (1 1 1), (2 0 0) and (2 2 0) facts of silver respectively (Fig. 15a). For AuNPs, the XRD peak corresponding to four peaks (JCPDS, No. 89−3722) at 38.17˚, 44.36˚, 64.65˚ and 77.59˚ were found which may be indexed to (1 1 1), (2 0 0) (2 2 0) and (3 1 1) and found to be identical with those reported for the standard gold metal (Au˚) (Fig. 15b). It suggests that the prepared AgNPs and AuNPs were biphasic in nature. The slight shift in the peak positions indicated the presence of strain in the crystal structure which is the characteristic of nanocrystallites.

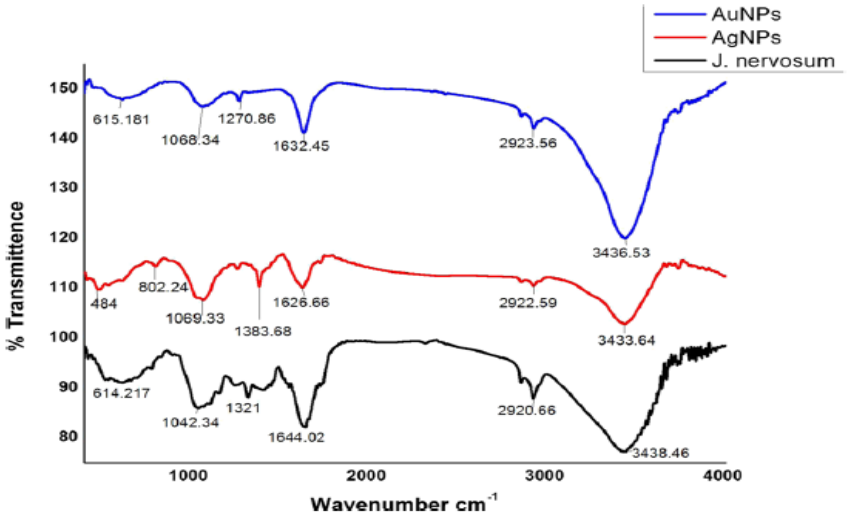


Figure 14: Fourier transform infrared (FTIR) spectrum of aqueous and synthesized AgNPs and AuNPs using *J. nervosum* leaf extract showing the interaction of nanoparticles with water soluble phenolic biocompounds

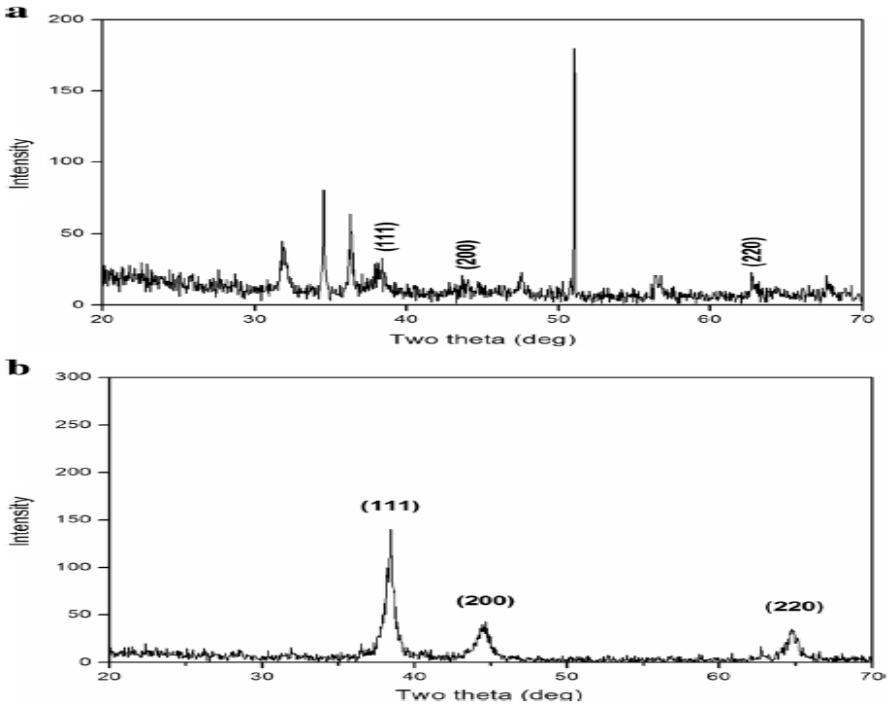


Figure 15 : X-ray diffraction (XRD) pattern of synthesized: a) silver nanoparticles b) gold nanoparticles from aqueous leaf extract of *J. nervosum*

5.7.2.3 Transmission Electron Miscroscope (TEM) analysis

While the absorption spectra provide solid evidence of nanoparticle formation and their growth kinetics, the shape and size of the resultant particles were elucidated with the help of TEM. NPs observed from the micrograph majority are spherical AgNPs, with a small percentage of elongated particles, and ranged in size between 4–22 nm with an average size of 10 ± 2 nm (Fig. 16a). Interestingly, TEM micrograph of AuNPs displays both triangle and spherical NPs with size ranging from 2-20 nm with an average size of 9.4 ± 4 nm (Fig. 16b). It was also found that both the AgNPs and AuNPs has a thin layer of biomolecule on its surface which prevents the aggregation and the particles were polydispersed and stable for long period of time. Thus, the

XRD pattern proves to be strong evidence in favour of the UV–Vis spectra and TEM images for the presence of silver and gold nanocrystals.

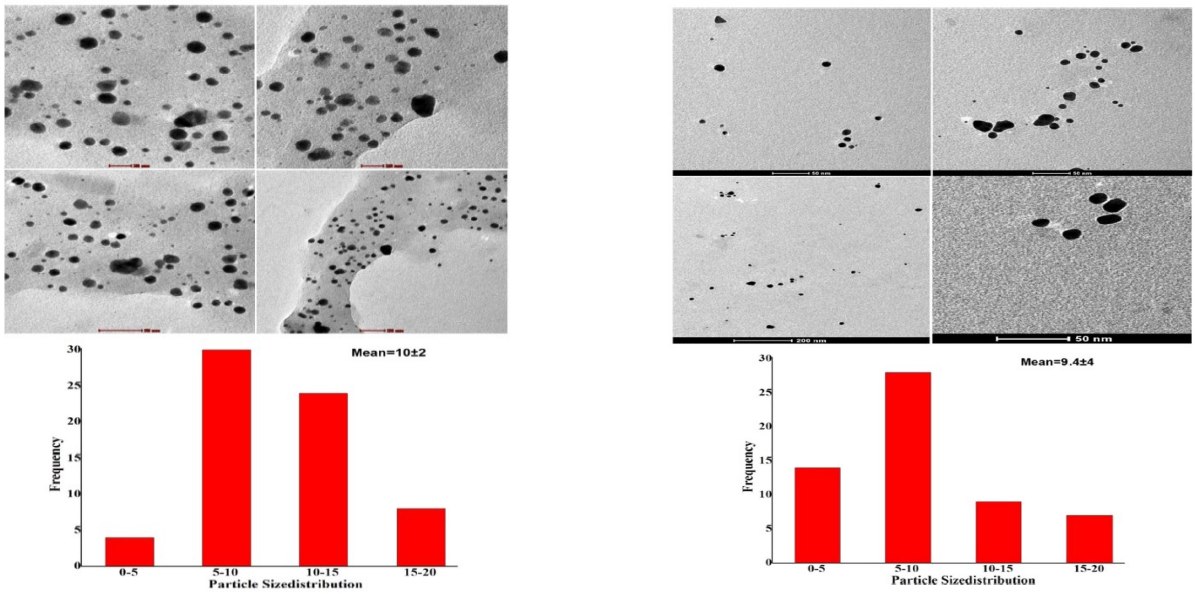


Figure 16: High resolution transmission electron microscopic images of *J.nervosum* displays well dispersed

a) AgNPs in the size range between 4-22 nm with the average size of 10±2 nm

b) AuNPs in the size range between 2-20 nm with the average size of 9.4±4 nm

Selected area electron diffraction pattern of synthesized AuNPs confirms the face centre cubic crystalline structure and DLS analysis shows the particle size distribution and hydrodynamic diameter of synthesized AgNPs and AuNPs.

5.8 Synthesis of AgNPs and AuNPs – Helicia robusta bark extract

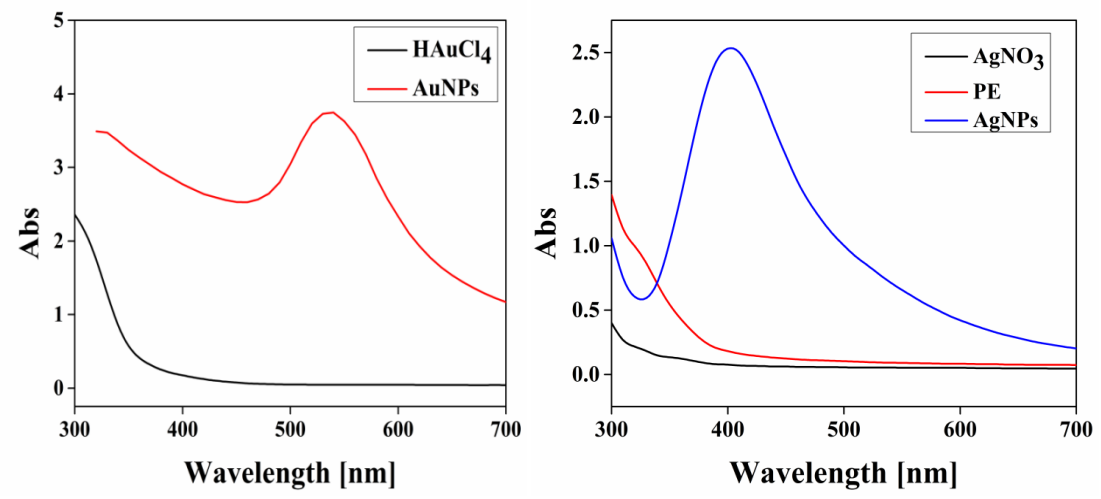
AgNPs and AuNPs were synthesized using aqueous bark extracts of *H. robusta*. The $AgNO_3$ and $HAuCl_4$ solutions turned to yellowish brown colour and pinkish violet colour with the addition of bark extract, respectively (Fig.17). Interestingly, both the nanoparticles were synthesized rapidly within 1 hr of incubation period.

Figure 17: a) Visible colour indicates the generation of nanoparticles of *H.robusta* (1.bark extract 2.AgNPs 3.AuNPs). b) Picture of stem bark of *H.robusta*.

5.8.1 Characterization of AgNPs and AuNPs - Helicia robusta bark leaf extract

5.8.1.1 UV-Visible spectrophotometer

Absorbance spectrum of the reaction mixture at different wavelengths ranging from 300 to 700 nm revealed a peak at 550 nm for AuNPs (Fig. 18a) and at 420 nm for AgNPs (Fig. 18b). The optimal condition to synthesis narrow size range nanoparticles with high stability was fixed at 70^0C temperature, pH = 7, 1mM concentration of metal ion, stoichiometric ratio of reaction mixture = 95 mL of $AgNO_3$ and $HAuCl_4$ with 5 mL of plant extract and 1 hr incubation time. Intensity of yellowish brown and pinkish violet colour increased in direct proportion to the incubation period. It was due to the excitation of surface plasmon resonance (SPR) effect and reduction of metal ions into nanoscale particles. The silver and gold surface plasmon resonance were observed at 550 nm and 420 nm, respectively, which steadily increased in intensity as a function of time of reaction (ranging from 10 min to 6 h) without showing any shift of the wavelength maximum.



a b

Figure 18: UV-Visbile spectroscopic analysis shows the intense absorbance spectra at (a) 550 nm and (b) 420 nm indicates the formation of AuNPs and AgNPs respectively. No SPR spectra were recorded for GERE and metal ion substrate ($AgNO_3$ and $HAucl_4$).

5.8.1.2 Fourier transform infrared (FTIR) and X-rayDiffraction (XRD) analysis

FTIR spectroscopy analysis was performed to ascertain the involvement of possible plant biocompound responsible for reduction of Ag^+ and Au^+ ions and capping and stabilization of bioreduced AgNPs and AuNPs synthesized by using plant extract. (Fig. 19) shows the aqueous and synthesized AgNPs and AuNPs using *Helicia robusta* bark extract where the absorption spectrum manifests prominent transmittance located at 3433, 2922, 1644, 1632.45, 1383, 1069 and 1042 cm^{-1} in the region 500 – 4000 cm^{-1}. The strong bands at 1644 cm^{-1} corresponded to the –C=C– stretches (flavanones) and broad peaks at 3433 and 2922 cm^{-1} indicating the –N-H– stretches (amide group) and cyclic CH2 stretches (aliphatic group). The prominent band at 1383 may be attributed to –C–O stretching mode showing the presence of carbonyl groups. The band at 1069 cm^{-1} corresponded to the presence of fatty acids . A number of Bragg reflections with 2θ values of 38.13◦, 43.92◦ and 64.51◦ sets of lattice planes are observed which may be indexed to

the (1 1 1), (2 0 0) and (2 2 0) facts of silver respectively. For AuNPs, the XRD peak corresponding to four peaks (JCPDS, No. 89−3722) at 38.17˚, 44.36˚, 64.65˚ and 77.59˚ were found which may be indexed to (1 1 1), (2 0 0) (2 2 0) and (3 1 1) and found to be identical with those reported for the standard gold metal (Au˚) (Fig 20). It suggests that the prepared AgNPs and AuNPs were biphasic in nature. The slight shift in the peak positions indicated the presence of strain in the crystal structure which is the characteristic of nanocrystallites .

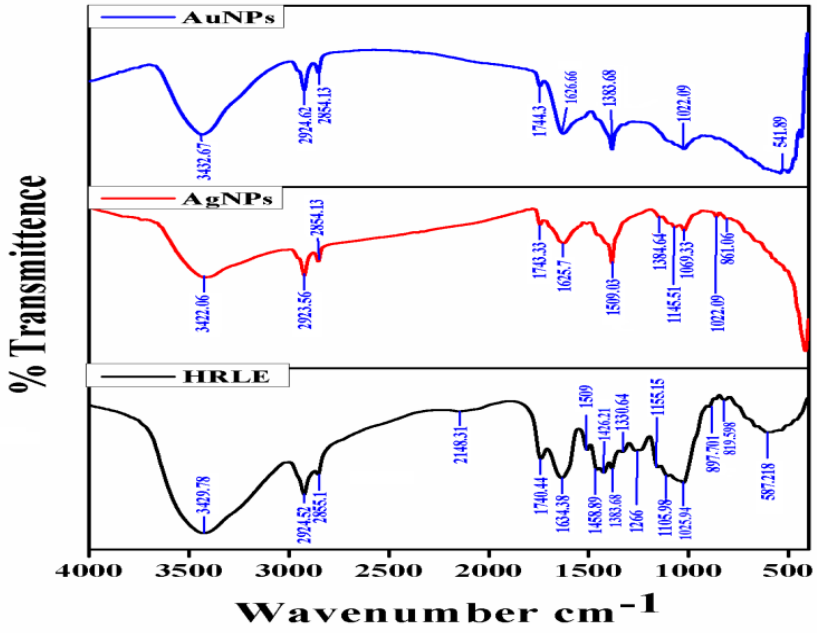


Figure 19: Fourier transform infrared spectroscopic analysis of synthesized AgNPs and AuNPs generates the transmittance corresponding to O-H, C=O and C-H functional group denotes the active role of CERE metabolites in reduction and stabilization.

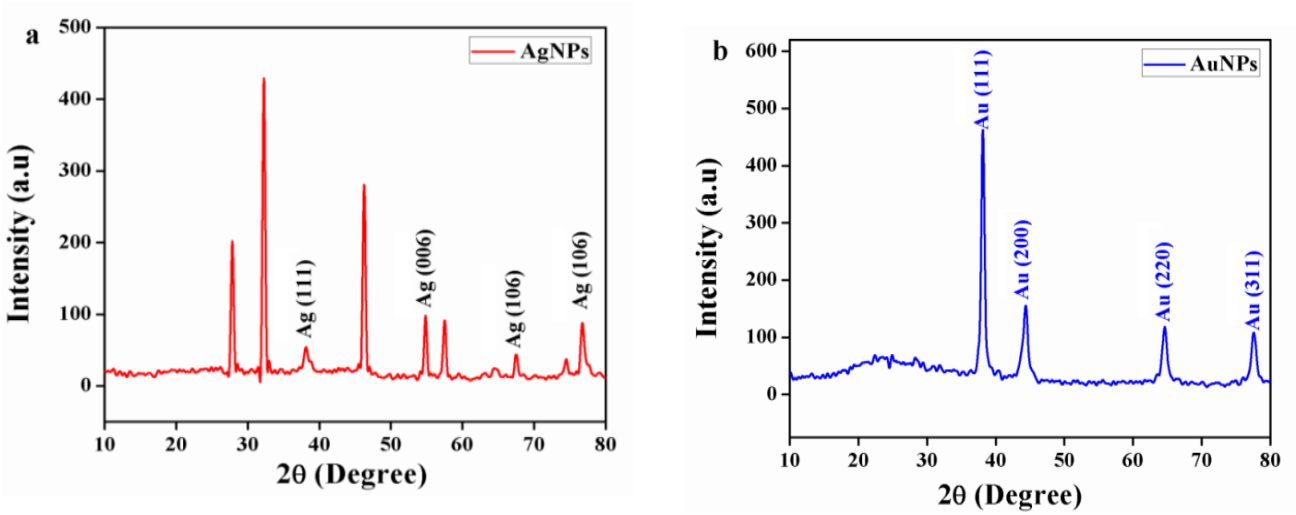


Figure 20: a) X-ray Diffraction analysis of synthesized AgNPs shows the planes corresponds to both cubic and b) hexagonal crystalline structure whereas AuNPs possess the planes for face centred cubic (fcc) crystalline structure from aqueous leaf extract of *H.robusta.*

5.8.1.3 Transmission Electron Miscroscope (TEM) analysis

While the absorption spectra provide solid evidence of nanoparticle formation and their growth kinetics, the shape and size of the resultant particles were elucidated with the help of TEM. NPs observed from the micrograph ,majority are spherical AgNPs, with a small percentage of elongated particles, and ranged in size between 4.5-56.4 nm with an average size of 21±11 nm (Fig. 21a). Interestingly, TEM micrograph of AuNPs displays both triangle and spherical NPs with size ranging from 4.5-30.2 nm with the average size of 12.8 ± 6 nm (Fig. 19b). It was also found that both the AgNPs and AuNPs has a thin layer of biomolecule on its surface which prevents the aggregation and the particles were polydispersed and stable for long period of time. Thus, the XRD pattern proves to be strong evidence in favour of the UV–Vis spectra and TEM images for the presence of silver and gold nanocrystals.

