In vitro antioxidative and antimicrobial activity of silver nanoparticles synthesized from *Glycyrrhiza glabra* root extract

By

ISWARYA, A.

(16PBC004)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore-641043

In partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN BIOCHEMISTRY

April 2018

In vitro antioxidative and antimicrobial activity of silver nanoparticles synthesized from *Glycyrrhiza glabra* root extract

By

ISWARYA, A.

(16PBC004)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women

Coimbatore-641043

In partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN BIOCHEMISTRY

April 2018

10/4/18

Signature of the
Head of the Department

Signature of the Guide

ACKNOWLEDGEMENT

*It is with the choice of blessings and the divine grace of **LORD ALMIGHTY** that any human endeavour is achieved. In addition to the will of supreme divinity, the willingness of many subject experts and erudite scholars to extend their assistance and help for the completion of a work plays, indeed a vitally important role.*

*I take the opportunity of expressing my sincere thanks to **Dr**. **(Thiru) P.R. Krishna Kumar**, chancellor, Avinashilingam Institute for Home Science and Higher Education for women, Coimbatore, for providing the opportunity and infrastructure to undertake this investigation.*

*I immensely thank **Dr. Premavathy Vijayan**, Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing the entire facilities essential to carry out and complete the study.*

*I record my sincere thanks to **Dr. S. Kousalya**, Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for timely help rendered to carry out the work.*

*My sincere thanks to **Dr. A. Parvathi**, Dean, Faculty of Science, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her guidance and affectionate support, expert suggestions throughout the project.*

*I express my special gratitude to **Dr. S. Annapurani,** former professor and Head of the Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her sustained support throughout the study.*

*I record my sincere thanks to **Dr. P.R. Padma**, Professor and Head of the Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing the opportunity and timely help rendered to carry out the work successfully.*

I owe my indebtedness, profound and deepest thanks to my guide ***S. Velvizhi*** *Assistant Professor, Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her excellent guidance, immense tolerance, meticulous care, good support, creative influence, thoughtful advice, steady encouragement, motherly love throughout the research and motivation right from the selection of topic to the compilation and completion of the work efficiently and effectively.*

My special thanks to ***Dr. Anitha Subash,*** *Tutor and Professor , Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, who helped me to complete the work successfully and brought out the good ideas in me.*

I submit my sincere thanks to all ***The Staff Members****, of department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for lending a helping hand and invaluable guidance during the course of this thesis work.*

I would like to express my special thanks to my lovable parents ***Mr. P. Ayyasamy, Mrs. A.Maheswari*** *and my sister* ***A. Saranya, A. Kowsalya, A. Bharathy*** *for their loving care and enthusiastic support without which I could not complete my work.*

I am very much thankful to ***my friends*** *and* ***Classmates, my entire well-wishers*** *for their constant encouragement, moral support and help in the successful completion of the study.*

ISWARYA A

CONTENTS

S.NO	TITLE	PAGE NO
	LIST OF TABLES	
	LIST OF FIGURES	
1	**INTRODUCTION**	**1**
2	**REVIEW OF LITERATURE**	**6**
3	**MATERIALS AND METHODS**	**26**
4	**RESULTS AND DISCUSSION**	**34**
5	**SUMMARY AND CONCLUSION**	**52**
	BIBLIOGRAPHY	
	APPENDICES	

LIST OF TABLES

S.NO	TITLE	PAGE NO
1	**Assessment of Preliminary Phytochemical Analysis**	**30**
2	**Qualitative Phytochemical Analysis In Differennt Extract in *Glycyrriza glabbra***	**36**
3	**Enzymatic antioxidant activity of *Glcyrrhiza glabra* root extract**	**39**

LIST OF FIGURES

S.NO	TITLE	PAGE NO
1	Glycyrrhizin	8
2	Licorinne Pharmacology activity	10
3	*Glycyrrhiza glabra* root and leaves	12
4	Imbalance between oxidant and antioxidant	13
5	Silver nanoparticles synthesis	21
6	Various modes of action of silver nanoparticles on bacteria	24
7	Root sample and powder sample	34
8	Preparation of Different Extracts	35
9	Quantitative Phytochemical Analysis	38
10	Ascorbic Acid, Reduced Glutathione	40
11	α-Tocopherol	41
12	DPPH	42

LIST OF FIGURES

S.NO	TITLE	PAGE NO
13	ABTS And Superoxide Generation	42
14	Hydroxyl Radical Activity	43
15	Nitric Oxide and Hydrogen Peroxide Scavenging Activity	44
16	X-ray diffraction (XRD)	45
17	Zeta-Potential	46
18	UV-Visible Spectroscopy	47
19	FT-IR (Fourier Transform Infrared Spectroscopy)	48
20	SEM A) 20,000 B) 30,000 C) 40,000 D) 55,000	49
21	EDX (Eergy Dispersive X-ray analysis)	50
22	Antimicrobial Activity A) Methanol extract in *Bacillus* B) Silver nanoparticles in *Bacillus* C) Methanol extract in *S-aureus* D) Silver nanoparticles in *S-aureus*	51

LIST OF APPENDIX

S.NO	TITLE	PAGE NO
1	Estimation of Superoxide dismutase	65
2	Estimation of Catalase activity	66
3	Estimation of Peroxidase activity	67
4	Estimation of Glutathione Peroxidase activity	68
5	Estimation of Glutathione-s-transferase	68
6	Estimation of α-Tocopherol	69
7	Estimation of Ascorbic Acid	70
8	Estimation of Reduced Glutathione	71
9	DPPH Radical Scavenging activity	72
10	ABTS Radical Scavenging activity	72
11	Superoxide Generation Scavenging activity	73

LIST OF APPENDIX

S.NO	TITLE	PAGE NO
12	Hydroxyl Radical Scavenging activity	74
13	Hydrogen peroxide Scavenging activity	75
14	Nitric oxide scavenging activity	75
15	Qualitative Phytochemical Analysis	76
16	Estimation of Total Flavanoids	78
17	Estimation of Total phenol	79
18	Estimation of Total Alkaloids	80
19	Well diffusion method	81

1.0 INTRODUCTION

India is known as the botacial garden of the world as it is the lead producer of medicinal plants. India has more than 2,500 medicinal plants many of which are yet to be investigated for their medicinal values. From ancient times different parts of plants are send for the treatment and curing of various diseases in the from of curde extracts or combination of many extracts. But now a days number of drugs derived from plants are effective against many diseases and for this chemical costituents present in the plants be isolated. About 25% of medicinal drugs in developing countries depend on the derivaties of plants and are mainly used for rural peoples from many years (Puneetha, *et al.,* 2016).