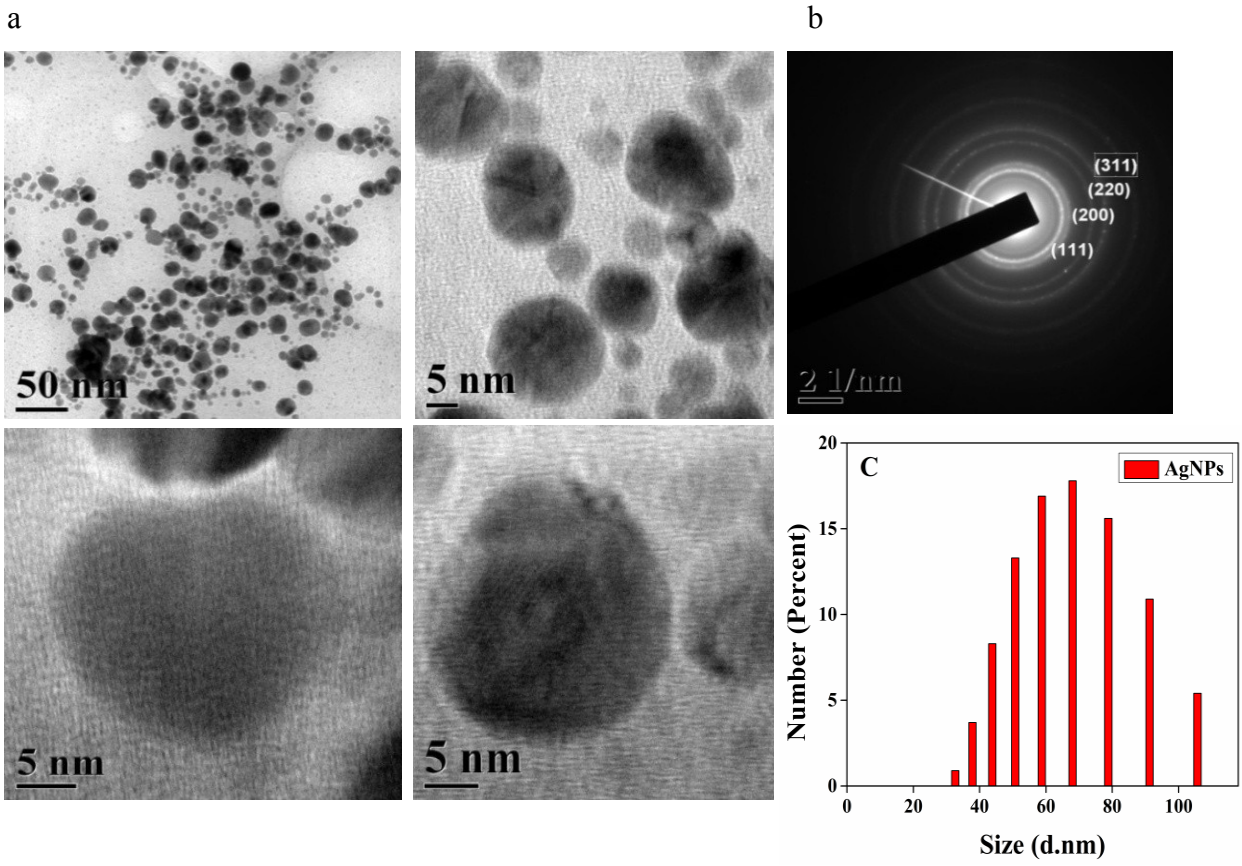


Figure 21a : (a) High resolution transmission electron microscopic images displays well dispersed AgNPs in the size range between 4.5-56.4 nm with the average size of 21±11 nm

(b) SAED Pattern of synthesized AgNPs confirms the mixed phase of both cubic and hexagonal crystalline structure

(c) DLS analysis shows the particle size distribution and hydrodynamic diameter of synthesized AgNPs.

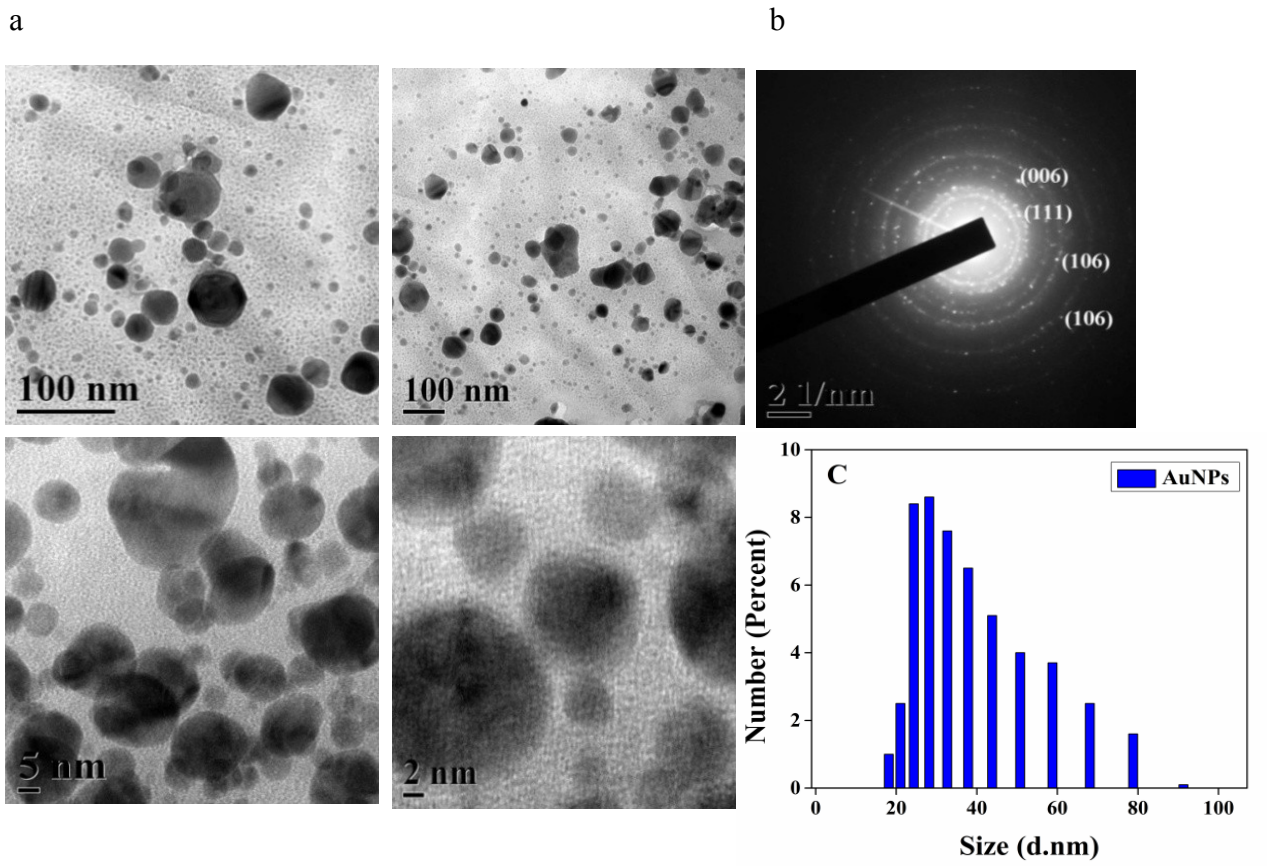


Figure 21b: (a) High resolution transmission electron microscopic images displays well dispersed AuNPs in the size range between 4.5-30.2 nm with the average size of 12.8±6 nm

(b) Selected area electron diffraction pattern of synthesized AuNPs confirms the face centered cubic crystalline structure

(c) Dynamic light scattering analysis shows the particle size distribution and hydrodynamic diameter of synthesized AuNPs.

5.9 Acute Toxicity Study

The present study conducted as per the OECD guidelines 423 revealed that the *J. nervosum* leaf extract, *H. robusta* bark extract, silver and gold nanpaticle from *J. nervosum* leaf extract and *H. robusta* bark extracts did not produce any mortality throughout the study period of

14 days even when the limit dose was maintained at 400 mg/kg body weight. The oral LD_{50} was indeterminable being in excess of 400 mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-toxic. Table 1 indicates the parameters observed before and after the administration of the six test substances - *J. nervosum* leaf extract, *H. robusta* bark extract, silver and gold nanpaticle from *J. nervosum* leaf extract and *H. robusta* bark extracts. The writhing reflux was observed immediately upto 15 min after administration of the test substance at all administered doses, whereas all the other parameters observed were normal even at the highest dosage of 400 mg/kg body weight of the test animal. This clearly indicated that the above test substances do not produce oral toxicity. The medium lethal dose (LD_{50}) of the extracts is higher than 400 mg/kg body weight and hence, in a single dose administration, the plant extracts and nanoparticles had no adverse effect. From the statistical analysis of the dosage administered to the animals, it was found that the values are significant at 5% . Macroscopic examination of the organs of the animals treated with extract and nanoparticle showed no changes in color compared to control. Autopsy at the end of the experiment period revealed no apparent changes in the liver, kidney, lungs, heart and spleen organs from both control and treated rats in the histopathology analysis. The microscopic examination revealed that, all the organs from the extract and nanoparticle treated rat did not show any alteration in cell structure or any unfavorable effects when viewed under the light microscope using multiple magnification power. The structure or coordination of cells in extract treated organs is more or less similar compared with the control organs.

Table 10: Potential toxic effects of the crude extract of *H.robusta*, *J.nervosum*, Gold and and Silver nanoparticles of *H. robusta*, Gold and Silver nanoparticles of *J. nervosum*.

Control	**crude extract of *H.robusta*** 3000 mg/kg	Control	**crude extract of *J.nervosum*** 3000 mg/kg
0/6[a]	0/6[a]	0/6[a]	0/6[a]
Control	**Silver nanoparticles of *H.robusta*** 100 mg/kg	Control	**Gold nanoparticles of *H.robusta*** 100 mg/kg
0/6[a]	0/6[a]	0/6[a]	0/6[a]
Control	**Silver nanoparticles of *J.nervosum*** 100 mg/kg	Control	**Gold nanoparticles of *J.nervosum*** 100 mg/kg
0/6[a]	0/6[a]	0/6[a]	0/6[a]

[a]Number of dead rats/number of rats used.

Table 11: General appearance and behavioral observations for control and treated groupsof the crude extract of *H. robusta*, *J. nervosum*, Gold and Silver nanoparticles of *H. robusta*, Gold and Silver nanoparticles of *J. nervosum*.

Observation	Control group		Test groups of the crude extract of *H.robusta*, *J.nervosum*, Gold and Silver nanoparticles of *H.robusta*, *J.nervosum*	
	6 hr	14 hr	6 hr	14 hr
Skin and fur	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal
Mucous membrane	Normal	Normal	Normal	Normal
Behavioral patterns	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Lethargy	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal
Diarrhea	Normal	Normal	Normal	Normal
Coma	Normal	Normal	Normal	Normal
Tremors	Normal	Normal	Normal	Normal

5.10 Gross Evaluation of Gastric Lesions

a) *Helicia robusta* methanolic bark extract

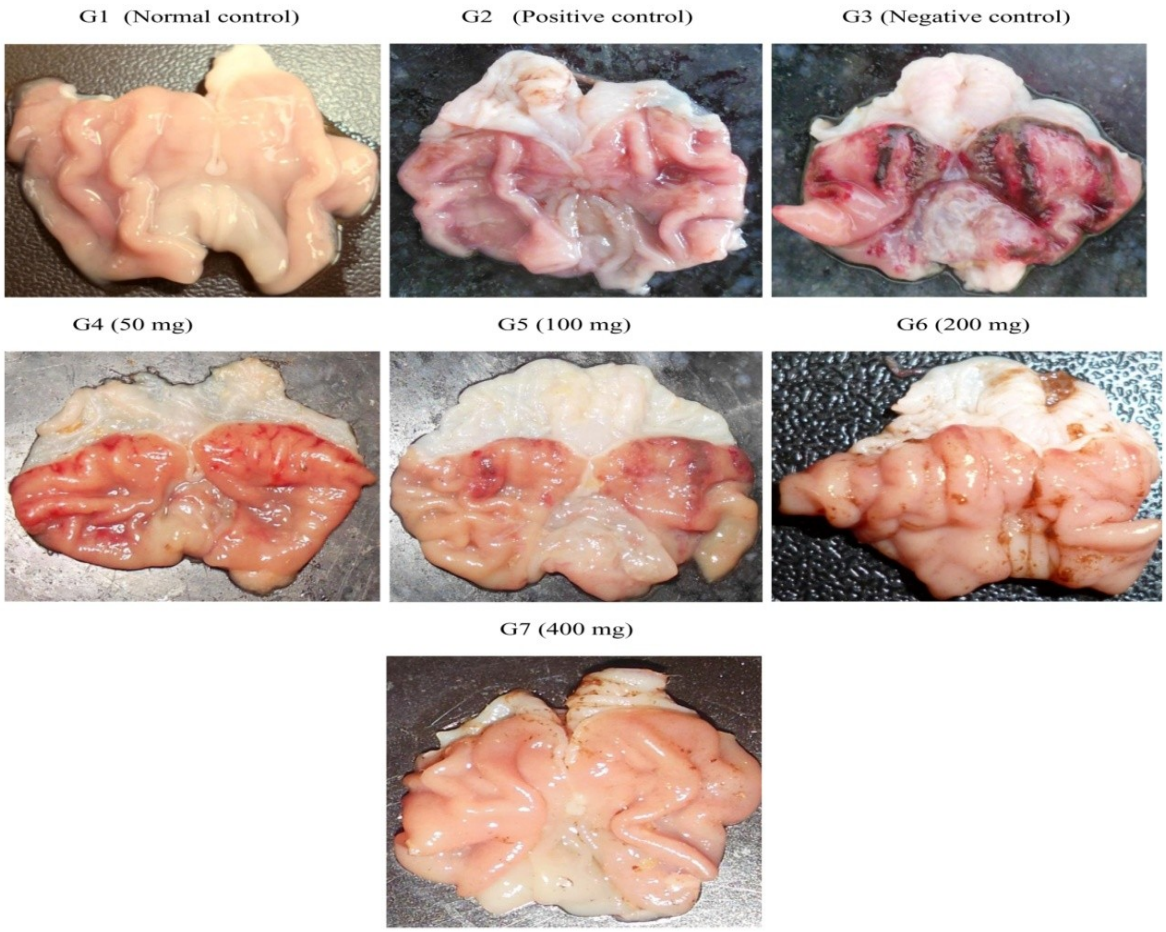


Figure 22: The effect of *Helicia robusta* bark on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(G1) (Normal control group); (G2) (Ulcer control group); (G3) (Omeprazole); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Helicia robusta* extract

Table 12: Effects of *Helicia robusta* bark methanolic extract on the gastric mucosa of rats.

S. No	Animal group	Gastric pH	Ulcer index (mm^2)	Inhibition (%)	Total acidity (mEq/ltr)
1	Normal control	6.88 ± 0.13^{a}	---	---	195.25 ± 2.64^{e}
2	Ulcer control	5.26 ± 0.23^{b}	248.01 ± 1.26^{a}	---	476.70 ± 2.15^{a}
3	Omeprazole	6.72 ± 0.16^{a}	92.80 ± 1.72^{f}	71.25 ± 1.42^{a}	219.40 ± 1.84^{d}
4	H.R (50mg)	4.14 ± 0.11^{c}	142.32 ± 1.53^{b}	57.40 ± 1.37^{d}	321.50 ± 2.63^{b}
5	H.R(100mg)	5.28 ± 0.13^{b}	107.81 ± 1.82^{c}	61.38 ± 1.39^{c}	255.75 ± 1.28^{c}
6	H.R(200mg)	5.72 ± 0.08^{b}	102.29 ± 1.35^{cd}	68.15 ± 1.28^{c}	246.15 ± 1.74^{c}
7	H.R(400mg)	6.06 ± 0.12^{a}	72.06 ± 1.38^{e}	83.17 ± 1.32^{ab}	$209.95\ 1.82^{d}$

- H.R - *Helicia robusta*

b) *Jasminum nervosum* methanolic leaf extract

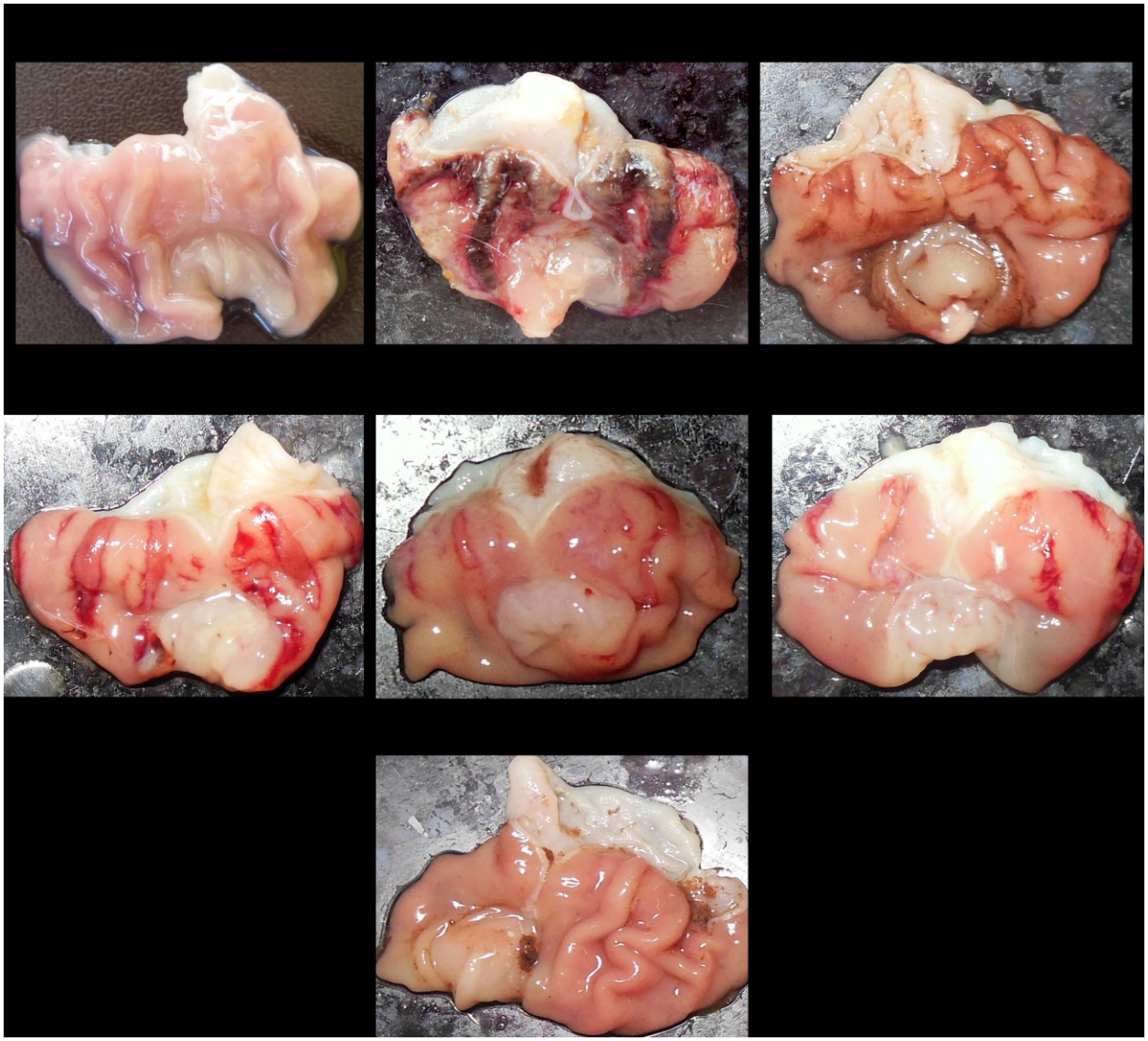


Figure 23: The effect of *Jasminum nervosum* leaf on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control group); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Jasminum nervosum* extract

Table 13: Effects of *Jasminum nervosum* leaf methanolic extract on the gastric mucosa of rats.

S.No	Animal group	Gastric pH	Ulcer index (mm^2)	Inhibition (%)	Total acidity (meq/ltr)
1	Normal control	6.65 ± 0.17^{a}	---	---	182.25 ± 2.84^{g}
2	Ulcer control	4.85 ± 0.12^{c}	247.5 ± 1.38^{a}	---	1421.55 ± 2.95^{a}
3	Omeprazole	6.72 ± 0.16^{a}	139.2 ± 1.92^{f}	71.04 ± 1.31^{ab}	279.7 ± 2.51^{f}
4	J.N(50mg)	5.30 ± 0.12^{b}	178.3 ± 1.62^{b}	59.67 ± 1.85^{e}	510.3 ± 2.76^{b}
5	J.N(100mg)	5.41 ± 0.19^{b}	154.3 ± 1.35^{c}	61.13 ± 1.32^{d}	400.95 ± 2.81^{c}
6	J.N(200mg)	5.37 ± 0.09^{b}	146.9 ± 1.25^{d}	67.28 ± 1.94^{c}	291.6 ± 2.56^{d}
7	J.N(400mg)	6.96 ± 0.06^{a}	129.6 ± 1.84^{e}	77.14 ± 1.47^{a}	255.15 ± 2.73^{e}

- J.N - *Jasiminum nervosum*

c) Gold and Silver Nanoparticles of *Helicia robusta* methanolic bark extract

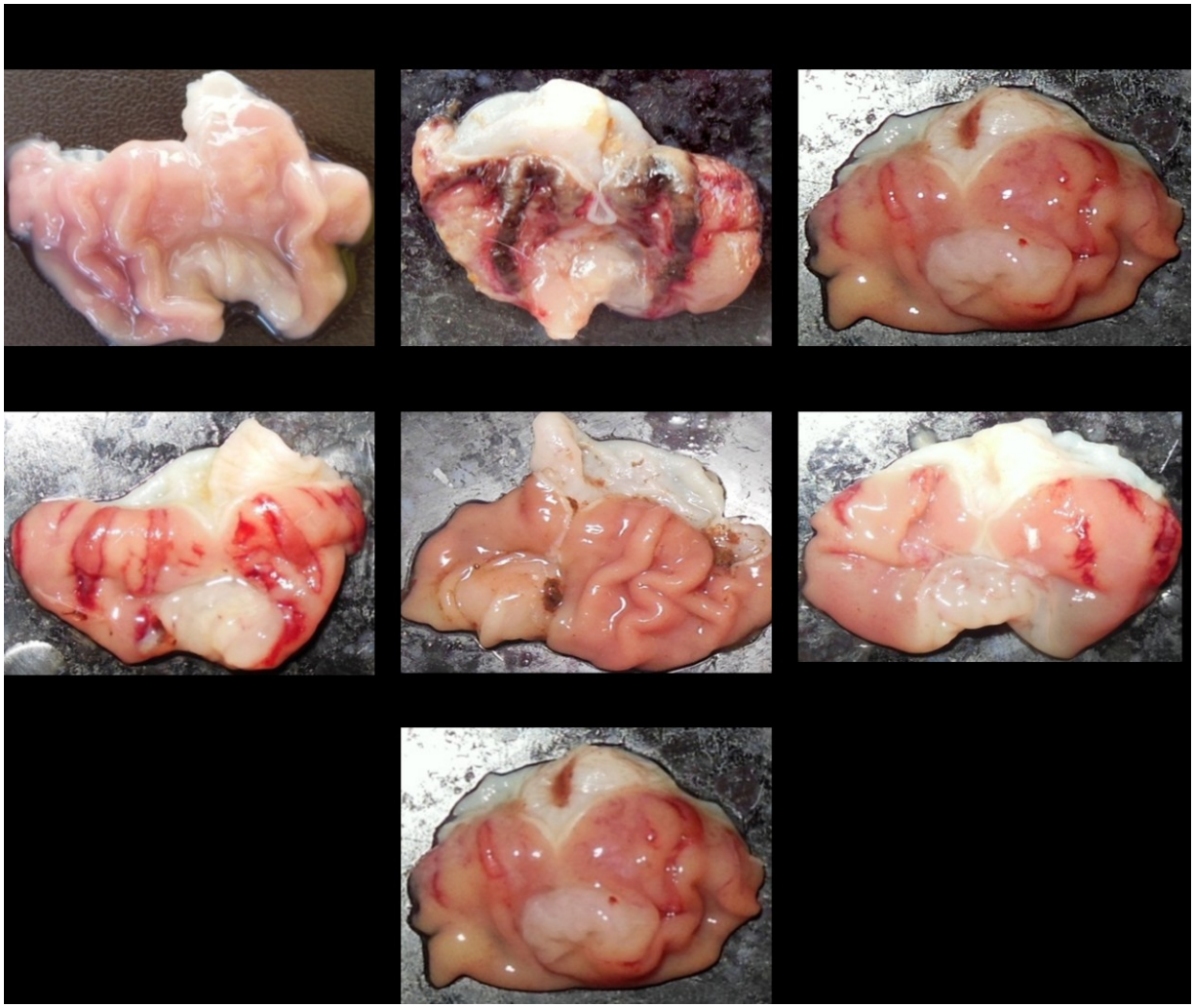


Figure 24: The effect of gold and silver nanoparticles of *Helicia robusta* on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in albino rats.

(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control group); (G4) (0.09Au mg/kg), (G5) (0.45Au mg/kg), (G6) (0.09Ag mg/mg), (G7) (0.45Ag mg/kg) *Helicia robusta* extract.

Table 14: Effects of gold and silver nano particle of *Helicia robusta* bark methanolic extract on the gastric mucosa of albino rats.

S.no	Animal group	Gastric pH	Ulcer index (mm^2)	Inhibition (%)	Total acidity (meq/ltr)
1	Normal control	6.65 ± 0.12^{a}	---	---	182.25 ± 1.35^{g}
2	Ulcer control	4.85 ± 0.11^{c}	242.5 ± 1.72^{a}	---	1421.55 ± 2.94^{a}
3	Omeprazole	6.72 ± 0.16^{a}	134.2 ± 1.27^{d}	68.04 ± 1.42^{b}	273.7 ± 1.64^{f}
4	H.R(0.09mg)Au	5.30 ± 0.19^{b}	143.2 ± 1.48^{d}	66.67 ± 1.53^{b}	301.95 ± 2.53^{c}
5	H.R(0.45mg)Au	5.41 ± 0.15^{b}	122.3 ± 1.28^{b}	77.13 ± 1.62^{c}	255.15 ± 1.84^{e}
6	H.R(0.09mg)Ag	5.37 ± 0.16^{b}	134.9 ± 1.23^{c}	64.28 ± 1.43^{c}	356.3 ± 2.49^{b}
7	H.R(0.45mg)Ag	6.96 ± 0.07^{a}	129.6 ± 1.42^{e}	67.14 ± 1.12^{a}	281.6 ± 1.53^{d}

- H.R – *Helicia robusta*

d) Gold and Silver Nanoparticle of Jasminum nervosum methanolic leaf extact

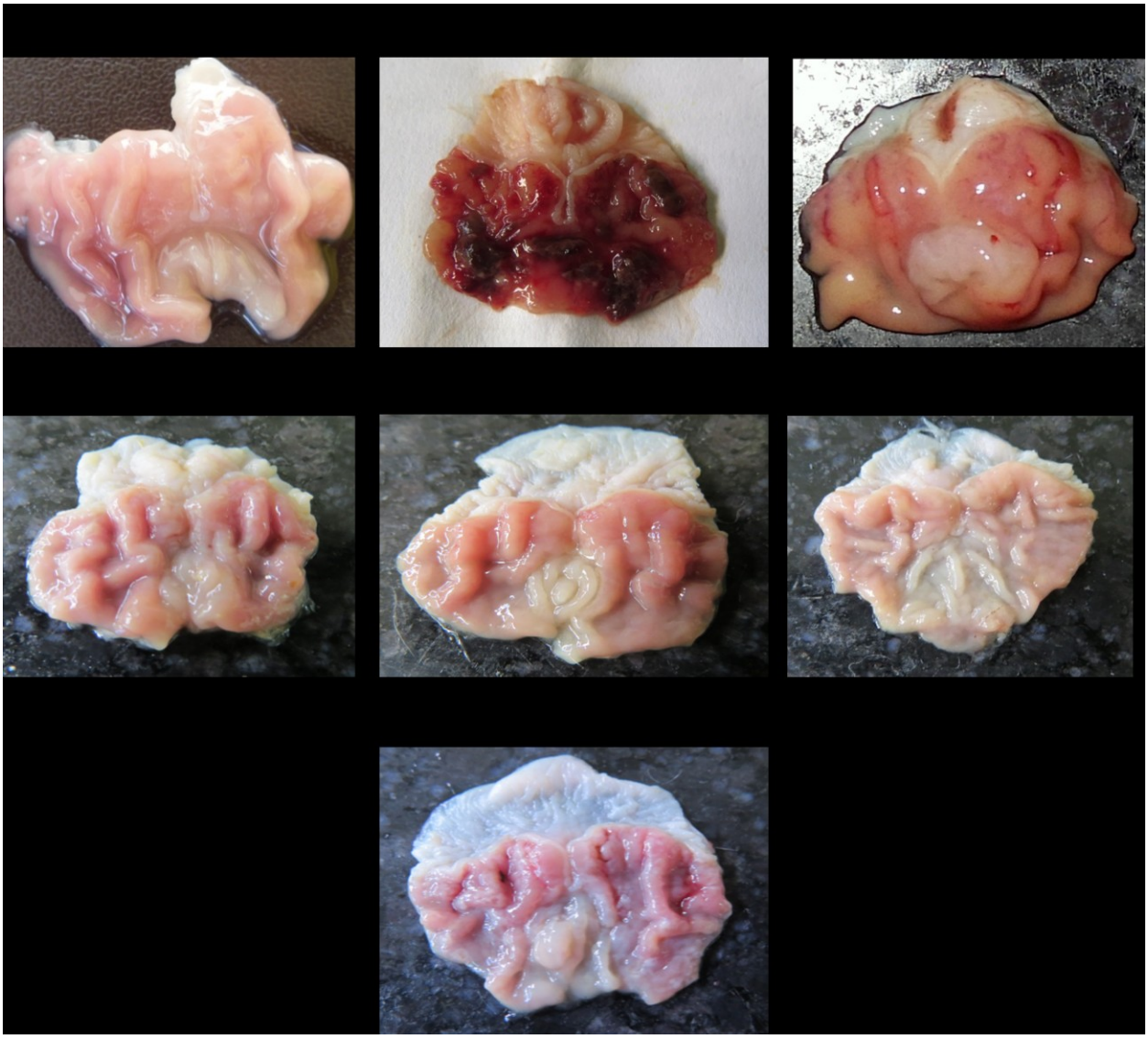


Figure 25: The effect of gold and silver nanoparticles of *Jasmimum nervosum* on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats.

(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control); (G4) (0.09Au mg/kg), (G5) (0.45Au mg/kg), (G6) (0.09Ag mg/kg), (G7) (0.45Ag mg/kg) *Jasmimum nervosum* extract

Table 15: Effects of gold and silver nanoparticle of *Jasminum nervosum* methanolic extract on the gastric mucosa of rats.

S.no	Animal group	Gastric pH	Ulcer index (mm^2)	Inhibition (%)	Total acidity (meq/ltr)
1	Normal control	6.72 ± 0.15^{a}	---	---	179.32 ± 1.73^{f}
2	Ulcer control	4.98 ± 0.05^{d}	249.5 ± 1.63^{a}	---	1387.95 ± 2.94^{a}
3	Omeprazole	6.52 ± 0.14^{a}	146.2 ± 1.49^{c}	64.12 ± 1.29^{b}	208.1 ± 1.57^{e}
4	J.N (0.09mg)Au	5.42 ± 0.18^{c}	121.0 ± 1.52^{d}	74.62 ± 1.32^{b}	423.75 ± 2.41^{c}
5	J.N (0.45mg)Au	5.75 ± 0.14^{b}	92.7 ± 1.44^{b}	85.16 ± 1.49^{c}	214.96 ± 1.39^{e}
6	J.N (0.09mg)Ag	5.95 ± 0.07^{b}	115.5 ± 1.37^{c}	78.41 ± 1.63^{c}	529.8 ± 2.63^{b}
7	J.N (0.45mg)Ag	6.85 ± 0.12^{a}	127.9 ± 1.24^{e}	72.46 ± 1.23^{a}	317.1 ± 2.15^{d}

- J.N – *Jasminum nervosum*

5.11 Histological Evaluation of Gastric Lesions

a) *Helicia robusta* methanolic bark extract

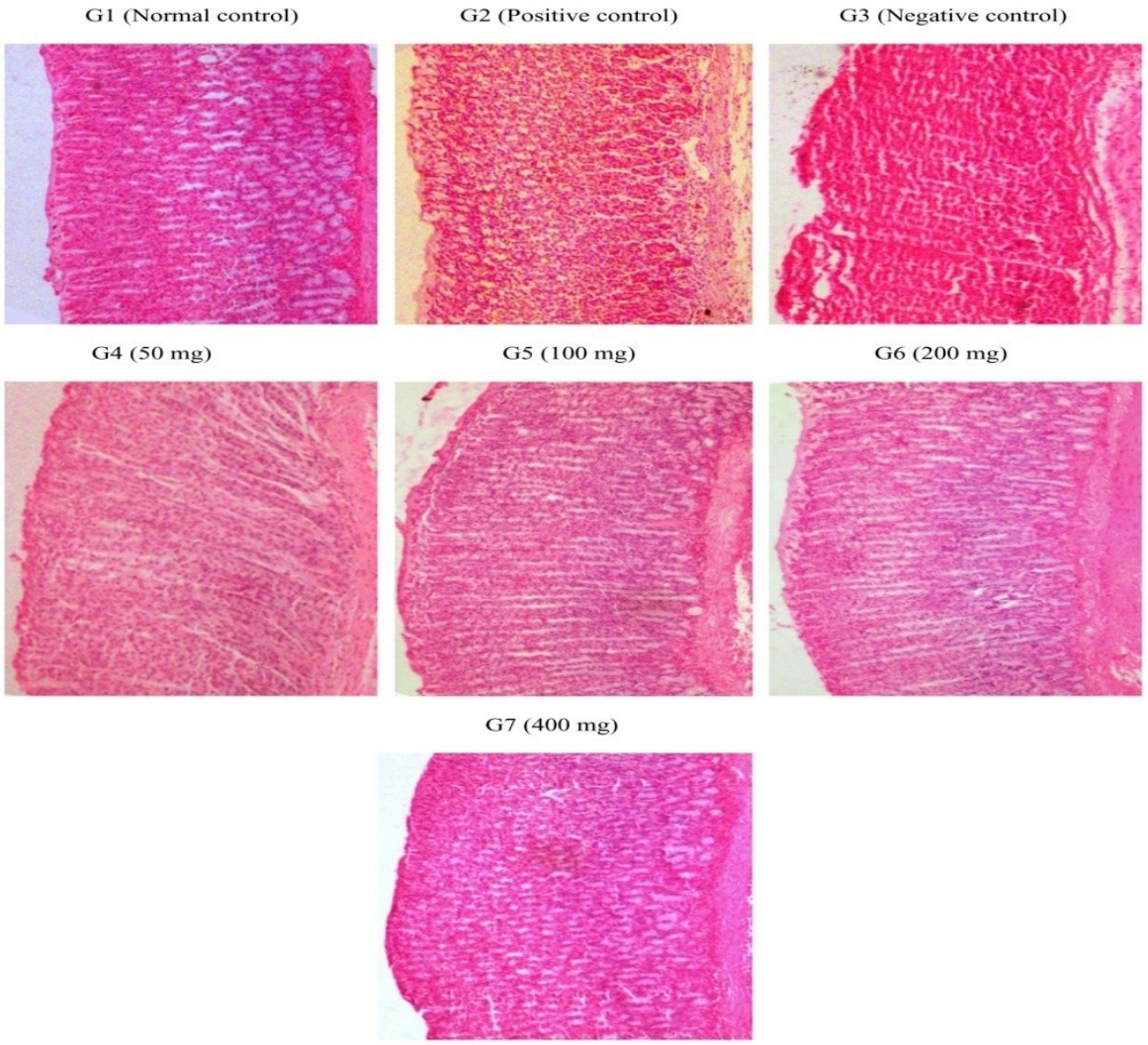


Figure 26: The effect of *H. robusta* on the histology of ethanol-induced gastric mucosal damage in rats. (G1) (Normal control group); (G2) (Ulcer control group); (G3) (Omeprazole); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Helicia robusta* extract.