The use of different parts of several medicinal plants to cure specific ailments has been vogue from ancient time. India is a best country where vide variations in climate, soil, altitude and latitude are available. Nature has best owned on us a very rich botanical wealth and a large number of diverse types of plant grow wild in different parts of the country (Kumar and Nautiyal, 2013).

Medicinal plants are recognized by their large number of bioactive secondary metabolites. They have been used to treat several human ailments and are believed to possess many biological activities (Duque *et al.,* 2017**).**

Herbal plants cosidered to be repository for many bioactive compounds possess various therapeutic properties (raina *et al.,* 2014). Many natural compods have been isolated from various parts such as leaves, roots, stems, fruits and seeds have been shown to possess excellent medicinal value (Sudhakar *et al.,* 2014). During the times of "Charaka"and "Sushrta" traditional medicinal preparations has been used (Adtani *et al.,* 2014). The importance of these medicinal plants lies in the subtances which is present in the plants that produce a distinct physiological action of human body (Dais *et al.,* 2014)

In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medines because of their less harmful effects. There is a wide diversity of compounds, especially secondary metabolites, found and isolated from plants and

studies have shown that these compounds have anticancer, antimicrobial, analgesic, anti-inflammatory, anti tumor, anti viral and many other activities to a greater or leaser extent (Iqbal *et al.,* 2015).

The effects of free radicals on human beigns are closely related to toxicity, disease and aging. Most living species have an efficient defense system to protect themselves against the oxidative stress induced by reactive oxygen species. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different diseases including cancer, antherosclerosis and the aging process. The antioxidants can interfere with the oxidation process by reaction with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers (Shareef *et al.,* 2014).

Plants are rich in secodary metabolites and essential oils of therapeutic imporatnce. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safe besides being economical, effective and their easy availability (Bilal *et al.,* 2012).

Natural products such as herbs, fruits and vegetables become popular in recent years due to public awareness and increasing interest among consumers and scientific community Natural products which contain antioxidant properties such as phenolics, include flavonoids and phenolic acids (Hakiman and Maziah, 2009).

Glycyrrhiza glabra Linn belongs to the family of Leguminosae, is a genus of perennial herbs and under shrubs distributed in the sub tropical and warm temperate regions of the world. *Glycyrrhiza glabra* Linn. Commonly known as licorice and sweet wood in english, Mulethi in Hindi, Madhuka in Sanskrit, Jashtimadhu, in Bengali, Yashtimadhukam in Telugu, Jethimadhu in Gujarati and Athimaduram in Tamil (Badkhane *et al.,* 2014).

Licorice is known for its phyoestrogen because it contains isoflavanes. Glycyyrhizic acid is so powerful to inhibit the liver damage and protect the body from three main toxic chemicals that are known endocrine disruptors. Therefore the present study is focused on one such very effective and potential medicinal herb licorice. *Glycyrrhiza glabra* is an age old plant used in traditional medicine across the globe for its ethnopharmacological values to cure varieties of ailments from simple cough to hepatitis to more complex like SARS and various diseases (Saxena, 2005)

Antioxidant effects play a vital role in the diseases related to oxidative stress. Oxidative stress is often defined as an imbalance between free radicals and antioxidants defense system. These radiclas contributes to the development of many diseases which can be neutralized by antioxidant defence system (Zengin, 2010).

Plants endowed with free radical scavenging molecuules such as vitamins, terpenoids, penolic acids, lignins, tannins, flavanoids, alkaloids, amines, betalains and other metabolites which are rich in antioxidant property. Studies have shown that these antioxidant compounds possess ani-inflammatory, anti atherosclerotic, anti tumar, antimutagneic and antiviral activities (Olayinka and Okoh, 2010).

It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of prooxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases (Hazra *et al.,* 2008).

The reactive oxygen species are unstable, highly active chemical entities capable oxidizing essential cellular components which results in cell damage. Normally human body defence system capable to prevent oxidative cell damage to some extent. But when the reactive oxygen species or free radicals produced in excess beyond the limit the human body will become unable to restore and leads to certain chronic diseases (Hannan *et al.,* 2012).

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and the metabolism free radicals contribute to more than one hundreds of diseases or disorders in humans including atherosclerosis, arthritis, ischemia (Baskar *et al.,* 2009). Plants containing beneficial phytochemical may supplement the needs of the human body by acting as natural antioxidants. Various studies have shown that many plants are rich source of antioxidants. For instance, vitamins A, C, E, and phenolic compounds such as flavonoids, tannins, and lignins, found in plants, all act as antioxidants (Altemimi *et al.,* 2017).

Glycyrrhiza glabra, also known as licorice and sweet wood, is native to the Mediterrannean and certain areas of Asia. Licorice has been used as a a medicine for more

than 4000 yrs. In traditional siddha system of medicine, it is used as a demulcent, expectorant, anti-tussive, laxative and sweetener (Anil and Jyotsna, 2012). Glabridin, a prenylated isoflavanoid of *Glycyrrhiza glabra* root has been associated with many biological properties such as antioxidants, antiatherogenic and estrogenic compounds. Due to this high biological property this root sample of *Glycyrrhiza glabra is* selected for the investigation.

Nanoparticles of noble metals such as gold, silver and palladium have drawn immense attention due to the wide range of new applications in various fields of industry. Particularly, silver nano particles have significant interest in medical applications such as very effective antibacterial agents without the toxic effects, and industry application such as containing well uniform dispersions of nano sized silver particles that are useful for producing electronic circuits. It is important that the silver nano particles require not only the particles to be of nano size, but also synthesis of the nanoparticles to be produced easily and at low cost. Over the past few decades, many synthetic methods of silver nanoparticles have been studied (Natsuki *et al.,* 2015)

Among the various metal nanoparticles synthesized (such as silver, gold, zinc, palladium or platinum), now a days, silver nanoparticles (AgNPs) are one of the promising nanopartciles in the nanotechnology field, because of their applications in various disciplines like biomedical, catalysis, energy and materials (Ashokkumar *et al.,* 2015). Right from olden days, humans have been in contact with silver, with brazing or soldering, coins, tableware, jewellery and dental filling (Hadrup and Lam, 2014). Silver nanoparticles were observed as a safe inorganic and non-toxic agents, possessing antibacterial and antifungal effects. Silver, in the nano size, encompasses a wide range of applications in biomedical field.

Production of nanoparticles can be acheived through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas (Song and Kim, 2009).

Nanomaterials display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface to volume ratio and increased percentage of atoms at the grain boundaries (Raffi *et al.,* 2008).

Several studies have reported that silver nanoparticles have potent antimicrobial effect. The nanoparticles are known to attach to the surface of the cell membrane or bind to the enzymes and proteins within the bacteria, which severely damages the cell and its major functions such as permeability, regulation of enzymatic signaling activity, cellular oxidation and respiratory processes, resulting in the bacterial death .They also penetrate into the cell easily due their small size and larger surface area, which unique character helps to kill the microorganisms. Silver nanoparticles with the size range from 20-25 nm were the most effective in suppressing the growth of clinically relevant bacteria with moderate to high antibiotic resistance (Aziz *et al.,* 2014)

The licorice plant has been used by physician and herbalists since the earliest of times. Man of the claims for the effectiveness of licorice extracts have been shown by modern science to be credible, a root component *Glycyrrhizin* being generally regarded as the major biological active principles. These extracts have been widely used in pharmaceuticals and confectioner industries (Nitalikar *et al.,* 2010).

In folk medicine it is used as a laxative, emmenagogue, galactagoge, anti-asthmatic drug and antiviral agent (Dhanakar *et al.,* 2000). The glbaridin and glabrene possess estrogen like activity (Schitra and Shaknthala, 2014). Licorice can reduce serum testosterone probably due to the block of 17 hydroxyl steroid dehydrogenase (Decio *et al.,* 2007).

Glycyrrhizin exhibits anti-inflammatory, antimicrobial, hepatoprotective, antitumor, antiulcer and immunomodulatory activity (Astaf'eve and Sukhenko, 2014).

Based on these background information the present study entitled **"*In vitro* antioxidative and antimicrobial activity of silver nanoparticles synthesized from *glycyrrhiza glabra* root extract"** was formulated with the following objectives

- To determine the antioxidant and free radical scavenging activity of *Glycyrrhiza glabra* root extract
- To synthesize and characterize Silver nanopaericles from *Glycyrrhiza glabra* root extract
- To evalute the antimicrobial effect of Silver nanopartocles of *Glycyrrhiza glabra* root extract

2.0 REVIEW OF LITERATURE

Natural products have provided us some of the important life saving drugs used in the modern medicine. However, among the estimated 250,000 - 400,000 plant species, only 6% have been studied for biological activity, and 15% have been investigated phytochemically. This shown a need phytopharmological evaluation of herbal drugs (Lakshmi and Geetha, 2011).

The review of literature pertaining to the study entitled **"*In vitro* antioxidative and antimicrobial activity of silver nanoparticles synthesized from *glycyrrhiza glabra* root extract"**is discussed below

2. 1 Role of medicinal plants

2. 2 *Glycyrrhiza glabra*

2.3.1 Scientific Classification

2.3.1 Medicinal use

2.3 Oxidative stress

2.4 Antioxidant

2.4.1 Enzymatic antioxidant

2.4.2 Non- Enzymatic antioxidant

2.5 Free radicals

2.6 Phytochemical

2.7 Silver nanoparticles

2.7.1 SEM - EDX

2.7.2 UV - Visible

2.7.3 Fourier Transform Infrared (FTIR) Spectroscopy

2.7.4 Zeta potential

2.7.5 X- Ray diffraction

2.8 Antimicrobial Activity

2.8.1 Nanoparticles as anti microbial agents

2.1 ROLE OF MEDICINAL PLANT

Traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and there are medicinal plants in order to meet health care needs. Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002).

The term medicinal plants include a various types of plants used in herbalist and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Medicinal plants have many characteristics when used as a treatment follows:

- Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
- Support of official medicine- In the treatment of cancer diseases the components of the plants proved to be very effective.
- Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Hassan, 2012).