b) *Jasminum nervosum* methanolic leaf extract

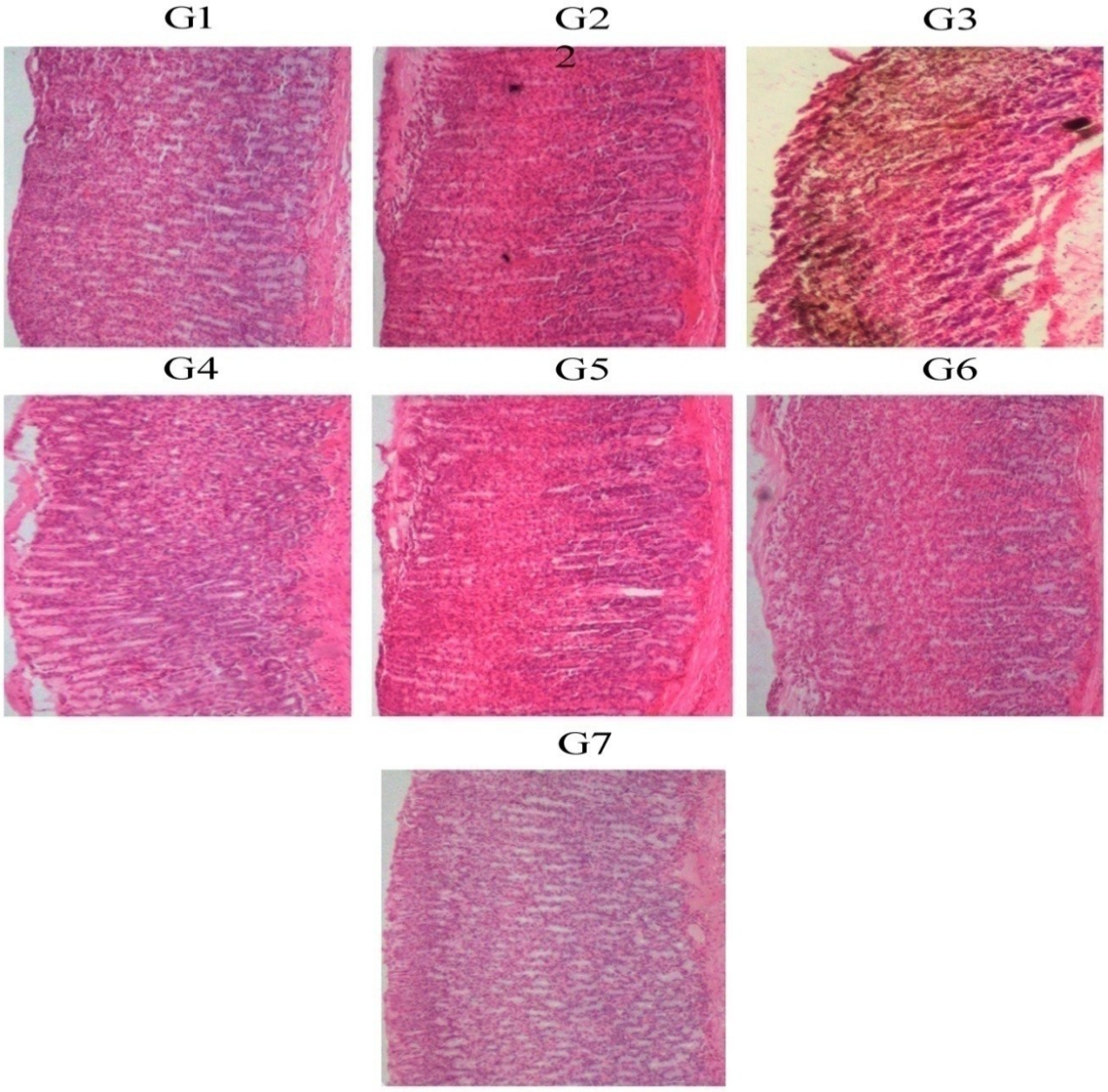


Figure 27:The effect of *J. nervosum* on the histology of ethanol-induced gastric mucosal damage in rats.

(G1) (Normal control group); (G2) (Ulcer control group); (G3) (Omeprazole); (G4) (50 mg/kg), (G5) (100 mg/kg), (G6) (200 mg/kg), (G7) (400 mg/kg) *Jasminum nervosum* extract

c) Gold and Silver Nanoparticle (*Helicia robusta* bark extract)

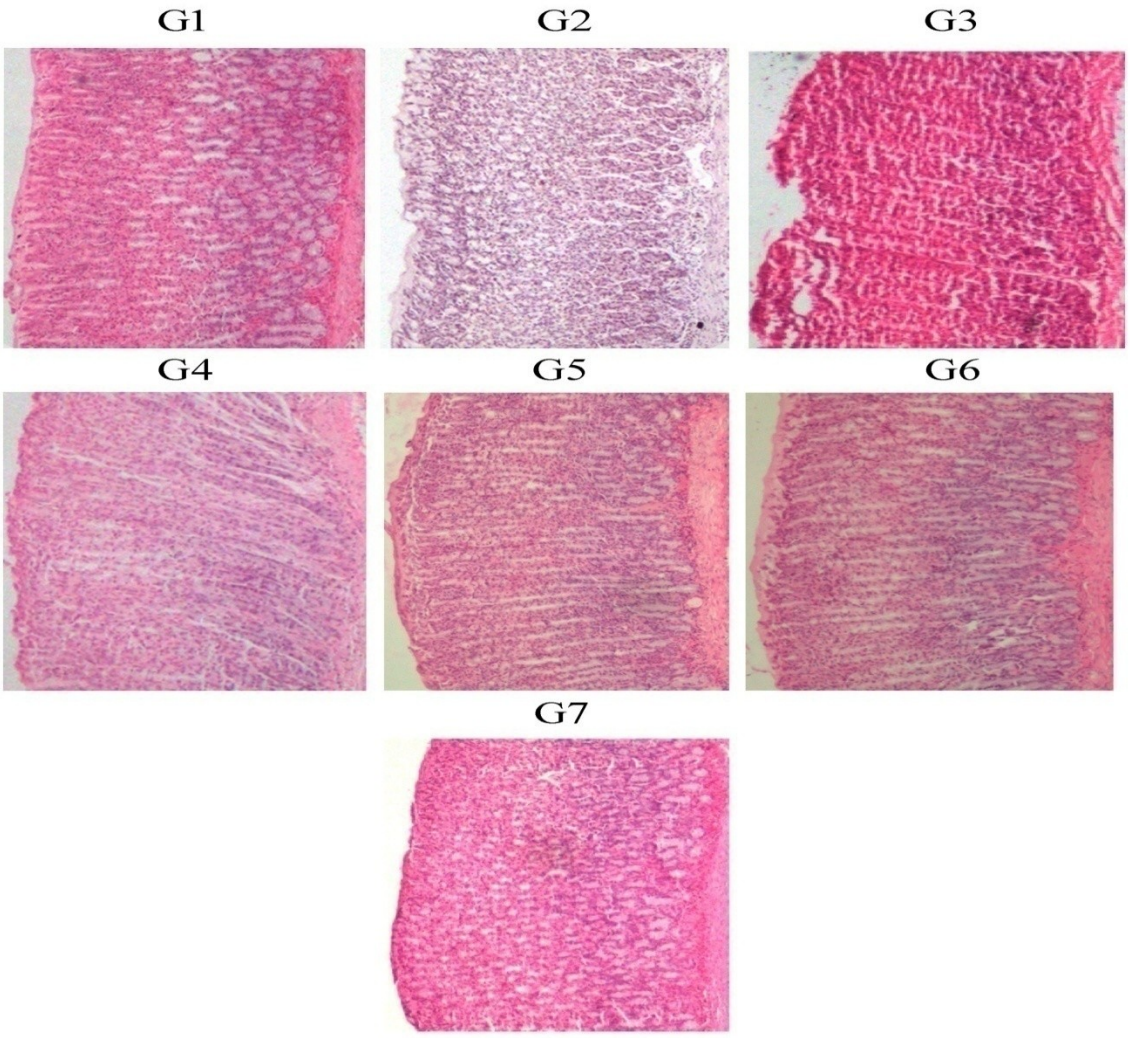


Figure 28: The effect of gold and silver nanoparticle of *H. robusta* on the histology of ethanol-induced gastric mucosal damage in rats.

(G1) (Normal control group); (G2) (Positive control group); (G3) (Negative control group); (G4) (0.09Au mg/kg), (G5) (0.45Au mg/kg), (G6) (0.09Ag mg/kg), (G7) (0.45Ag mg/kg) *Helicia robusta* extract.

d) Gold and Silver Nanoparticle (*Jasminum nervosum* leaf extract)

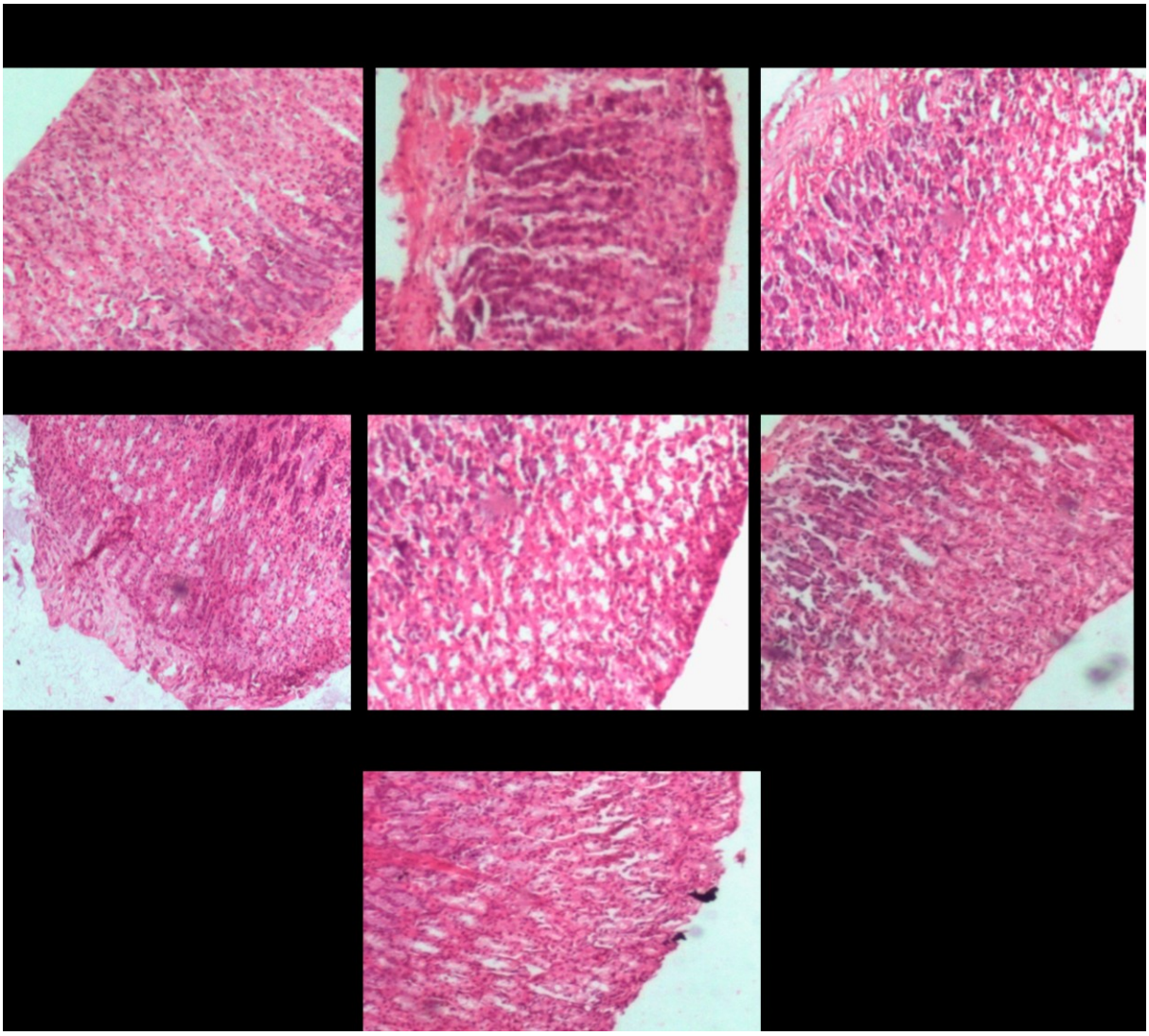


Figure 29: The effect of gold and silver nanoparticle of *J.nervosum* on the histology of ethanol-induced gastric mucosal damage in rats.

(G1) (Normal control group); (G2) (Negative control group); (G3) (Positive control); (G4) (0.09Au mg/kg), (G5) (0.45Au mg/kg), (G6) (0.09Ag mg/kg), (G7) (0.45Ag mg/kg) *Helicia robusta* extract.

CHAPTER 6
DISCUSSION

Helicia robusta (Roxb.) an evergreen tree of sub-tropical forests have been found to be present in countries like Bangladesh, Myanmar, Malaysia, Maghalaya and Andamans. They are found at an elevation of about 1200 m. The young shoots are cooked and eaten as a vegetable, since the wood is heavy and hard it is used in house construction and for the shafts of axes however its medicinal uses have not been reported from these countries. While in Mizoram it is found in areas like Ngopa, Sialsuk, Biate, N.Vanlaiphai forest at an elevation of 700- 1400 m. The local herbal practitioners have been using the bark of this plant as a great traditional values in case for treatment for colic/stomach ulcers, strenghthening the function of uterus and for many other ailments for quite sometime, however the use of its leaves, flowers and fruits are still unknown. The local people named it as Pasaltakaza and according to Lalawmpuii *et al*., 2014 she have identified pasaltakaza as *Helicia nilagirica* which was collected from Southern part of Mizoram. Certain cases occurred depending on the location and the local people that certain local names have been identical while the plant varies. However, since sharing the same local name and same antioxidant assays, the paper was then used for comparing the various results.

The methanolic extract was subjected to phytochemical screening and the presence of steroids, flavonoids, glycosides, and carbohydrates were detected. In our cases,the presence of alkaloids, flavonoids, amino acids, steroids, tannins and carbohydrates were detected. Wide ranges of phytoconstituents are responsible for anti-inflammatory activity including phenolics, alkaloids, and terpenoids . Flavonoids exhibit varied biological activities that include analgesic, anti-

inflammatory, antioxidant, hepatoprotective, and antiulcer activities. The presence of a phytochemical of interest may lead to its further isolation, purification, and characterization. It can then be used as the basis for a new pharmaceutical product. DPPH is a free radical which is stable at room temperature, and this method is often employed to determine the antioxidant activity of many plant extracts. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen-or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore, radical scavengers and *H.nilagirica* exhibited only 3.92 µg/ml while *H.robusta* exhibited 49.4 µg/ml. Then flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are especially distinct for flavonols. The total flavonoid content of *H.nilagirica* was found to be 56 mg QE/g dry weight of the sample while for H.helicia it was found to be 83.46 mg QE/g dry weight of the sample. Then finally, the reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. Fe3+ reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. In this assay, the presence of antioxidant in the extract resulted into reduction of the ferric cyanide complex (Fe3+) to the ferrous cyanide form (Fe2+). Higher is the absorbance of the reaction mixture, higher would be the reducing power. The reducing power of the extract increases as the concentration increases suggesting that some compound in the extract may be able to terminate the radical chain reaction. The reducing power of the extract of *H. nilagirica* was lower when compared to the standard Ascorbic acid. However, the antioxidant present in the extract cause the

reduction of Fe3+ to Fe2+ and thus proved its reducing power. Similarly in case of *H.robusta* the reducing power of the extract was lower in when compared to the standard Ascorbic acid i.e 0.2415 > 0.1175, thus proved its reducing power. Thus, after comparing the two species of *Helicia*, we can conclude that both the plants proved to possess the potent antioxidant substances which may be responsible for its anti-inflammatory, anti-ulcer etc. However, between the two species *H.robusta* proved to have higher antioxidant properties than that of *H.nilagirica*.

On the other hand, *Jasminum nervosum* Lour. is a natural scandent shrub of 1-5 m height, belonging to the Oleaceae family. It is found in the tropical dense evergreen forest of Bhutan, Bangladesh, Myanmar at an elevation of below 2000 m. It has a great medicinal values to the local people of these countries. An infusion of the leaves is administered to women after childbirth. A decoction of the fresh leaves is used to wash wounds and against skin problems. A poultice of the crushed leaves is used to treat ulcers and mastitis . The dried young shoots are used in the treatment of lymphadenopathy, metritis, galactophoritis, leucorrhoea, rheumatism, ostalgia, impetigo, dysmenorrhoea and haematometra. An extract of the pounded root is used to treat quotidian fever. The plant has shown antibiotic activity against Staphylococcus aureus and Streptococcus hemolyticus. Other uses included the stem is used for wickerwork and binding. It is used to make ropes and pot mats. In Mizoram, locally it is named Hruikha and found in the tropical evergreen forest of Mampui forest, Lawngtlai District, Mizoram at an altitude of between 250-1000 m. The local people have been using it as a traditional medicine in certain cases. The leaves and the stems are widely used for various applications in traditional medicine, exhibiting a remedy for diarrhea, malaria, sores and ulearations. The local people took the infusion of the leaves internally for stomachache and diarrhea. It is taken at a tablespoon (10 ml) thrice daily.