The desire to capture the wisdom of traditional healing systems has led to a interest in herbal medicines. Particularly in Europe and North America, where herbal products have been incorporated into so-called 'alternative', 'complementary', 'holistic' or 'integrative' medical systems. (Tyler, 2000)

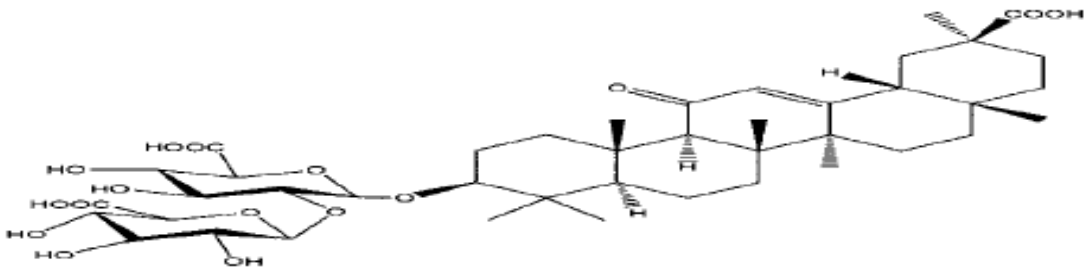
2.2 *Glycyrrhiza glabra*

Glycyrrhiza glabra Linn commonly known as sweet wood in English is the most valuable medicine of that belongs to pea family “Legumiosea". *Glycyrrhiza* is derived from two greek words “glcos” which means sweet and “rhiza” means root. *G. glabra* consists of unpeeled dried and stolon. It is an important medicinal herb which is known since ancient time and it is also one of most widely used herbs in different traditional system of medicines worldwide. It is widely cultivated in Chinna, France, Grermany, India, Italy, Russia, United Kingdom and United State of America (Husainn *et al.,* 2015). Licorice has been used in medicine for more than 4000 years. The earliest record of its use in medicine is found in ‘code Humnubari’. (Sofia and Walter, 2013).

Medicinal uses of Licorice include cough suppression, gastric ulcer treatment, treatment of early Addison disease, treatment of liver disease, and as a laxative. The antiulcerative activity has been demonstrated extensively and in China and Japan, licorice is clinically used for the treatment of stomach ulcers. Its preparations are used as a conditioning and flavouring agent in tobacco products (Nitalikar *et al.,* 2010).

The underground unpeeled or peeled stems or roots are used for its sweet teste. Licorice flavonoid oil (LFO) suppresses abdominal fat accumulation by regulation of rate-limiting enzyme activities related to fatty acid synthesis and oxidation in the liver (Nakagawa et al., 2004). The extract and its primary constituent glycyrrhizin are extensively used among US population and considered as Generally Recognised as Safe (GRAS) for use in foods by U.S FDA (Isbrucker and Burdock, 2006).

FIGURE 1



Glycyrrhizin

Glycyrrhizin is the main biological active constituent of licorice and extracted from the roots of licorice species *G.glabra.* (Thakur *et al.,* 2015). Secondary metalbilites like flavonoids, alkaloids, terpenoids, glycosides, and steroids were measured and it was found that highly polar solvents ike ethyl alcohol and ethyl acetate extract more metabolites as compared to less polar solvents. *G.glabra* linn, also known as sweet wood, is present in central and south west Asia, mediterranean basin of Africa, south Europe and certain areas of India (Iqbal *et al.,* 2017)

Different species of Licorice have been used medicinally since at least 500 BC and licorice has been described as the "The Grand Father of Herbs" (Ody, 2000). Licorice is also used for the treatment of disorders in lungs, respiratory tracts, stomach, kidney and other organs. In the past century, a number of components, including both complexes of biologically active substances and hundreds of individual compounds belongs to various chemical class and represents groups which possess different pharmacotherapeutic properties were isolated from the licorice (Ammar *et al.,* 2012).

It is considered as one of the best remedies to remove the irritating effects of acids in better way than alkalies (Sharma *et al.,* 2013). The thrombin induced platelet aggregation was found to be inhibited by the action of Glycyrrhizin (Silva *et al.,* 2003).

Glycyrrhizic acid which is present in the plant inhibits the growth of virus and inactivates the virus paricles there for this is a potential source of immunomodulator (Arora *et al.,* 2011).

2.2.1 Scientific Classification

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledoneae

Order: Rosales

Family: Leguminosae

Genus: Glycyrrhiza

Species: glabra Linn

FIGURE 3

***Glycyrrhiza glabra* root and leaves**

It is perennial, generally glandular herbs, with sweet root. Stems several from the crown, 2-4 feet or high, erect, stiff, solid, strongly, striates, shortly pubescent branched. Leaves alternate, spreading, large, stalked, with very minute deciduous stipules, impair pinnate, leaflets opposite in 4-7 pairs. The root is said to be good for sore throat. The root in combination with other drug is prescribed for the snake bite treatment (Prajapati and Patel, 2013)

Glycyrrhiza glabra is used as a medicine as well as a flavoring agent. The *Glycyrrhiza glabra* roots contain an important active connstituent *Glycyrrhizin*, a saponin which is repored to be 60 times sweeter than cane sugar. The roots are used in traditional medicine of medicine including folk medicines. It is popular remedy in traditional Ayurvedic, Chinese and Unani system of medicine (Parvaiza *et al.*, 2014).

2.2.1 Medicinal use

Glycyrrhiza glabra roots and rhizomes are important in drug development with various pharmacological activities. The root has also been shown to have a hormonal effect similar to the ovarian hormone (Badkhane *et al.*, 2015).

In traditional medicine, Licorice has been recommended as a prophylactic agent for gastric and duodenal ulcers. It is employed in dyspepsia as antioxidant and anti-inflammatory agent during allergic reactions (Ammosov and Litvienko, 2003). It is an important ingredient in medicinal oils used for the treatment of rheumatism, Hemorrhagc diseases, epilepsy and paralysis (kaur *et al.*, 2013). In the condition in anaemia, the decoction of *Glycyrrhiza glabra* or its powder was prescribed as laxative. The root decoction is good wash for falling and gray hair (Anil and Jyotsna, 2012).

For the treatment of chronic fatigue stress *Glycyrrhiza glabra* can be used as an alternative for conventional medicines (Trivedi and Sharma, 2011). It is related to be an effective pigment lightening agent. Glabridin in the hydrophobic fraction of licorice extract inhibits tyrosinase activity in cultured B16 murine melanoma cells. Isoliquiritin is responsible inhibition of tyrosinase activity (Cronin and Draellos, 2010).

Licorice decreases serum testosterone level in women. It is beneficial in aplastic anaemia and Addison's disease. *Glycyrrhiza glabra* in combination with zinc has shown good potential of immunomodulator activity in both humoral as well as cellular arms of the immune system (Mazumder *et al.*, 2012)

The effect of Licorice was investigated on androgen metabolism in nine healthy women of 22-26 years age, in the lutealphase of the cycle. They were given 3.5g of a commercial preparation of licorice daily for two cycles. Plasma rennin activity, serum adrenal and serum testosterone decreased gradually with in two months. (Goswami, 2012).

FIGURE 2

Licorice Pharmacology activity (Kalsi *et al.*, 2016)

2.3 OXIDATIVE STRESS

Oxidative damage to cellular biomolecules such as lipids, proteins and DNA is thought to play a crucial role in the incidence of several chronic diseases. Flavonoids are a group of polyphenolic compounds found abundantly in the plant kingdom. Antioxidants are radical scavenger which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's diseases, mongolism, ageing process and dementia (Sumathi *et al.,* 2016)

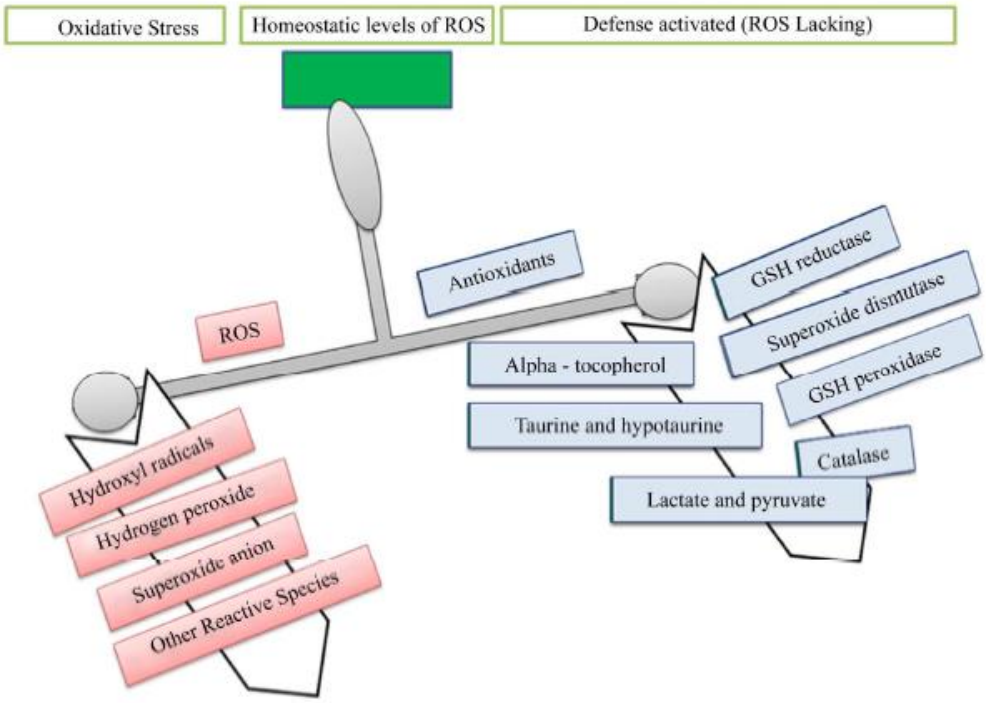
Free radicals or Reactive Oxygen Species (ROS) are formed in our body as a result of biological oxidation and metabolism. Free radicals are molecules that have lost an electron and try to replace it by reacting with other molecules. Most of the reactive free radicals are scavenging by endogenous defence systems such as catalase, superoxide dismutase and peroxidase, glutathione system (Madhu, 2013).

A plant based diet with high intake of fruits, vegetables and other nutrient rich foods ma reduce the risk of oxidative stress related diseases (Johnson *et al.,* 2004). It is reported

that the oxidative stress and apoptosis are closely related physiological phenomenon and are implicated in diseases including autoimmune diseases (Dianzai *et al.*, 2003).

To combat the adverse effects of the Reactive Oxygen Species (ROS) which induces oxidative damages, exogenous antioxidants or increasing endogenous antioxidant defences the body in a potential way. The plants have the ability to synthesize an inclusive range of non-enzymatic antioxidants which is capable of attenuating ROS induced oxidative damage. Antioxidants can be synthesized *in vivo* or from the dietary intake (Rajan *et al.*, 2017).

FIGURE 4



Imbalance between oxidant and antioxidant (Rahman *et al.*, 2012)

Oxidative stress is known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies. Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction (yoshikawa *et al.*, 2002). The current concept of "oxidative stress" should also include the pathways related to the "oxidative stress" and, for their implication in cellular and

extracellular metabolic events, to the “metabolic stress”. Reactive oxygen intermediate (ROI) and reactive nitrogen intermediate (RNI) are constantly produced under physiological conditions, is the crucial event in living organisms (Rahman *et al.*, 2012).

Oxidative stress is increasingly being recognized as central to the underlying pathophysiology of critical illness, especially the development of organ failure. Reactive oxygen species and Reactive nitrogen-oxygen species have clearly identified roles in modulating cell signalling, proliferation, apoptosis, and cell protection (Daren *et al.*, 2005).

2.4 ANTIOXIDANT

Antioxidant is the molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Antioxidant compounds have the ability to capture, deactivate and repair the damage caused free radicals (Pratibha *et al.*, 2010). Antioxidant compounds from plants can minimize the generation of free radicals and alleviate disease caused by oxidative stress (Diaz, *et al.*, 2012).

The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Javanmardi *et al.*, 2003). Nature has endowed living biological systems with antioxidants and free radical scavenger compounds such as dietary antioxidants (ascorbic acid, β-carotene, glutathione, α-tocopherol, and uric acid), hormones (angiotensin, estrogen) and endogenous enzymes (Bajpai *et al.*, 2015).