Other uses included the making of ropes and mats. Since, no work have been reported from this particular plant, I took up a plant that belongs to the same family named *Jasminum grandiflorum* lour. The leaves of *Jasminum grandiflorum L* is used in folk medicine for treating ulcerative stomatitis, skin diseases, ulcers, wounds etc. According to M. Umamaheswari *et al*., 2006 he studied the antiulcer and in vitro antioxidant activities of *Jasminum grandiflorum.L* where we could compare some of the assays.

Preliminary phytochemical screening of the powdered leaves was performed and shows the presence of phenolics, flavonoids and carotenoids. In our cases, alkaloids, flavanoids, amino acids, tannins and carbohydrates were found to be present. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen-or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore, radical scavengers and *J.grandiflorum* exhibited 19.5 µg/ml while *J.nevosum* exhibited 59.2 µg/ml. The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron. Fe3+ reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action . In this assay, the presence of antioxidant in the extract resulted into reduction of the ferric cyanide complex (Fe3+) to the ferrous cyanide form (Fe2+). Higher is the absorbance of the reaction mixture, higher would be the reducing power. The reducing power of the extract increases as the concentration increases suggesting that some compound in the extract may be able to terminate the radical chain reaction. The reducing power of the extract of *J.grandiflorum* was lower when compared to the standard Butylated hydroxy toluene (BHT). Similarly in case of *J.nervosum* the reducing power of the extract was lower when compared to the standard Ascorbic acid i.e 0.2415

> 0.1143. However, the antioxidant present in the extract cause the reduction of Fe3+ to Fe2+ and thus proved its reducing power.

The phytoconstituents like flavonoids, tannins, terpenoids, and saponin have been reported in several anti-ulcer literatures as possible gastro protective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed (Borelli *et al*., 2000). Flavonoids have been reported to act in the gastrointestinal tract, having antispasmodic,anti-secretory, antidiarrheal,antiulcer, and antioxidant properties. Flavonoids protect the gastric mucosa against a variety of ulcerogenic agents via several mechanisms of action, mainly free-radical scavenging and antioxidant properties, increased mucus production, antisecretory action, and inhibition of the *Helicobacter pylori* growth. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants (Berenguer *et al*., 2005; Nwafor *et al*., 1996). The phytoconstituents found in *H.robusta* and *J.nervosum* were alkaloids, flavonoids, carbohydrates, tannins, amino acids and steroids. These phytoconstituents present in these plants could be the possible agents in the prevention of ulcer development.

For the separation and identification of these valuable compounds, 16 different standards of phenolic compounds were administered through Column Chromatography where only 5 standards showed good peak with retention time namely ferulic acid, lutein, gallic acid, chebulagic acid and catechin. Among these five standards for *H.robusta* bark extract, Ferulic acid (4.9 mg/ml), Gallic acid (9.2 mg/ml) and Lutein (20.1 mg/ml) were found to be present while in *J.nervosum* leaf

extract only Lutein (28.1 mg/ml) was found to be present. Though only few of the standards were found to be present yet it clearly shows that these compounds play a great valuable role in the mechanism of preveting ulcer formation. Therefore, from all the results and information obtained from this study, we can conclude that the selected medicinal plants have a great antioxidant and antiulcer properties which is a great findings potential of using it as a remedy for gastric ulcers.

Using plants in the biosynthesis of AgNPs and AuNPs has gained importance as viable alternative to chemical and physical methods. Bioreduction of metal NPs using a combination of biomolecules found in plant extract is environmentally benign yet chemically complex. Extracts from plants may act as both reducing and capping agents in NPs synthesis (Barman *et al*., 2013). In the present study the AgNPs and AuNPs were synthesized by using aqueous leaf extracts of *J. nervosum* and bark extracts of *H. robusta*. The antiulcer activity of synthesized AgNPs and AuNPs were tested against the Wistar albino rats.

Generally, UV-VIS spectroscopy can be used to examine size and shape of the controlled AgNPs and AuNPs in aqueous suspense (Gnanadesigan *et al*., 2011; Veerakumar *et al*., 2013; Velayutham *et al*., 2013; Soni and Prakash, 2012). UV–Vis spectrograph of the colloidal solution of Ag and Au NPs has been recorded. Absorption spectra of Ag and Au nanoparticles formed in the reaction media have an absorbance peak at 420 and 550 nm, respectively (Naresh Kumar *et al*., 2013; Veerakumar *et al*., 2014). The sharpness in the absorption peak increases with an increase in the concentration of leaves extract of *J. nervosum*, thus being sharper with a higher concentration. AgNPs and AuNPs can be synthesized by reducing Ag^{+} and Au^{+} metal ions using chemicals reductant. In green synthesis, it is believed that natural material extract act as a reducing agent for the generation of metal NPs. The results of the UV-VIS absorbance showed

increasing colour intensity with increased time intervals and this might be due to the production of the AgNPs and AuNPs (Shrivastava and Dash, 2010) and the formation of the brownish yellow colour and pinkish violet colour might be due to the excitation of the surface plasmon vibration of the synthesized AgNPs and AuNPs (Krishnaraj *et al*., 2010).

XRD method can be used to quantify the relative phase abundances and to determine quantitative phase amounts in multiple phase mixtures in AgNPs and AuNPs (Walenta and Fullmann, 2004). In order to verify the result of the UV–Vis analysis, the sample of the Ag^{+} and Au^{+} exposed to the leaf broth of *J. nervosum* was examined by XRD to investigate the composition and phase structure of prepared AgNPs and AuNPs. The broadening of the peaks was observed due to the effect of NPs. The noise observed might be due to the presence of various crystalline biological macromolecules in the aqueous extract of *J. nervosum*. The result showed that the Ag^{+} of silver nitrate and Au^{+} of chloroauric acid had reduced to Ag° and Au° by *J. nervosum*, respectively. The results of (Sathyavathi *et al*., 2010) are in concordance with the results of present study with the diffraction peaks at 38.13◦, 43.92◦ and 64.51◦ corresponding to the (1 1 1), (2 0 0) and (2 2 0) facets of Ag° and 38.17°, 44.36°, 64.65° and 77.59° indexed to the (1 1 1), (2 0 0) (2 2 0) and (3 1 1) facets of Au°. The result of the XRD pattern indicates the presence of sharp bands of Bragg peaks and this might be due to the stabilization of the synthesized AgNPs and AuNPs by the leaf extract of the *J. nervosum* reducing agents, and thus confirming the crystallization of the bioorganic phase occurs on the surface of the silver and gold nanoparticles (Jayaseelan *et al*., 2011).

FTIR can be used to quantitate the components from an unknown mixture (Stuart 2004; Vilchis-Nestora *et al*., 2008). The biological molecules could possibly perform dual functions of

reduction and stabilization of AgNP and AuNP in the aqueous medium and were observed to be stable in solution and showed very little aggregation (Ahmad *et al*., 2010). When metal NPs form in solution, coagulation might occur if they are not stabilized against the van der Waals forces of attraction and at the same time the physisorbed surfactant and polymers may cause steric or electrostatic barriers around the particle surface and may thereby provide stabilization (Mulvaney 1996). A few unassigned peaks were also noticed in the vicinity of the characteristic peaks (Sathishkumar *et al*., 2009). Absence of secondary metabolites was observed in FTIR spectroscopy after the bioreduction of AgNPs and AuNPs. This may be due to the fact that the polyols are mainly responsible for the reduction of Ag^{+} and Au^{+} ions, whereby they themselves get oxidized to unsaturated carbonyl groups leading to a broad peak at 1626.66 cm^{-1} and 1632.45 cm^{-1}, respectively (Jain *et al*., 2009). Earlier authors reported that the biosynthesis of AgNPs using leaf extract of *Acalypha indica* is a source of bioreductant and stabilizers (Krishnaraj *et al*., 2010). FTIR studies and XRD analysis showed the presence of bioorganic components which acted as a probable stabilizer for the synthesized AgNPs (Prathna *et al*., 2010). In the present study, FTIR was also used for quantitative analysis, since the strength of the transmittance is proportional to the concentration. The prominent peaks of the FTIR results are showing the correspond values to the amide group (N-H stretching-3433), aliphatic group (Cyclic CH2 -2922), flavanones (1644), alkene (CC-1632.45 and 615), carbonyl groups (CO-1383), fatty acids (1069) and ether groups (COC-1042). The observed peaks are considered as major functional groups in different chemical classes such as flavonoids, triterpenoids and polyphenols (Asmathunisha *et al*., 2010). Hence, the terpenoids are proved to have good potential activity to convert the aldehyde groups to carboxylic acids in the metal ions. Further, amide groups are also responsible for the presence of the enzymes and these enzymes are responsible for the reduction synthesis and

stabilization of the metal ions, further, polyphenols are also proved to be a potential reducing agent in the synthesis of the silver and gold nanoparticles (Prasad and Elumalai 2011). FTIR peaks from AgNPs synthesized by *Sesuvium portulacastrum* leaf extracts related to aromatic rings, geminal methyls, and ether linkages indicated the presence of flavones and terpenoids, which might be responsible for the stabilization of the nanoparticle (Nabikhan *et al*., 2010). (Kumar and Yadav 2009) and (Kumar *et al*., 2010) have synthesized AgNPs using *Syzygium cumini* leaf and seed extract as reducing and stabilizing agent.

The size and shape of NPs plays an important role in many of biological applications especially in health management program. It has been confirmed that the AgNPs and AuNPs mostly exist in spherical shape which was confirmed by TEM. However, a high concentration of *J. nervosum* extract leads to strong interaction between biomolecules and surfaces of the nanoparticles, preventing nascent AgNPs and AuNPs from rapid sintering. Higher concentration of extract offers to more number of spherical particles with sizes from 4–22 nm with an average size of 10 ± 2 nm for AgNPs and between 2-20 nm with an average size of 9.4 ± 4 nm for AuNPs with various spherical shapes, which falls closer to many of the silver and gold nanoparticles produced by other plant materials (Soni and Prakash, 2014). The shape of plant-mediated AgNPs was mostly spherical with the exception of *Azadirachta indica* which yielded both spherical and flat plate-like morphology (Shankar *et al*., 2004). SEM images of AgNPs from *Emblica officinalis* were also predominantly spherical ranging from 7.5 to 25 nm with an average size of 16.8 nm (Ankamwar *et al*., 2005).

A simple and efficient biological method was developed for the synthesis of AgNPs and AuNPs using *J. nervosum* aqueous extract, which was further used for toxicity studies against the filarial and arboviral vector. In the present study, it is evident that the percentage of mortality of *C.*

quinquefasciatus after the treatment of AgNPs and AuNPs increased as the concentration was increased, and the mortality was high in AgNPs when compared with the later ones. This is in accordance with the report of (Litaiff *et al*., 2008) who showed that the percentage of mortality was increased as the concentration was increased. Low dose biosynthesized AgNPs and AuNPs resulted in high mortality.The study also deals with the larvicidal activity in relation to lethal dose and lethal time of *J. nervosum*. It is widely distributed throughout India and is used as a folk medicine in the treatment of several diseases. Aqueous leaf extract of *J. nervosum* exhibited excellent larvicidal activity on the filarial vector, *C. quinquefasciatus*. In the NPs treatment, the third instar larval stages of the *C. quinquefasciatus* are very much susceptible when compared with that of the aqueous leaf extract treatment.

In conclusion, green synthesis shows that the environmentally safe and renewable source of *J. nervosum* and *H. robusta* can be used as a potent reducing agent for the synthesis of AgNPs and AuNPs. Thus, biological reduction of AgNPs and AuNPs would be a boon for the development of clean, non-toxic, and environmentally acceptable green approach to produce nanoparticles involving plants. This is the first report on the antiulcerogenic activity of synthesized NPs from *J. nervosum* and *H. robusta* . The formed AgNPs and AuNPs are highly stable and have significant antiulcer activity against Wistar albino rats.

Peptic ulcers are caused by an imbalance between the protective and the aggressive mechanisms of the mucosa, and are the result of the association of several endogenous factors and aggressive exogenous factors that are related to living conditions (Demir *et al*., 2003). In the HCl/ethanol-induced gastric ulceration model, HCl causes severe damage to gastric mucosa (Yamahara *et al*., 1988), whereas ethanol produces necrotic lesions by direct necrotizing action which in turn

reduces defensive factors like the secretion of bicarbonate and production of mucus (Marhuenda *et al*., 1993). Ethanol-induced gastric lesions impaired gastric defensive factors such as mucus and mucosa circulation (Ferreira *et al*., 2008). Ethanol causes necrotic lesions of the gastric mucosa in a multifactorial way. It can reach the mucosa by disruption of the mucus-bicarbonate barrier and cause cell rupture in the wall of blood vessels. These effects are probably due to biological actions, such as of lipid peroxidation, formation of free radicals, intracellular oxidative stress, changes in permeability and depolarization of the mitochondrial membrane prior to cell death (Sannomiya *et al*., 2005). Oral administration of absolute ethanol is noxious to the stomach since it affects the gastric mucosa topically by disrupting its barrier and provoking pronounced microvascular changes within a few minutes after its application (Moleiro *et al*., 2009). In addition, it produces linear hemorrhagic lesions, extensive submucosal edema, mucosal friability, inflammatory cells infiltration, and epithelial cell loss in the stomach, which are typical characteristics of alcohol injury (Jelski *et al*., 2009). The pathogenesis of ethanol-induced gastric mucosal damage occurs directly and indirectly through various mediators such lipoxygenase, cytokines, and oxygen-derived free radicals (Abdel-Salam *et al*., 2001). Mucus secretion is regarded as a crucial defensive factor in the protection of the gastric mucosa from gastric lesions (Oluwole *et al*., 2008).

In the present study, acute toxicity test did not show any signs of toxicity and mortality. Behavioural changes like irritation, restlessness, respiratory distress, abnormal locomotion, and catalepsy over a period of 15 days were not observed. Phytotheraputic products from medicinal plants have become universally popular in primary healthcare, particularly in developing countries, and some have been mistakenly regarded as safe just because they are a natural source. Nevertheless, these bioactive products from medicinal plants are presumed to be safe without any

compromising health effect, and thus widely used as self medication (Vaghasiya *et al*., 2011). However, there is a lack of proven scientific studies on the toxicity and adverse effect of these remedies. Therefore, further acute oral toxicity study is vitally needed not only to identify the range of doses that could be used subsequently, but also to reveal the possible clinical signs elicited by the substances under investigation. It is also a useful parameter to investigating the therapeutic index of drugs and xenobiotics (Rang *et al*., 2001). In general *in vivo* toxicity study is the toxicological analysis of many medicinal plants and its potency to evaluate qualitatively and quantitatively by histopathology and oral acute toxicity studies. Oral acute toxicity testing in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose (Sasidharan *et al*., 2008). A toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. Toxicity results from animals will be crucial in definitively judging the safety of medicinal plants if they are found to have sufficient potential for development into pharmacological products (Moshi *et al*., 2011). As use of medicinal plants increases, experimental screening of the toxicity of these plants is crucial to assure the safety and effectiveness of those natural sources. However, acute toxicity studies do not detect effects on vital functions like the cardiovascular, central nervous, and respiratory systems which are not usually assessed during the study and these should be evaluated prior to human exposure (Syahmi *et al*., 2011). Moreover, acute toxicity is mainly to obtain an appropriate dose for long-term toxicity tests and to find out the affected organs at the end of the treatment. The previous study on preliminary toxicity analysis of plant extracts by using brine shrimp lethality test have documented the seeds extract are not toxic and shows that, the extract can further explored for the development of natural product based pharmaceutical products (Lachumy *et al*., 2010). Hence, the present study was particularly

designed to further investigate toxicity of methanolic extracts of *J. nervosum* and *H. robusta* and nanoparticles by using acute oral toxicity analysis.

In this study, the rats in the control and treated groups were administrated with vehicles and methanolic extracts of *J. nervosum* and *H. robusta* and nanoparticles, respectively. The rats were monitored daily until day fourteen for any toxic signs and mortality. The clinical symptom is one of the major important observations to indicate the toxicity effects on organs in the treated groups (Eaton *et al*., 1996). During the 14 days of period acute toxicity evaluation, rats which are orally administrated with methanolic extracts of *J. nervosum* and *H. robusta* and nanoparticles at single dose 400 mg/kg showed no overt signs of distress, and there were no observable symptoms of either toxicity nor deaths. All of the rats gained weight and displayed no significant changes in behavior. Apart from that, the physical appearance features such as skin, fur and eyes were found to be normal and whilst the body weight of the mice showed as increase, this indicates that the administration of the methanolic extracts of *J. nervosum* and *H. robusta* and nanoparticles has negligible level of toxicity on the growth of the animals. Furthermore, determination of food intake and water consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drugs tested (Steven *et al*., 1994; Iversen *et al*., 2003). In this study, the food intake and water consumption also was not affected by the administration of methanolic extract of *J. nervosum* and *H. robusta* and nanoparticles and it did not induce appetite suppression and had no deleterious effects. Thus, this indicates there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen *et al*., 2001).

In the case of *H.robusta,* the experimental rats exhibited maximal protective effect at 400 mg/kg against ethanol-induced gastric ulcers over omeprazole (20 mg/kg) and negative control (N.C).

This is evidenced in the ulcer index (400 mg/kg, UI =72.06; omeprazole = 92.80 and N.C=201.01),with the percentage ulcer preventive index (400 mg/kg CSE=89%; omeprazole =77% and N.C=0%) while the minimal protective effect is at 50 mg/kg with ulcer index of 142.32 and the percentage ulcer protective index of 58%. In the negative control, there were severe and generalized erosions of the gastric epithelium with massive disorientation of the villi and crypts. The damage affected the submucosa and muscularis propria (Fig. 22; Table 12). From the synthesized gold and silver nanoparticle extract of this plant, significant protective effects against ulcers were observed.(Fig. 24; Table 14) The experimental rats showed maximal protective effect at 0.5 mg/kg AuNPs and minimal protective effect at 0.1 mg/kg AuNPs over omeprazole (20 mg/kg) and negative control. This is also evidenced in the ulcer index (0.5 mg/kg AuNPs =122.3; 0.1 mg/kg AuNPs = 154.3; omeprazole =134.2 and N.C= 203.5), with the percentage ulcer preventice index (0.5 AuNPs mg/kg = 76% ; 0.1 mg/kg AuNPs = 64% ; omeprazole= 68% and N.C= 0%). Similarly, in the histological evaluation, Group 7 (400 mg/ml *H.robusta* bark extact) showed mild edema and leucocyte infiltration of the submucosal layer, but no disruption on the epithelium surface and . Group 4 (50 mg/ml *H.robusta* bark extract) exhibited moderate disruption of the surface epithelium, with edema and leucocyte infiltration of the submucosal layer. Omeprazole exhibited a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration, while the histological examination showed extensive damage to the gastric mucosa in Group 3 (negative control) with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer. In the case of nanoparticles also ,we can see that from Fig. 28, Group 2 (negative control) exhibited histological examination showed extensive damage to the gastric mucosa with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of

the submucosal layer Group 3 (positive control or omeprazole) showed a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration and group 5 (.5mg/ml AuNPs of *H.robusta* bark extract) showed a mild disruption of the surface epithelium with edema and leucocyte infiltration into the submucosal layer but very less disruption of the surface epithelium while while the Group 4 (0.1 mg/ml AuNPs of *H.robusta* bark extract) exhibited moderate disruption of the surface epithelium, with edema and leucocyte infiltration of the submucosal layer.