The many number of medicinal plants are used in the cellular and metabolic disease treatment such as diabetes, obesity and cancer. There are some speculations that the generation of free radicals inside the body in some physiological conditions is resulted in the cellular changes and development of diseases. And this could be neutralized by the antioxidants from different medicinal plants. Several studies have shown that plant derived antioxidant neutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects (sathisha *et al.*, 2010)

Antioxidant is capable of exerting protective effects against oxidative stress in biological system. Antioxidant based drugs and formulations for the prevention and treatment of complex diseases have appeared during the last three decades. In recent herbal drugs scenario plant derived antioxidants are gaining importance due to their potential health benefits, no toxicity and side effects over synthetic antioxidants like butylhydroxy anisole and butylhydroxy toluene (Onocha *et al.,* 2010).

Antioxidants are used by the food industry to delay the oxidation process. Many different methods have been used to measure the resistance of a lipid to oxidation when in the presence of potential antioxidants. The importance antioxidants present in food can limit the damage of somatic cells and pathogenesis of diseases by acting directly on reactive species or by stimulating endogenous defence systems of antioxidant constituents in maintenance of health attracted research in plant based antioxidants. Antioxidant activity of plant extracts was determined by *in vitro* methods such as, the DPPH free radical scavenging assay and reducing ability methods (Yasodamma *et al.,* 2013).

The intracellular concentration of ROS depends on the production and/or removal by the antioxidant system. Cells contain a large number of antioxidants to prevent or repair the damage caused by ROS, as well as to regulate redox-sensitive signaling pathways. Additional methods that can be used to determine SOD activity are the cytochrome c assay or the potassium superoxide-based direct assay.

2.4.1 Enzymatic Antioxidants

The body possess defence mechanism against free radicals induced oxidative stress, which involves prevention mechanism, repair mechanism, physical mechanism and antioxidant defence. Enzymatic antioxidant defence include catalase, superoxide dismutase and glutathione peroxidase (Ali *et al.,* 2010). These are considered to be the main protective enzymes being engaged in the removed of free radical and active oxygen species (Blokhina *et al.,* 2003).

2.4.1.1 Catalase

Catalase enzyme is present in the peroxisome of aerobic cells and is very efficient in promoting the conversion of hydrogen peroxide to water and molecular oxygen (Rahman, 2007). Catalase and hydroperoxidase enzymes covert hydrogen peroxide and hydroperoxides

to non radical forms and function as natural antioxidants in human body. Plants possess a protective system composed of antioxidant such as peroxidase and catalase. Catalae as a primary hydrogen peroxide scavenger in the peroxisomes and the mitochondria (Zolfaghar *et al.,* 2010).

2.4.1.2 Superoxide dismutase

Superoxide dismutase is one of the most effective intracellular enzymatic antioxidants and it catalyzes the conversion of superoxide anion to oxygen and hydrogen peroxide (Rahman, 2007).

2.4.1.3 Peroxidise

Peroxidase, which is an enzmatic antioxidant prevents the free radicals damages caused in humans (Dinesh *et al.,* 2009). The degradationn of hydrogenn peroxide is an importannt in certain redox signaling pathway (Ali *et al.,* 2010). In the presence of the hydrogen donor pyrogallol, peroxidase converts H2O2 to water and oxygen.

2.4.1.4 Glutathione-s-transferase

Glutathione-s-transferase comprised of many functional enzymes that enhances te conjugation to GSH of large variety of electrophilic alkylating compounds, some of which are products of oxidative damage of biological membranes and macromolecules (Carone *et al.,* 2003)

2.4.1.5 Glutothione peroxidase

Glutathione peroxidase is the most abundant enzyme, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. Glutathione peroxidase as high preference for lipid hydroperoxides, it is expresses in nearly every mammalian cell, though at much lower levels (Muller, *et al.,*2007).

2.4.2 Non – Ezymatic Antioxidant

The non-enzymatic antioxidant substances taking part in the first line of defence belong to preventive antioxidants and in blood plasma are represented by ceruloplasm, ferritin, transferrin and albumin. These proteins inhibits the formation of new reactive species

by binding transition metal ions. Also metallothionein plays an essential role in the prevention against reactive species.

The dietary antioxidant such as Vitamin E, C, carotenoids, some minarals and polyphenols can affect the activity of endogenous antioxidants (Chodakowska, *et al.,* 2018).

2.4.2.1 Ascorbic acid

Ascobric is a water soluble vitamin. It is essential for the biosynthesis of collegen, caritine and neurotransmitters. Health benefits of vitamin C are antioxidant, antiathrogenic and immunomodulator (Li *et al.,* 2010). Vitamin C cooperates with vitamin E to regenerate α-tocopherol radicals in membranes and lipoprotein and also raises intracellular glutathione level this playing an important role in protein thiol group production against oxidative stress (Rahman, 2007).

2.4.2.2 α – Tocopherol

Tocopherols are class of Organic chemical compouunds, mainly which in have Vitami E activity. Vitamin E is a lipophilic antioxidant sthesized all plants (Kiffin *et al.,* 2006). Vitamin E is a chiral componds with eight stereo isomers such as α, β, γ, ε-tocoperol etc. (Nguyen *et al.,* 2006). α-Tocopherolis the main source found in spplements.

2.5 FREE RADICALS

Free radicals are atoms or groups containing one unpaired electron making them high reactive species. These species like superoxide anions, hydroxyl and hydrogen peroxide, nitric oxide can induces oxidative damage to the biomolecules which includes diseases like diabetes mellitus, cancer, DNA damage (Afsar *et al.,* 2012).

It is responsible for several disorders in man body. Oxidative process is one of the most important ways to produce free radicals in food, drug and even in living system. In human body the free radicals have adverse effects on immune system. Consumption of natural oxidant as free radicals scavenger may turn necessary to develop the depleted immune system (Saikia and Upadyaya, 2011).

2.5.1 DPPH

DPPH (1,1-diphenyl-2-picryl hydrazine) free radical-scavenging capabilities of methanolic extracts were evaluated the many samples. Decreased absorbance of the sample indicates DPPH free radical scavenging capability (Sindhu *et al.,* 2014). Antioxidants react with DPPH and convert it to diphenyl-picryl hydrazine by donating its OH group. The degree of discoloration from purple to yellow colour can be measured at 519nm, which is a measure of the radical scavenging potential of the extracts (Mensor *et al.,* 2001).

2.5.2 ABTS

ABTS is a chromogen, which changes into a coloured mono-cation radical form (ABTS+) in the presence of oxidative agent and the ABTS+ has an absorption peak at 750nm. Antioxidants will reduce ABTS+ into its colourless form and the extent of decolourisation corresponds to the per cent reduction of ABTS+ (shirwaikar *et al.,* 2006). ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation de-colourisation assay was employed to assess the radical-scavenging effect of the root extracts of the candidate plant.

2.5.3 Superoxide generation

The extent of superoxide generation was studied on the basis of inhibition of the production of nitroblue tetrazolium formazon of the superoxide ion by the plant extracts and is measured spectrophotometrically at 560nm (winterbourn *et al.,* 1975)

2.5.4 Hydroxyl radical scavenging activity

Hydroxyl radicals are generated from a Fe2+/ascorbate/EDTA/H2O2 system, which attacks deoxyribose and eventually produces thibarbituric acid reactive substances (TBARS). The ability of the plant extracts to inhibit TBARS formation is measured spectrophotometrically at 532nm (Rao *et al.,* 1990).

2.5.6 Hydrogen peroxide

Hydrogen peroxide is a chemical compound of hydrogen and oxygen. Pure anhydrous hydrogen peroxide is a colourless liquid that it rapidly decomposes into oxygen and water.

Hydrogen peroxide is a freely miscible with water and apparently able to cross cell membranes readily (Halliwell, *et al.,* 2000).

2.5.7 Nitric oxide

Nitric oxide was generated from sodium nitroprusside and measured by the greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by use of greiss reagent. (Baskar and Balakirshnan, 2009).

2.6 PHYTOCHEMICALS

Phytochemicals are the chemicals that present naturally in plants. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer. Plants are recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. The biologically active compounds present in plants are called phytochemicals. These phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, barks, roots and pulps (Banu *et al.,* 2015).

Medicinal plants are one of the main sources for new pharmaceutical and health care products as most of the plants contain phytochemicals which have curative/protective properties against various diseases. Most phytochemicals, especially phenolics have been proved to benefit health of the human beings by scavenging free radicals or quenching reactive oxygen species (Prabhavathi *et al.,* 2016)

Medicinal plants are of prime importance to the health of individuals and communities and the medicinal values of these economically important plant species is due to presence of some chemical substances which produce a definite physiological action on human body like alkaloids, tannins, flavonoids, terpinoids, phenols, carbohydrates, phytosterols and saponin (Khan *et al.,* 2011)

2.6.2.1 Phenolics

Phenol a family of organic compounds characterized by a hydroxyl group attached to a carbon atom that is part of an aromatic ring. Phenols are similar to alcohols but form stronger hydrogen bonds.

2.6.2.2 Flavanoids

Flavanoids are a diverse group of phytonutrients found in almost all fruits and vegetables. Along with caroteoids, they are responsible for the colours in fruits and vegetables. Flavanoids is group of natural substances with various phenolic structures, are found in fruits, vegetables, grains, seeds, roots, barks, stems, flowers, tea ad wine (Panche *et al.*,2016)

2.6.2.3 Alkaloids

Alkaloids, any of a class of naturally occuring organic nitrogen containing bases. Alkaloids have diverse and important physiological effects on humans and other animals. Alkaloids are derived from various amino acids, such as Ornithine,Lysine,Phenylalanine etc., Alkaloids are the secondary metabolites of plants.

2.7 SILVER NANOPARTICELS

Nanotechnology is one of the emerging fields of science interfacing life sciences, material science and information technology. The National Nanotechnology Initiative (NNI) has defined nanotechnology as “working at the atomic, molecular and supra molecular levels, in the length scale of approximately 1–100 nm range in order to understand and create materials, devices and systems with fundamentally new properties and functions because of their small structure” (www.nano.gov).

The use of nanoparticles (NPs) have greater scope in many fields like electronic, medicine, pharmaceuticals, engineering and agriculture. The effective use of nanotechnology in agriculture sector is multi directional and has the potential to change the entire scenario of agriculture with the help of new tools for rapid detection of pathogens and pesticides molecules using nano based kits, treating against pest and disease problems including weeds, improving the ability of plants to absorb nutrients, genetic improvement of plants; conservation of soils and to maintain their structure and fertility of soil (Allah Ditta, 2012).

Metal nanoparticles are intensely studied due to their unique optical, electrical and catalytic properties. To utilize and optimize chemical or physical properties of nanosized metal particles, a large spectrum of research has been focused to control the size and shape, which is crucial in tuning their physical, chemical and optical properties (Bar *et al.*, 2009).