On the other hand, *J.nervosum* leaf extract also proved to have a good protective effect againt ulceration. The experimental rats also exhibited maximal protective effect at 400 mg/kg against ethanol-induced gastric ulcers over omeprazole (20 mg/kg) and negative control (N.C). This is evidenced in the ulcer index (400 mg/kg, UI =129.6 ± 1.84; omeprazole = 139.2 ± 1.92 and N.C = 203.5 ± 1.38),with the percentage ulcer preventive index (400 mg/kg CSE=77%; omeprazole =71% and N.C=0%) while the minimal protective effect is at 50 mg/kg with ulcer index of 178.3 ± 1.53 and the percentage ulcer protective index of 52%. In the negative control, there were severe and generalized erosions of the gastric epithelium with massive disorientation of the villi and crypts (Fig. 23; Table 13). From the synhesized gold and silver nanoparticle extract of this plant, significant protective effects against ulcers were observed (Fig. 25; Table 15). The experimental rats showed maximal protective effect at 0.5 mg/kg AuNPs and minimal protective effect at 0.1 mg/kg AgNPs over omeprazole (20 mg/kg) and negative control. This is also evidenced in the ulcer index (0.5 AuNPs mg/kg = 92.7 ± 1.44 ; 0.5 AgNPs mg/kg = 136.9 ± 1.24 ; omeprazole = 146.2 ± 1.49 and N.C = 203.5 ± 1.72), with the percentage ulcer preventive index (0.5 Au mg/kg = 85% ; 0.5 AgNPs mg/kg = 72 % ; omeprazole = 64% and N.C= 0%). The histological evaluation also clearly shows that from Fig. 27, Group 2 (negative control) exhibited

histological examination showed extensive damage to the gastric mucosa with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer Group 3 (positive control or omeprazole) showed a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration. Group 7 (400mg/ml *J.nervosum leaf extract*) showed mild edema and leucocyte infiltration of the submucosal layer, but no disruption of the surface epithelium while the Group 4 (50 mg/ml *J.nervosum leaf* extract) exhibited moderate disruption of the surface epithelium, with edema and leucocyte infiltration of the submucosal layer. Omeprazole exhibited a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration, while the histological examination showed extensive damage to the gastric mucosa in group 3 (negative control) with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer. In the case of NPs, we can see that from (Fig. 29), Group 2 (negative control) exhibited histological examination showed extensive damage to the gastric mucosa with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer Group 3 (positive control or omeprazole) showed a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration. Group 5 (0.5mg/ml AuNPs of *J.nervosum* leaf extract) showed less disruption of the surface epithelium with edema and leucocyte infiltration into the submucosal layer but very less disruption of the surface epithelium, while Group 7 (0.5mg/ml AgNPs of *J. nervosum* leaf extract) showed mild edema and leucocyte infiltration of the submucosal layer, and less disruption of the surface epithelium.

CHAPTER 7
SUMMARY

Based on the survey and literature, 8different traditional medicinal plants were selected at first for studying the antioxidant potential and anti-ulcer activity. But depending on the availability, location and knowledge of the medicinal uses of the local people only two plants were further selected for this study namly *Helicia robusta (Roxb.)R.Br.ex.Blume* (Pasaltakaza), *Jasminum nervosum* Lour (Hruikha).

The selected medicinal plants were identified and authenticated as per Lalramnghinglova (1998) and Sawmliana (2003).

Helicia robusta belongs to the family Protoceae locally named Pasaltakaza/Sialhma. It is a small to medium-sized evergreen tree of sub-tropical forests and found to be present in Ngopa, Sialsuk, Biate, N.Vanlaiphai forest etc in Mizoram at an altitude of 700-1400 m. It is also found in other countries like Bangladesh, Myanmar, Malaysia,Maghalaya and Andamans.

Jasminum nervosum Lour. belongs to the Oleaceae family locally named Hruikha. It is a natural scandent shrub of 1-5 m height, found in the tropical dense evergreen forest of Mampui village, Lawngtlai District, Mizoram at an altitude of 250-1000 m. It is also found in other countries like Bhutan, Bangladesh, Myanmar.

Gelsemium elegans belongs to Gelsemiaceae family locally named Hnamtur. It is an evergreen shrub found in sub-tropical of a tropical hill forests areas of Biate, Sialhawk, Vantlang

in Mizoram above an altitude of 800 m. It is also found in other countries like South China, Myanmar.

The study involves both in-vitro and in-vivo studies where in-vitro studies includes various techniques like phytochemical screening of phytoconstituents, evaluation of antioxidant activity of the plants by using assays like total β-carotene content, total flavonoid content, DPPH radical scavenging activity and reducing power (iron chelation). The in-vivio studies includes various techniques like collection of albino rats, gastric ulcer Induction by ethanol, study of acute toxicity, measurement of gastric juice acidity, histological examination of the gastric mucosa.

However, Gelsemium elegans popularly known for its poisonous properties was later discarded from the study for precaution and safety because it was too dangerous for humans and the rats without proper safety measures and instruments.

In the in-vitro studies, *H.robusta* bark and *J.nervosum* leaves were first extracted using Soxhlet apparatus where pet ether, chloroform and methanol were used as an extracting agents. After screening of the phytoconstituents, we found out that methanolic extracts of both the plants showed the maximum presence of certain important phytoconstituents like alkaloids, flavonoids, amino acids, steroids, tannins and carbohydrates and was then prepared to be used for all the necessary remaining techniques. Comparing the two plants, *H.robusta* proved to have higher antioxidant properties than *J.nervosum* but we also proved that both the plants have higher antioxidant properties than other plants like *H.niligarica* and *J.grandiflorum* belonging to the same family of each where the some similar techniques were used. Firstly, the β-carotene content was tested where in *H.robusta* it was 2.86 mg/100ml while in *J.nervosum* it was only 1.11

mg/100ml. Secondly, the flavonoid content was tested where in *H.robusta* it was 83.46 mg (QE/g) while in *J.nervosum* it was only 68 mg (QE/g) which is still higher than that of *H.niligarica* (56 QE/g) and *J.grandiflorum* (51 QE/g). Thirdly, the DPPH radical scavenging activity was tested where in *H.robusta* it was 49.4 µg/ml while in *J.nervosum* it was 59.2 µg/ml, and that of *H.niligarica* was 62.3 µg/ml and *J.grandiflorum* was 69.1 µg/ml where in this case the lesser the value the stronger the radicl scavenging activity. Lastly, the reducing power which is often used to evaluate the ability of an antioxidant to donate an electron. The presence of antioxidant in the plants extract resulted into reduction of the ferric cyanide complex (Fe3+) to the ferrous cyanide form (Fe2+). We found that in *H.robusta* it was 0.1175 which is slightly highr than that of *J.nervosum* which is 0.1143.

The phytoconstituents like flavonoids, tannins, terpenoids, and saponin have been reported in several anti-ulcer literatures as possible gastro protective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which anti ulcerogenic efficacy has been extensively confirmed (Borelli *et al*., 2000). Therefore, our findings from the study also let us to summarized that the selected two plants are a great potential candidate for modern drug discovery etc.

While in the in-vivo studies, the fully mature 150 albino rats were used and were divided into 7 groups where each group were carefully given a standard doses of both the plants extract, omeprazole, ethanol etc. Ulcers were induced using ethanol where omeprazole (20mg/kg) was used as a positive control. Then, after administering all the standard doses, we found that *H.robusta* proved to have a better protection against ulcer development in certain doses than that

of *J.nervosum*. For comparing the two plants we took the highest dose and lowest dose which were at 400mg/kg and 50mg/kg respectively. In *H.robusta* the ulcer index at 400mg/kg was 72.06 ± 1.38 ; omeprazole = 92.80 ± 1.72 and negative control = 248.01 ± 1.26, with the percentage ulcer preventive index (400 mg/kg= 92%; omeprazole =81% and negative control = 0% and at 50 mg/kg the ulcer index was 142.32 ± 1.53 and the percentage ulcer index of 58% while in *J.nervosum* the ulcer index at 400 mg/kg was 129.6 ± 1.84; omeprazole = 139.2 ± 1.92 and negative control = 247.5 ± 1.38, with the percentage ulcer preventive index (400 mg/kg = 71%; omeprazole = 63% and negative control = 0% and at 50 mg/kg the ulcer index was 178.32 ± 1.53 and the percentage ulcer index of 52%.

An additional important technique was carried out in our in-vivo study where gold and silver nanoparticles were synthesized from both the plants methanolic extract and were designated as AuNPs and AgNPs. The same procedure was used for two different doses of 0.1mg/kg AuNPs and 0.5 AgNPs for both the plants. Then we found that these nanoparticles also play a significant role in preventing the ulcer development. However, unlike the plants extract, although both the nanoparticles of both plants showed great results, in this case *J.nervosum* nanoparticles showed better protection than *H.robusta.*In *H.robusta* the maximum protective effect was observed at 0.5 mg/kg AuNPs and minimal protective effect at 0.1 mg/kg AuNPs over omeprazole (20 mg/kg) and negative control where this is also evidenced in the ulcer index (0.5 mg/kg AuNPs =122.3 ± 1.28 ; 0.1 mg/kg AuNPs = 143.2 ± 1.48; omeprazole =134.2 ± 1.27 and negative control = 242.5 ± 1.72), with the percentage ulcer preventice index (0.5 AuNPs mg/kg = 76% ; 0.1 mg/kg AuNPs = 64% ; omeprazole= 68% and negative control = 0%). While in *J.nervosum* the maximal protective effect was observed at 0.5 mg/kg AuNPs and minimal

protective effect at 0.1 mg/kg AgNPs over omeprazole (20 mg/kg) and negative control. This is also evidenced in the ulcer index (0.5 AuNPs mg/kg = 92.7 ± 1.44 ; 0.5 AgNPs mg/kg = 127.9 ± 1.24 ; omeprazole = 146.2 ± 1.49 and negative control = 249.5 ± 1.72), with the percentage ulcer preventice index (0.5 Au mg/kg = 83% ; 0.5 AgNPs mg/kg = 73 % ; omeprazole = 56% and negative control = 0%). The histological studies also showed that in *H.robusta* Group 7 (400 mg/ml *H.robusta* bark extact) showed mild edema and leucocyte infiltration of the submucosal layer, but no disruption of the surface epithelium and . Group 4 (50 mg/ml *H.robusta* bark extract) exhibited moderate disruption of the surface epithelium, with edema and leucocyte infiltration of the submucosal layer. Omeprazole exhibited a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration, while the histological examination showed extensive damage to the gastric mucosa in group 3 (negative control) with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer. Similar ly in the case of *J.nervosum* Group 2 (negative control) exhibited histological examination showed extensive damage to the gastric mucosa with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer Group 3 (positive control or omeprazole) showed a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration. Group 7 (400mg/ml *J.nervosum leaf* extract) showed mild edema and leucocyte infiltration of the submucosal layer, but no disruption of the surface epithelium while the Group 4 (50 mg/ml *J.nervosum leaf* extract) exhibited moderate disruption of the surface epithelium, with edema and leucocyte infiltration of the submucosal layer. Omeprazole exhibited a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration, while the histological examination showed extensive damage to the gastric mucosa in group 3 (negative control) with

necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer.

In the case of nanoparticles also in *H.robusta* Group 2 (negative control) exhibited histological examination showed extensive damage to the gastric mucosa with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer Group 3 (positive control or omeprazole) showed a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration and group 5 (0.5mg/ml AuNPs of *H.robusta* bark extract) showed a mild disruption of the surface epithelium with edema and leucocyte infiltration into the submucosal layer but very less disruption of the surface epithelium while while the Group 4 (0.1 mg/ml AuNPs of *H.robusta* bark extract) exhibited moderate disruption of the surface epithelium, with edema and leucocyte infiltration of the submucosal layer. Similarly for *J.nevosum* Group 2 (negative control) exhibited histological examination showed extensive damage to the gastric mucosa with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the submucosal layer Group 3 (positive control or omeprazole) showed a mild disruption of the surface epithelium, with submucosal edema and leucocyte infiltration. Group 5 (0.5mg/ml AuNPs of *J.nervosum* leaf extract) showed less disruption of the surface epithelium with edema and leucocyte infiltration into the submucosal layer but very less disruption of the surface epithelium, while Group 7 (0.5mg/ml AgNPs of *J. nervosum* leaf extract) showed mild edema and leucocyte infiltration of the submucosal layer, and less disruption of the surface epithelium.

Lastly, we undertook a simple chromatogram to identify the possible compounds present in these methanolic plants extract using 16 standards. On doing so, only 5 standards were reported

to show good peak with retention time namely ferulic acid, lutein, gallic acid, chebulagic acid and catechin. Among these five standards for *H.robusta* bark extract, Ferulic acid (4.9 mg/ml), Gallic acid (9.2 mg/ml) and Lutein (20.1 mg/ml) were found to be present while in *J.nervosum* leaf extract only Lutein (28.1 mg/ml) was found to be present. Though only few of the standards were found to be present yet it clearly shows that these compounds play a great valuable role in the mechanism of preveting ulcer formation. Therefore, from all the results and information obtained from this study, we can summarized that the selected medicinal plants have a great antioxidant and antiulcer properties which is a great findings potential of using as a noble candidate for drug discovery like remedy for gastric ulcers etc.

CHAPTER 8
BIBILOGRAPHY

Abdel-Salam O. M. E., Czimmer J., Debreceni A., Szolcsányi J. and Mózsik G. (2001). Gastric mucosal integrity: gastric mucosal blood flow and microcirculation. An overview. Journal of Physiology, Paris, 1–6, 105–127.

Abdulla M.A., Ali H.M., Ahmed KA-A., Noor S.M., Ismail S. (2009). Evaluation of the anti-ulcer activities of *Morus alba* extracts in experimentally-induced gastric ulcer in rats. Biomedical Research-India, 20: 35–39.

Abdulla M. A., Ahmed K. A., Al-Bayaty F. H. and Masood Y. (2010). Gastroprotective effect of *Phyllanthus niruri* leaf extract against ethanol-induced gastric mucosal injury in rats. African Journal of Pharmacy and Pharmacology, 226–230.

Abidi,S.L. (1991). High-performance liquid chromatography of phosphatidic acids and related polar lipids. Journal of Chromatography, 587: 193-203.

Adesokan A.A., Yakubu M.T., Owoyele B.V., Akanji M.A., Soladoye A., Lawal O.K..(2008). Effect of administration of aqueous and ethanolic extracts of *Enantia chlorantha* stem bark on brewer's yeast-induced pyresis in rats. African. Journal of Biochemistry Research, 165-169.

Ahmad N., Malik M.A., Al-Nowaiser F.M., Khan Z. (2010). A kinetic study of silver nanoparticles formation from paracetamol and silver (I) in aqueous and micellar media. Colloids Surface B, 78:109–114.

Akerele O. (1988). Medical Plants and Primary Health Care: An Agenda for Action *Fitoterapia Lix,* 356.

Al-Attar A. M. (2011). Protective effect of *Avicennia alba* leaves extract on gastric mucosal damage induced by ethanol. Research Journal of Medicinal Plant, 477–490.

Ame S.N., Shigrenaga M.K. and Hagen T.M. (1993). Oxidants, antioxidants and degenerative diseases of aging. Proceedings of the National Academy of Science USA, 90: 7915-22.

Ankamwar B., Damle C., Ahmad A., Sastry M. (2005). Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. Journal of Nanoscience nad nanotechnology, 5: 1665–1671.

Anoosh E and Fatemeh S. (2010). Determination of total phenolic and flavonoids contents in methanolic and aqueous extract of *Achillea Millefolium*. Journal of Organic Chemistry. 2010, 81-84.

Anonymous (2007a). Medicinal, Culinary and Aromatic Plants in the near East-Medicine. http://www.faO.Org/dorep/x5402e16.htm.

Aquila S., Giner R.M., Recio M.C., Spegazzini E.D. and Rios J.L. (2009). Anti-inflammatory activity of flavonoids from *Cayaponia tayuya* roots.Journal of Ethnopharmacology,121: 333-337.

Asmathunisha N., Kathiresan K., Anburaj K. (2010). Synthesis of antimicrobial silver nanoparticles by callus leaf extracts from saltmarsh plant *Sesuvium portulacastrum* L. Colloids Surf B Biointeraction, 79:488–493.

Ayerton J. (1981). Assay of ceftazidime in biological fluids using high-pressure liquid chromatography. Journal of Antimicrobial Chemotherapy, 8: 227-231.

Balick J.M and Cox P.A. (1996). Plants, People and Culture: the Science of Ethnobotany. New York: Scientific American Library.

Bardi D. A. A., Sarah Khan M. A., Sabri S. Z. (2011). Anti-ulcerogenic activity of *Typhonium flagelliforme* aqueous leaf extract against ethanol-induced gastric mucosal injury in rats. Scientific Research and Essays, 3232–3239.

Barman G., Maiti S., Laha J.K. (2013). Bio-fabrication of gold nanoparticles using aqueous extract of red tomato and its use as a colorimetric sensor. Nanoscale Research Letters, 8: 181.

Behmer O., Tolosa E., Freitas-Neto A. (1976). Manual para histologia normal e patológica. São Paulo, 239.

Berenguer B., Sanchez L.M., Qulilez A., Lopez barreiro Galvez J., Martin M.J. (2005). Protective and antioxidant effects of *Rhizophora mangle* L. against NSAID-induced gastric lesions. Journal of Ethnopharmacology , 104-200.

Bergh J. J and Breytenbach J. C. (1987). Stability-indicating High-performance Liquid-chromatographic Analysis of *Trimethoprim* in Pharmaceuticals. Journal of Chromatogrphy. 387: 528-531.

Blois M.S. (1958). Antioxidant determination by the use of stable free radicals. Nature, 1199-2000.

Borelli F., Izzo A.A. (2000). The plant kingdom as a source of anti-ulcer remedies. Phytotherapy Research, 14:581–91.

Bounine J.P.,Tardif B.,Beltran P.,Mazzo D.J. (1994). High-performance Liquid- chromatographic Stability-indicating determination of Zopiclone in tablets. Journal of Chromatography, 677: 87-93.

Bowden R.E. and Madsen P.O. (1986). High- pressure liquid chromatographic assay of sulbactam in plasma, urine and tissue. Antimicrob. Agents Chemotherapy, 30: 31-233.

Brooks F. P. (1985). The pathophysiology of peptic ulcer disease. Digestive Diseases and Sciences, 15–29.

Brown J.E. and Rice-Evans C.A. (1998). Luteolin rich artichoke extract protects low-density lipoprotein from oxidation in vitro. Free Radical Research, 29:247-255.

Brzozowski T., Konturek P. C., Konturek S. J. (1998). Involvement of endogenous cholecystokinin and somatostatin in gastroprotection induced by intraduodenal fat. Journal of Clinical Gastroenterology, 125–137.

Brzozowski T., Tarnawski A., Hollander D., Sekhon S., Krause W.J., Gergely H. (2005). Comparison of prostaglandin and cimetidine in protection of isolated gastric glands against indomethacin injury. Jounal of Physiology and Pharmacology, 56: 75-88.

Carvalho A.C.B., Nunes D.S.G., Baratelli T.G., Shuqair N.S.M.S.A.Q., Machado Netto E. (2007). Aspectos da legislação no controle dos medicamentos fitoterápicos. T&C Amazônia , 5:26-32.

Carvalho C.R., Gonçalves V.N., Pereira C.B., Johann S., Galliza I.V., Alves T.M.A. (2007). The diversity, antimicrobial and anticancer activity of endophytic fungi associated with the medicinal plant *Stryphnodendron adstringens* (Mart.) Coville (*Fabaceae*) from the Brazilian savannah. Symbiosis, 57: 95-107.

Chan F.K.L and Graham D.Y. (2004). Review article: prevention of non-steroidal anti-inflammatory drug gastrointestinal complications—review and recommendations based on risk assessment. Alimentary Pharmacology and Therapeutics, 1051–1061.

Chaturvedi A., Kumar M.M., Bhawani G., Chaturvedi H., Kumar M., Goel K.R. (2007). Effect of ethanolic extract of *Eugenia Jambolana* seeds on gastric ulceration and secretion in rats. Indian Journal of Physiology and Pharmacology, 51: 131-140.

Chen H.Y. and Yen G.C. (2005). Antioxidant activity and free-radical scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chemistry, 101: 689-694.

Cowman M.M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Review, 12:564-82.

Debjit B, Chiranjib C, Tripathi K.K, Pankaj, and Sampath Kumar K.P. (2010). Recent trends of treatment and medication peptic ulcerative disorder. International Journal of PharmTech Research, 970–980.

Demir S., Yilmaz M., Köseoğ lu M., Akalin N., Aslan D. and Aydin A. (2003). Role of free radicals in peptic ulcer and gastritis. Turkish Journal of Gastroenterology, vol. 14, no. 1, pp. 39–43, 2003.Dermott J.H. Mc. Journal of American. Pharmacists. Association, 40**:** 785**.**

Di-Matteo V. and Exposito E. (2003). Biochemical and therapeutic effects of antioxidants in the treatement of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Current Neural Disorders, 2: 95-107.

Eaton D.L., Klaassen C.D.(1996). Principles of toxicology. In Casarett and Doull's Toxicology: TheBasic Science of Poisons, 5th ed.; Klaassen, C.D., Ed.; McGraw-Hill: New York, USA, 13.

Editorial committee of the National Chinese Medicine Administrative bureau. (2005). Chinese *Materia medica.* Shanghai Scientific and Technical Education Publishing House, Shanghai.

Elham R., Soheil Z.M., Omer A.H., Mehran F., Maryam H., Khalijah A., Chung L., Mahmood A. and Zahurin M. (2014). Evaluation of acute toxicity and gastroprotective activity of *Curcuma purpurascens* BI. rhizome against ethanol-induced gastric mucosal injury in rats. BMC Complementary and Alternative Medicine, 378.

Fabricant D.S and Farnsworth N.R. (2001). The value of plants used in traditional medicine for drug discovery. Environmental Health Perspective, 109: 69–75.

Ferreira M. D. P., Nishijima C. M., Seito L. N. (2008). Gastroprotective effect of *Cissus sicyoides* (Vitaceae): involvement of microcirculation, endogenous sulfhydryls and nitric oxide. Journal of Ethnopharmacology, 70–174.

Funari C.S. and Ferro V.O. (2005). Ethical use of the Brazilian biodiversity: necessity and opportunity. Revista Brasileira Farmacognosia,15:178-82.

Fredj G., Paillet Aussel M. F., Brouard A., Barreteau H., Divine C., Micaud M. (1986). Determination of sulbactam in biological fluids by high-performance liquid chromatography. Journal of Chromatography, 383: 218-222.

Gerber M., Boutron-Ruault M.C., Hercberg S., Riboli E., Scalbel, A. and Siess M.H. (2002). Food and cancer: state of the art about the protective effect of fruits and vegetables. Bulletin of Cancer, 89: 293-312.

Gil M.I., Ferreres F., Tomas-Barberan F.A . (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. Journal of Agricultural and Food Chemistry, 47:2213-2217.

Glavin G. B and Szabo S. (1992). Experimental gastric mucosal injury: laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. The Journal of Federation of American Societies for Experimental Biology, 825–831.

Gnanadesigan M., Anand M., Ravikumar S., Maruthupandy M., Syed Ali M., Vijayakumar V., Kumaraguru A.K. (2012). Antibacterial potential of biosynthesised silver nanoparticles using *Avicennia marina* mangrove plant. Applied Nanoscience, 2:143-147.

Golbabapour S., Hajrezaie M., Hassandarvish P., Abdul Majid N., Hadi A.H.A. (2013). Acute toxicity and gastroprotective role of *M. Pruriens* in ethanol-induced gastric mucosal injuries in rats. BioMed Research International.

Griffiiths D.W and Moseley G. (1980). The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of rats.Journal of Science Food and Agriculture, 31: 255-259.

Haginaka J., Yasuda H., Uno T., Nkagawa T. (1984). Alkaline degradation and determination by high-performance by high-performance liquid chromatography. Chemical and Pharmaceutical Bulletin, 32: 2752-2758.

Halliwell B., Gutterridge J.M., Cross C.E. (1992). Free radicals, antioxidants and human disease: where are we now? Journal of Laboratory and Clinical Medicine, 119: 598-620.

Halliwell B. and Grootveld, M. (1987). FEBS Letters. 9:213.

Halliwell B., Aeschbach R., Loliger J. and Aruoma O.I. (1995). The characterization of antioxidants. Food and Chemical Toxicology, 33: 601-617.

Halliwell B. (1996) Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radical. Research,. 25: 1-32.

Hara Y. and Honda M. (1992). Inhibition of rat small intestinal sucrose and alpha- glucosidase activities by tea polyphenols.Bioscience Biotechnology and Biochemistry, 57: 123-124.

Harborne,J.B. (1998). Phytochemical methods:A guide to modern technique of plant analysis. Chapman and Hall, London, UK. Journal of Biological Chemistry, 266: 4648-4653.

Hernandez-Munoz R., Montiel-Ruiz C. and Vazquez-Martinez O. (2000). Gastric mucosal cell proliferation in ethanol-induced chronic mucosal injury is related to oxidative stress and lipid peroxidation in rats. Laboratory Investigation, 1161–1169.

Hoogerwerf W. A. and Pasricha P. J. (2001). Agents used for control of gastric acidity and treatment of peptic ulcers and gastro esophageal reflux diseaseedition, McGraw-Hill, New York, USA, 10th edition, 15–19.

Hongxia Yu., Jing C., Cui Y., Shang H., Ding Z., Jin H. (2004). Application of toxicity identification evaluation procedures on wastewaters and sludge from a municipal sewage treatment works with industrial inputs. Ecotoxicology and Environmental Safety, 57: 426-430.

Ingle A., Rai M., Gade A., Bawaskar M.J. (2009). *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles. Journal of Nanoparticle Research, 11:2079–2085.

Iversen P.O., Nicolaysen G. (2003). Water for life. Journal of Norwegian Medical Association, 123: 3402-3405.

Jablonski N.G, Chaplin G. (2004). The evolution of skin coloration. Journal of Human Evolution, 39:57–106.

Jain S.K. (2009). Medicinal plant India, Land and Land People, National Book Trust of India, 1-3.

Jain S.K. (1968). Medicinal Plants.National Book Trust, India.

Jayaprakash G.K and Rao L. J. (2000). Phenolic constituents from lichen *Parmontrema stuppeum*. Food Control, 56: 1018-1022.

Jayaseelan C., Marimuthu S., Rahuman A.A., Rajakumar G., Santhoshkumar T., Kirthi A.V. (2011). Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Research, 108:1541–1549.

Jin W and Jan G.F. (2006). Biological activities of iridoids, Herald of Medicine., 25: 530–533.

Jelski W., Kozlowski M., Laudanski J., Niklinski J. and Szmitkowski M. (2009). The activity of class I, II, III, and IV Alcohol Dehydrogenase (ADH) Isoenzymes and Aldehyde Dehydrogenase (ALDH) in esophageal cancer. Digestive Diseases and Sciences, 725–730.

John Africa L.B ., Yahaya T.A ., Isimi C.Y. (2014). Anti-ulcer and wound healing activities of *Sida corymbosa* in rats. Afican Journal of Tradit Complement Alternative Medicine, 11: 87-92.

Kahkonen M.P., Hopia A.I., Vuorela H.J., Rauha J.P., Pihlaja K., Kujala T.S., Heinonen M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry. 47:3954-3962.

Kala C.P. (2000). Status and conservation of rare and endangerred medicinal plants in the Indian trans-Himalaya. Biological Conservation, 93:371-379.

Kala C.P. (2005). Indigenous uses, population density and conservation of threatened medicinal plants in protected areas of the Indian Himalayas. Biological Conservation,19:368-378.

Kala C.P. (2005). Current status of medicinal plants used by traditional Vaidyas in Uttaranchal state of India. Ethnol Research and. Application, 3:267-278.

Kala C.P. (2006). Medicinal plants of the high altitude cold desert in India: Diversity, distribution and traditional uses. International Journal of Biodiversity Science and Management, 2:1–14.

Kala C.P., Dhyani P.P., Sajwan B.S. (2006). Developing the medicinal plants sector in northern India: challenges and opportunities. Journal of Ethnobiology and Ethnomedicine.

Karuppusamy S. (2009). A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. Journal of Medicinal Plants Research., 3: 1222-1239.

Kato S., Kawase T., Alderman J., Inatomi N. and Lieber C. S. (1990). Role of xanthine oxidase in ethanol-induced lipid peroxidation in rats, Gastroenterology, 203–210.

Kavitha., Bommana., Yasodamma., Nimmanapalli., Alekhya Cheruku. (2014). In-vitro anti-bacterial and phytochemical studies of “Pterolobium hexapetalum (Roth.) Sant. and Wagh.” A Medicinal Plant of Nallamalai Hills, Andhra Pradesh. Journal of Pharmacy Research, 5: 2750-2757.

Kim J.W. (2008). NSAID-induced gastroenteropathy. Korean Journal of Gastroenterology, 52: 134-141.

Klaassen C.D. (2001). Principles of Toxicology. In Casarett and Doull's Toxicology: The Basic Science of Poisons, 5th ed.; McGraw-Hill: New York, USA, 13.

Kris-Etherton P.M., Hecker K.D., Bonanome A., Coval S.M., Binkosi A.E., Hilpert K.F. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine, 113: 71-88.

Krishnaraj C., Jagan E.G., Rajasekar S., Selvakumar P., Kalaichelvan P.T., Mohan N. (2010). Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces,76:50-56.

Ku C. S, Mun S. P. (2007). Antioxidant activities of ethanol extracts from seeds in fresh *Bokbunja* (Rubus coreanus Miq.) and wine processing waste. Bioresource Technology, 99: 2852–2856.

Kumar V, Yadav S.K. (2009). Plant-mediated synthesis of silver and gold nanoparticles and their applications. Journal of Chemical Technology and Biotechnology, 84:151–7.

Kumar V., Yadav S.C., Yadav S.K. (2010). *Syzygium cumini* leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization. Journal of Chemical Technology and Biotechnology.

Kwok Y. C., Hsieh D.P. H., Wong P. K. (2006). Toxicity identification evaluation (TIE) of pore water of contaminated marine sediments collected from Hong Kong waters. Marine Pollution Bulletin, 51: 1085-1091.

Lachumy S.J.T., Zuraini Z., Sasidharan S. (2010). Antimicrobial activity and toxicity of methanol extract of *Cassia fistula* seeds. Research Journal of Pharmaceuical, Biological and Chemical Science, 1: 391-398.

Lai P. K. and Roy J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. Current Medical Chemistry. 11: 1451-1460.

Lalawmpuii P.C., Malsawmtluangi C., Zothanpuia,, Kakoti B.B. (2014). Phytochemical screening and determination of antioxidant activity of *Helicia nilagirica* bedd., an Ethnomedicinal plant of Mizoram, INDIA .Asian Journal of Pharmaceutical and Clinical Research.

Lalramnghinglova, H. (2003). Ethno-medicinal plants of Mizoram. Bishen Singh Mahendra Pal Singh, Dehra Dun, 333.

Lalramnghinglova and Sha L.K. (1998). Ethnobotanical plants among the hill tribes of Mizoram In prospects of medicinal plants (Eds. P.L.Gautam, R.Rains, Umesh Srivastava, S.P. Raychaudhury and B.B.Singh, Indian Society of Plant Genetic Resources ,New Delhi, 67-86.

Lau J. Y., Sung J., Hill C., Henderson C., Howden C. W. and Metz D. C. (2011). Systematic review of the epidemiology of complicated peptic ulcer disease: incidence, recurrence, risk factors and mortality. Digestion, 102–113.

Lauback R. G., Rice J. J., Bleiberg B., Muhammad N., Hanna S. A. (1984). Specific high-performance liquid-chromato- graphic determination of ampicillin in bulks, injectables, capsules and oral suspensions by reversed-phase ion-pair chromatography. Journal of Liquid Chromatography, 7: 1243-1265.

Li H, Hao Z, Wang X, Huang L, Li J. (2009). Antioxidant activities of extracts and fractions from *Lysimachia foenum-graecum* hance. Bioresource Technology, 100: 970–974.

Li H., Song F., Ren-You Gan., Zhang R., Qin X. and Kuang L. (2010). Total phenolic contents and antioxidant capacities of selected Chinese medicinal plants. International Journal of Moleculer Science, 11: 2362-2372**.**

Li X. Q., Andersson T. B., Ahlström M. and Weidolf L. (2004). Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. Drug Metabolism and Disposition, 821–827.

Litaiff E.C., Tadei W.P., Porto J.I.R. and Oliveira I.M.A. (2008). Analysis of toxicity on Bacillus sphaericus from amazonian soils to Anopheles darlingi and Culex quinquefasciatus larvae. Acta Amazonica, 38: 255-262.

Liu J., Andya J. D., Shire S. J. A. (2006). Critical review of analytical ultracentrifugation and field flow fractionation methods for measuring protein aggregation. Journal of the American Association of Pharmaceutical Scientists, 8:580–589.

Liu Y., Lee M.L. (2006). Ultrahigh pressure liquid chromatography using elevated temperature. Journal of Chromatography, 1104:198–202.

Lucy H and Edgar J.D. (1999). Medicinal Plants: A reemerging Health aid. Electronic Journal of Biotechnology, 2:1-15.

Luximon-Ramma A., Bahorun T., Soobratte M..A. and Aruoma O.I. (2002). Antioxidant activities of phenolic, pro anthocyanidins, and flavonoids components in extracts of *Cassia fistula*, Journal of Agriculture and Food Chemistry, 50: 5042-5047.

Maciel M.A.M., Pinto A.C., Veiga Jr. V.F., Grynberg N.F., Echevarria. (2002). A Medicinal plants: the need for multidisciplinary scientific studies. Quim Nova, 25:429-38.

McDaugall G.J. and Stewart D. (2005). The inhibitory effect of berry polyphenols on digestive enzymes. Biofactors, 23:189-195.

MacNeil L., Rice J. J., Muhammad N., Lauback R. G. (1986). Stability-indicating Liquid-chromatographic Determination of Cefapirin, Desacetylcefapirin and Cefapirin Lactone in Sodium Cefapirin Bulk and Injectable Formulations. Journal of Chromatography, 361: 285-290.

Mahesh B. and Satish S. (2008). World Journal of Agricultural Sciences, 4: 839 – 843.

Maheshwari J.K.., Singh K.K and Saha S. (1986). Ethanobotany of tribals of Mirzapur District, Uttar Pradesh, Economic Botany information Service, NBRI, Lucknow.

Mahmood A. A., Sidik K. and Fouad H. M. (2007). Prevention of ethanol-induced gastric mucosal injury by *Ocimum basilicum* seed extract in rats. Academy of Scinces Malaysia Science Journal, 1–6.

Mahmood A. A., Mariod A. A., Al-Bayaty F. and Abdel-Wahab S. I. (2010). Anti-ulcerogenic activity of *Gynura procumbens* leaf extract against experimentally-induced gastric lesions in rats. Journal of Medicinal Plant Research, 685–691.

Malyshenko O.S., Beloborodova E.I., Vavilov A.M., Lomivorotova G.V. and Kasperskaia V.I. (2005). Impact of age and type of behavior on the course of ulcer disease. Terapevticheskiĭ arkhiv, 77: 28-31.

Marhuenda E., Martin M. J. and Alarcon De La Lastra C. (1993). Antiulcerogenic activity of aescine in different experimental models. Phytotherapy Research, 13–16.

Marotta F., Tajiri H., Safran P., Fesce E., and Ideo G. (1999). Ethanol-related gastric mucosal damage: evidence of a free radical-mediated mechanism and beneficial effect of oral supplementation with Bionormalizer, a novel natural antioxidant. Digestion, 538–543.

Martin M., Guiochon, G. (2005). Effects of high pressures in liquid chromatography. Journal of Chromatography,7: 16-38.

Massignani J. J., Lemos M., Maistro E. L. (2009). Antiulcerogenic activity of the essential oil of *Baccharis dracunculifolia* on different experimental models in rats. Phytotherapy Research, 1355–1360.

Matsui T., Ueda T., Oki T., Sugita K., Terahara N. and Matsumoto K. (2001). Alpha-glucosidase inhibitory action of natural acylated anthosyanins. 1. Survey of atural pigments with potent inhibitory activity. Journal of Agriculture and Food Chemistry, 49: 1948-1951.

McDermott J.H. (2000). Antioxidant nutrients: current dietary recommendations and research update. Journal of American Pharmceutical Association, 40:785–799.

Moghadamtousi S. Z., Kamarudin M.N., Chan C.K., Goh B.H., Kadir H.A. (2014). Phytochemistry and biology of *Loranthus parasiticus* Merr, a commonly used herbal medicine. American Journal of Chinese Medicine, 42:23–35.

Mohammad A.E., Seyed F.N., Seyed M.N., Bahman E. (2010). Antihypoxic and antioxidant activity of *Hibiscus esculentus* sedes. Grasas y Aceites, 61: 30–36.

Moleiro F. C., Andreo M. A., Santos C. D. (2009). Mouriri elliptica: validation of gastroprotective, healing and anti-*Helicobacter pylori* effects. Journal of Ethnopharmacology, 359–368.

Moshi M.J. (2011). Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. African Jounal of Traditional, Complement and Alternative Medicine, 4: 219-225.

Mukunthan K.S., Elumalai E.K., Patel T.N. and Murty V.R. (2011). *Catharanthus roseus*: A natural source for the synthesis of silver nanoparticles. Asian Pacific Journal of Tropical Biomedicine, 1: 270-274.

Mulvaney P. (1996). Surface plasmon spectroscopy of nanosized metal particles.Langmiur, 12:788–800.

Nabikhan A., Kandasamy K., Raj A., Alikunhi N.M. (2010). Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* L. Colloids and Surfaces B Biointerfaces, 79: 488–93.

Nagata M.and Yamashita I.(1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaish, 39: 925–928.

Naresh kumar A., Jeyalalitha T., Murugan K., Madhiyazhagan P. (2013). Bioefficacy of plant-mediated gold nanoparticles and Anthocepholus cadamba on filarial vector, Culex quinquefasciatus (Insecta: Diptera: Culicidae). Parasitology Research, 112: 1053-1063.

Nascimento G.G.F., Locatelli J., Freitas P.C. and Silva G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian Journal fo Microbiology, 31: 247-256.

Nautiyal S., Kumar R and Husan. (2002). A status of medicinal plants in India:Some latest issues, Annals of Medicine, 10:181-190.

Niero R. (2010). Fitofármacos e fitoterápicos:abordagem econômica e de mercado. In: Bresolin TMB, Cechinel Filho V, editors. Fármacos e medicamentos. Uma abordagem multidisciplinar. São Paulo: Editora Santos, 1-15.

Nordmann R. (1994). Alcohol and antioxidant systems. Alcohol and Alcoholism, 513–522.

Nwafor P.A., Effrain K.D., Jack T.W. (1996). Gastroprotective effects of aqueous extract of *Khaya senegalensis* bark on indomethacin-induced ulceration in rats. West African Journal of Pharmacology and Drug Research,12: 46-50.

Oates P. J. and Hakkinen J. P. (1988.). Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroenterology, 94: 10–21.

Ode O. J. and Asuzu O. V. (2011). Investigation of *Cassia singueana* leaf extract for antiulcer effects using ethanol-induced gastric ulcer model in rats. International Journal of Plant, Animal and Environmental Sciences, 1: 1–7.

Okigbo R.N., Eme U.E., Ogbogu S. (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnology and Molecular. Biology Review, 3: 127-134.

Oluwole F. S., Ayo J. A., Omolaso B. O., Emikpe B. O. and Adesanwo J. K. (2008). Methanolic extract of *Tetracera potatoria*, an antiulcer agent increases gastric mucus secretion and endogenous antioxidants. Nigerian Journal of Physiological Sciences, 23: 79–83.

Oszoy N., Can A., Yanardag R., Akev N. (2008). Antioxidant activity of *Smilax excels* L leaf extracts. Food chemistry, 110: 571-583.

Othman A., Ismail A., Ghani A.N., Adenan I. (2007). Antioxidant capacity and phenolic content of cocoa beans. Food Chemistry, 100:1523–1530.

Owolabi J., Omogbai E. K. and Obasuyi O. (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelie africana*. (Bignoniaceae/0 Stem bark. African Journalof Biotechnology, 6: 882-85.

Oyaizu M . (1986). Studies on products of browning reaction prepared from glucoseamine. Japan Journal of Nutrition, 44:307-314.

Paganga G., Miller N., Rice-Evans C.A. (1999). The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute?. Free Radical. Research, 30: 153-162.

Parashar V., Parashar R., Sharma B., Pandey A.C. (2009). *Parthenium* leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. Digest Journal of Nanomaterials and Biostructures, 4: 45–50.

Pauli G.F., Jaki B.U., Lankin D.C. (2005). Quantitative H NMR: development and potential of a method for natural products analysis. Journal of Natural Products, 68: 133–149.

Prasad, T.N.V.K.V. and Elumalai E.K. (2011). Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine, 1: 439-442.

Prathna T.C., Lazar Mathew N., Chandrasekaran., Ashok M., Raichur and Amitava Mukherjee. (2010). Biomimetic Synthesis of Nanoparticles: Science, Technology & Applicability Biomimet. Lear. Nature, 25-40.

Rang, H.P.; Dale, M.; Ritter, J. (2001). Pharmacology, 4th ed.; Churchill Livingstone: New York,USA, 13.

Rashdi A.S., Salama S.M., Alkiyumi S.S., Abdulla M.A., Hadi A.H.A. (2012). Mechanisms of gastroprotective effects of ethanolic leaf extract of *Jasminum sambac* against HCl/Ethanol-induced gastric mucosal injury in rats. Evidence-Based Complementary and Alternative Medicine.

Repetto M. G and Llesuy S. F. (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazilian Journal of Medical and Biological Research, 523–534.

Rodenas V., Garcia M.S., Sanchez-Pedreno C., Albero M.I. (1997). Flow-injection spectrophotometric determination of frusemide or sulphathiazole in pharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis, 15: 1687-1693.

Sannomiya M., Fonseca V. B., Da Silva M. A. (2005). Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. Journal of Ethnopharmaclogy, 97: 1–6.

Sasaki Y.F., Kawaguchi S., Kamaya A., Ohshita M., Kabasawa K., Iwama K., Taniguchi K., Tsuda S. (2002). The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mutation Research/ Genetic Toxicology and Environmental Mutagenesis, 519: 103–119.

Sasidharan S., Darah I., Jain K. (2008). In vivo and in vitro toxicity study of *Gracilaria changii*. Pharmaceutical.Biology, 46: 413-417.

Sasmal D., Das S and Basu S.P. (2007). Phytoconstituents and therapeutic potential of *Nyctanthes arbortristis* Linn. Pharmacological Review, 1: 344-49.

Sathish kumar P., Paulsamy S., Ananda kumar A.M. and Senthil kumar P. (2009). Effect of habitat variation on the content of certain secondary metabolites of medicinal importance in the leaves of the plant, *Acacia caesia* Willd. Journal of Advance Plant Science, 22: 451-453.

Sathish R., Sahu A. and Natarajan K. (2011). Antiulcer and antioxidant activity of ethanolic extract of *Passiflora foetida* L. Indian Journal of Pharmacology, 43: 336–339.

Sathyavathi R., Krishna M.B.M., Rao S.V., Saritha R., Rao D.N. (2010). Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. Advanced Science Letters, 3 : 1–6.

Sawmliana, M. (2003). The Book of Mizoram Plants, Lois Bet, Chanmari, Aizawl.

Sener G., Paskaloglu K.. and Ayanoglu-Dülger G. (2004). Protective effect of increasing doses of famotidine, omeprazole, lansoprazole, and melatonin against ethanol-induced gastric damage in rats. Indian Journal of Pharmacology, 36: 171–174.

Serafini M., Bellocco R. and Ekstrom A. M. (2002). Total antioxidant potential of fruit and vegetables and risk of gastric cancer.Gastroenterology, 123: 985-991.

Shah A.J., Adlard M.W., Stride J.D. (1990). A sensitive assay for clavulanic acid and sulbactam in biological fluids by high-performance liquid chromatography and pre-column derivatization. Journal of Pharmaceutical and Biomedical. Analysis, 5: 437- 443.

Shankar S.S., Rai A., Ahmad A., Sastry M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using *Azadirachta indica* (Neem) leaf broth. Journal of Colloid Interface Science, 275: 496-502.

Shay H. (1945). A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology, 4: 43–61.

Shoji H., Yamashiro Y., Koletzko B. (2008). Oxidative stress and antioxidants in the perinatal period, In: Oxidative Stress and Inflammatory Mechanisms in Obesity, Diabetes, and the Metabolic Syndrome, L. Packer, H. SIES, eds., CRC Press Taylor & Francis Group, Boca Raton, Florida, 72.

Sies H. (1993). Strategies of antioxidant defence. European Journal of Biochemistry, 215:213-219.

Soares J.R., Dinis T.C.P., Cunha A.P. and Almeida L.M. (1997). Antioxidant Activities of some Extracts of *Thymus zygis*. Free Radical Research, 26: 469-478.

Soni N., Prakash S. (2012). Fungal-mediated nano silver: an effective adulticide against mosquito. Parasitology Research, 111: 2091-2098.

Soni N., Prakash S. (2014). Efficacy of Fungus Mediated Silver and Gold Nanoparticles against Aedes aegypti Larvae. Parasitol Research, 110:175-184.

Srivastava J., Lambert J. and Vietmeyer N. (1996). Medicinal plants: An expanding role in development. World Bank Technical , 320.

Shrivastava S., Dash D. (2010). Label-free colorimetric estimation of proteins using nanoparticles of silver. Nano-Micro Letters, 2: 164-168.

Steven K.R., Mylecrdfaine L. (1994). Issues in Chronic Toxicology. In Principles and Methods of Toxicology, 3rd ed.; Hayes, A.W., Editor.; Ravan Press: New York, USA, 673.

Stuart B. (2004). Infrared spectroscopy fundamentals and applications; J. Wiley: Chichester, England Hoboken, New Jersey.

Stubbs C., Kanfer I. (1990). Stability-indi- cating High-performance Liquid-chromato- graphic Assay of Erythromycin Estolate in Pharmaceutical Dosage Forms. International Journal of Pharmaceutics, 3: 113-119.

Sweeney A.P., Wyllie S.G., Shalliker R.A. and Markham J.L. (2001). Xanthine oxidase inhibitory activity of selected Australian native plants. Journal of Ethnopharmacology, 75: 273-277.

Syahmi A.R.M., Vijayarathna S., Sasidharan S., Yoga Latha L., Kwan Y.P., Lau Y.L., Shin L.N., Chen Y. (2010). Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis* Jacq., (OilPalm leaf) methanol extract. Molecules, 15: 8111-8121.

Tandon S. and Rane S. (2008). Decoction and hot continuous extraction techniques. In: Handa SS, Khanuja SPS, Longo G, Rakesh DD, editors. Extraction technologies for medicinal and aromatic plants. Italy: International. Centre for Science and High Technology, 93–106.

Tapsell L.C., Cobiac L., Sullivan D.R., Fenech M., Patch C.S., Roodenrys S., Keogh J.B., Clifton P.M., Williams P.G., Fazio V.A. and Inge K.E. (2006). Health benefits of herbs and spices: the past, the present, the future. Medical Journalof Australia, 185:1-24.

Tortora G.J. and Grabowski S.R. (2003). Principles of Anatomy and Physiology. 10th ed. John Wiley & Sons, Inc, New Jersey, 889.

Tseng T.H., Kao E.S., Chu C.Y., Chou F.P., Wu H.M.L. and Wang C.J. (1997). Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. Food Chemistry and Toxicology, 35: 1159-1164.

UNESCO. (1996). Culture and Health, Orientation texts- World Decade for Cultural development Documents CLT/DEC. PRO-1996, Paris, France, 29.

UNESCO. (1998). Terminal Report: Promotion of Ethnobotany and the sustainable use of Plant Resources in Africa, 60.

Van Wyk B.V., Gericke N. and Van Oudtshoorn B. (2000). Medicinal plants of South Africa. Briza Publications. Pretoria, South Africa, 274.

Vaghasiya Y.K., Shukla V.J., Chanda S.V. (2011). Acute oral toxicity study of *Pluchea arguta boiss* extract in mice. Journal of Pharmacology and Toxicology, 6: 113-123.

Veerakumar K., Govindarajan M., and Rajeswary M. (2013). Green synthesis of Silver naboparticles using *Sida acuta* (Malvaceae) leaf extract against Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti (Diptera:Culicidae).Parasitol Research, 112: 4073- 4085.

Veerakumar K., Govindarajan M., Rajeswary M., Muthukumaran U. (2014a). Low-cost and eco-friendly green synthesis of silver nanoparticles using *Feronia elephantum* (Rutaceae) against Culex quinquefasciatus, Anopheles stephensi, and Aedes aegypti (Diptera: Culicidae). Parasitol Research, 113: 1775-85.

Veerakumar K., Govindarajan M., Rajeswary M., Muthukumaram U. (2014b). Mosquito larvicidal properties of silver nanoparticles synthesized using *Heliotropium indicum* (Boraginaceae) against Aedes aegypti,Anopheles stephensi and culex quinquifasciatus (Diptera:Culicidae). Parasitol Reserch, 113: 2663-2673.

Velayutham K., Rahuman A.A., Rajakumar G,, Roopan S.M., Elango G., Kamaraj C., Marimuthu S., Santhoshkumar T., Iyappan M., Siva C. (2013). Larvicidal activity of green synthesized silver nanoparticles using bark aqueous extract of *Ficus racemosa* against Culex quinquefasciatu*s* and Culex gelidu*s*. Asian Pacific Journal of Tropical Medicine, 6 : 95–101.

Vilchis-Nestor A. R. (2008). Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. Materials Letters, 62: 3103–3105.

Vinson J.A., Jang J., Dabbagh Y.A., Serry M.M., Cai S. (1995). Plant polyphenols exhibit lipoprotein-bond antioxidant activity using an in vitro oxidation model for heart disease. Journal of Agricultural and Food Chemistry, 43:2798-2799.

Vyawahare N. S., Deshmukh V. V., Godkari M. R., and Kagathara V. G. (2009). Plants with anti-ulcer activity. Pharmacognosy Review, 3: 108–115.

Walenta G., Fullmann T. (2004). Advances in quantitative XRD analysis for clinker, cements, and cementitious additions. International Centre for Diffraction Data Advance X-ray Analysis, 47: 287- 296.

Walum, E., Nilsson, M., Clemedson, C., Ekwall, B. (1995). The MEIC program and its implications forthe prediction of acute human systemic toxicity. In Alternative Methods in Toxicology and the Life Sciences; New York, USA, 11: 275-282.

WHO. (1977). Resolution –Promotion and Development of Training and Research in Traditional Medicine. WHO document number, 30-49.

WHO. (2001). Legal Status of Traditional Medicine and Complementary/ Alternative medicine: A world wide review, WHO Publishing 1.

Wink M., Alfermann A.W., Franke R., Wetterauer B. and Distl M. (2005). Sustainable bioproduction of phytochemicals by plant In vitro cultures: Anticancer agents. Plant Genetic Resource Character Utility, 3: 90-100.

Wiklund A E., Dag B., Brita S. (2005). Toxicity evaluation by using intact sedi-ments and sedi-ment extracts. Marine Pollution Bulletin, 50: 660-667.

Yamahara J., Mochizuki M., Rong H. Q., Matsuda H. and Fujimura H. (1988). The anti-ulcer effect in rats of ginger constituents. Journal of Ethnopharmacology, 23: 299–304.

Zapata-Colindres J. C., Zepeda-Gómez S., Montaño-Loza A., Vásquez-Ballesteros E., De Jesús Villalobos J. and Valdovinos-Andraca F. (2006). The association of *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs in peptic ulcer disease," Canadian Journal of Gastroenterology, 20: 277–280.

Zhang, W.M., Li, B., Han, L., Zhang, H.D. (2009). Antioxidant activities of extracts from areca (*Areca catectu* L.) flower, husk and seed. African Journal of Biotechnology, 8: 3887-3892.

Zhang X., Yan S., Tyagi R.D., and Surampalli R.Y. (2011). Synthesis of nanoparticles by microorganisms and their application in enhancing microbiological reaction rates. Chemosphere, 82: 489–494.

PUBLISHED AND COMMUNICATED PAPERS

- H. Lallawmawma, G. Sathishkumar, S. Sarathbabu, Souvik Ghatak, S. Sivaramakrishnan, G. Gurusubramanian and N. Senthil Kumar (2015). Synthesis of silver and gold nanoparticles using *Jasminum nervosum* leaf extract and its larvicidal activity against filarial and arboviral vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Environmental Science and Pollution Research, 22:17753–17768.

- H. Lallawmawma, H. Lalhlenmawia, G. Gurusubramanian and N. Senthil Kumar (2015). In-vitro evaluation of antioxidant potential and HPLC analysis of methanolic extract of *Helicia robusta.* bark and *Jasminum nervosum.* leaves. Industrial Crops and Products (Communicated).

- H. Lallawmawma, G. Jeeva, Bavithran Moorthy, Vikas Kumar Roy, H. Lalhlenmawia, G. Gurusubramanian and N. Senthil Kumar (2015). Investigation of anti-ulcer activity of the crude extract and gold and silver nanoparticle of *Helicia robusta* and *Jasminum nervosum* using Wister strain Albino rats. PLoS ONE. (Communicated).