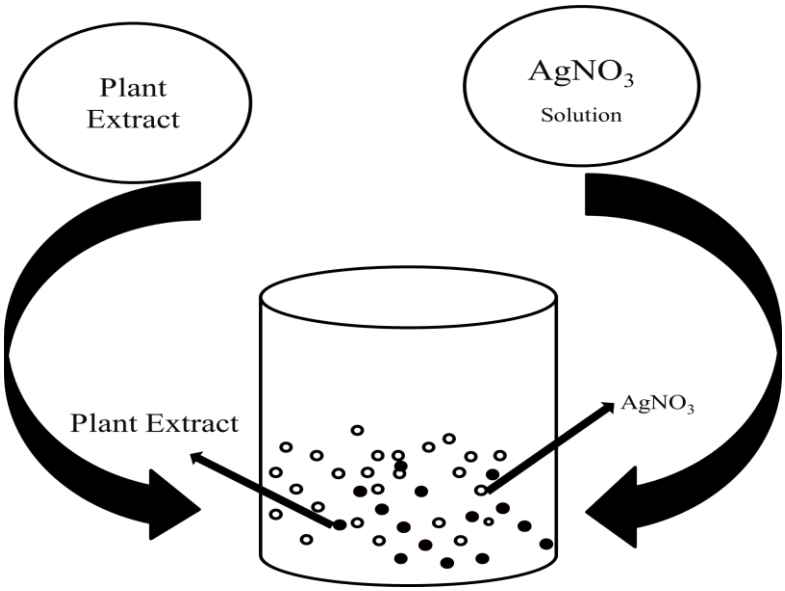
Silver nanoparticles can be synthesis by various chemical and physical methods such as UV irradiation, microwave irradiation, chemical reduction, photochemical method electron irradiation, and electrochemical Method (Shuman *et al.*, 2013).

The nanotechnology is a broad based science involving manipulation of atoms, electrons, protons and neutrons in a variety of ways to generate new understanding of how materials can be developed to solve many problems in various fields. Several nanodevices/nanomaterial/nanocides have been developed for plant disease prevention and their management (Patolsky *et al.*, 2006; Scrinis and Lyons, 2007 and Kawazoe and Meeche, 2005). Hence, the improved properties of the nanoparticles compared to the application of bulk materials have greater opportunity to reduce the load of unwanted chemicals especially plant protectants.

The application of nanoscale materials and structures is an emerging area of nanoscience and nanotechnology. Nanosized metal particles have been used widely in various fields including catalysis and photonics. Among various metals, silver nanoparticles (AgNPs) are of particular interest due to their remarkable antimicrobial and localized surface Plasmon resonance properties, which render them unique properties such as broad-spectrum antimicrobial Surface-Enhanced Raman spectroscopy (SERS) chemical /biological sensors and biomedicine materials and so on. Silver nanoparticles are usually ranging from 1 to 100 nm in size (Natshuki, *et al.,* 2015).

Nature has made noble metals part of our daily life. Recently there has been considerable interest in the development of techniques for the biosynthesis of metal-nanoparticles of well defined size, shape and composition, as they find applications in areas such as optics and electronics Among metal-nanoparticles, silver nanoparticles exhibit tremendous applications in spectrally selective coatings for solar energy absorption, optical receptors, bio-labeling, and intercalation materials for electrical batteries, filters, antimicrobial agents and sensors Silver nanoparticle-embedded antimicrobial paint is a promising area of environmentally friendly applications. Hence, a variety of techniques including physical and chemical methods have been developed to synthesize silver nanoparticles (Dinesh, *et al.,* 2012).

FIGURE 5



Silver nanoparticals Synthesis

Silver nanoparticles have attained a special focus. Conventionally silver nanoparticles are synthesized by chemical method using chemicals as reducing agents which later on become accountable for various biological risks due to their general toxicity; engendering the serious concern to develop environment friendly processes. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values (Ahmed *et al.*, 2016).

Nanoparticles usually referred as particles with a size up to 100 nm nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology, if compared with larger particles of the bulk material they are made of. Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles (Song *et al.,* 2009)

The silver nanoparticles show efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. (Rai *et al.,* 2009). While microorganisms such as bacteria, act in fungi continue to be investigated in metal nanoparticles synthesis, the use of parts of whole plants in similar nanoparticles synthesis methodologies is an exciting possibility that is relatively unexplored and under exploited. Even though gold nanoparticles are considered biocompatible, chemical

synthesis methods may still lead to the presence of some toxic chemical species absorbed on the surface that may have adverse effects in medical applications. Synthesis of nanoparticles using microorganisms or plants can potentially eliminate this problem by making the nanoparticles more biocompatible (Bar *et al.*, 2009).

Size control during synthesis of particles is an important criterion in the area of silver nanoparticle biosynthesis. Depending on the size of the nanoparticles, their applications branch out. To obtain the optimum concentration of AgNO3 that yields the maximum synthesis of nanoparticles and particle-size distribution, AgNO3, at concentrations ranging from 1 to 10mM, was added to the supernatant at pH 8.0 and temperature 300C (Gurunadhan *et al.*, 2009).

2.7.1 SEM and EDX

The EDX is an analytical technique used for the analysis of elemental composition and chemical nature of nanoparticles (Zaved *et al.*, 2012). The interactions between electromagnetic radiation and AgNPs emitting analytical rays in response to being hit with charged particles is the principle of EDX. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing X-rays that are characteristic of an elements atomic structure to be identified uniquely from one another.

2.7.2 UV – Visible Spectroscopy

The UV-Visible spectroscopy is a valuable tool used for confirming the synthesis of AgNPs which are stable in nature. It is well known that the optical absorption spectra of metal nanoparticles are dominated by surface plasmon resonances (SPRs) that shift to longer wavelengths with increasing particle size. Also, it is well known that the absorbance of AgNPs mainly depends on size and shape of particles (Jenkins and Snyder, 1996).

2.7.3 Fourier Transform Infrared (FTIR) Spectroscopy

The instruments FTIR is used for the identification of probable biomolecules responsible for acting as reducing, capping and stabilizing agents of AgNPs in the given samples (Vidhu et al., 2011).

Its principle is used to determine the reduction process with biomolecules while green synthesizing nanoparticles using aqueous plant extract and also bonding interaction between themselves. It is also helpful to detect the vibration characteristics of chemical functional groups of the green synthesized AgNPs by adsorbing infra red radiation in a specific wave number range.

2.7.4 Zeta potential

Zeta potential is the key of parameter that controls electrostatic interection in particle dispersions. Laser Doppler electophoreis is an accepted method fo the measuement of particle electrophoretic mobility and hence zeta potential of dispesions of colloidal size mateials. It can be used to optimize the fomulations of supensions and emulison and aid in prdicting long-term stabbility

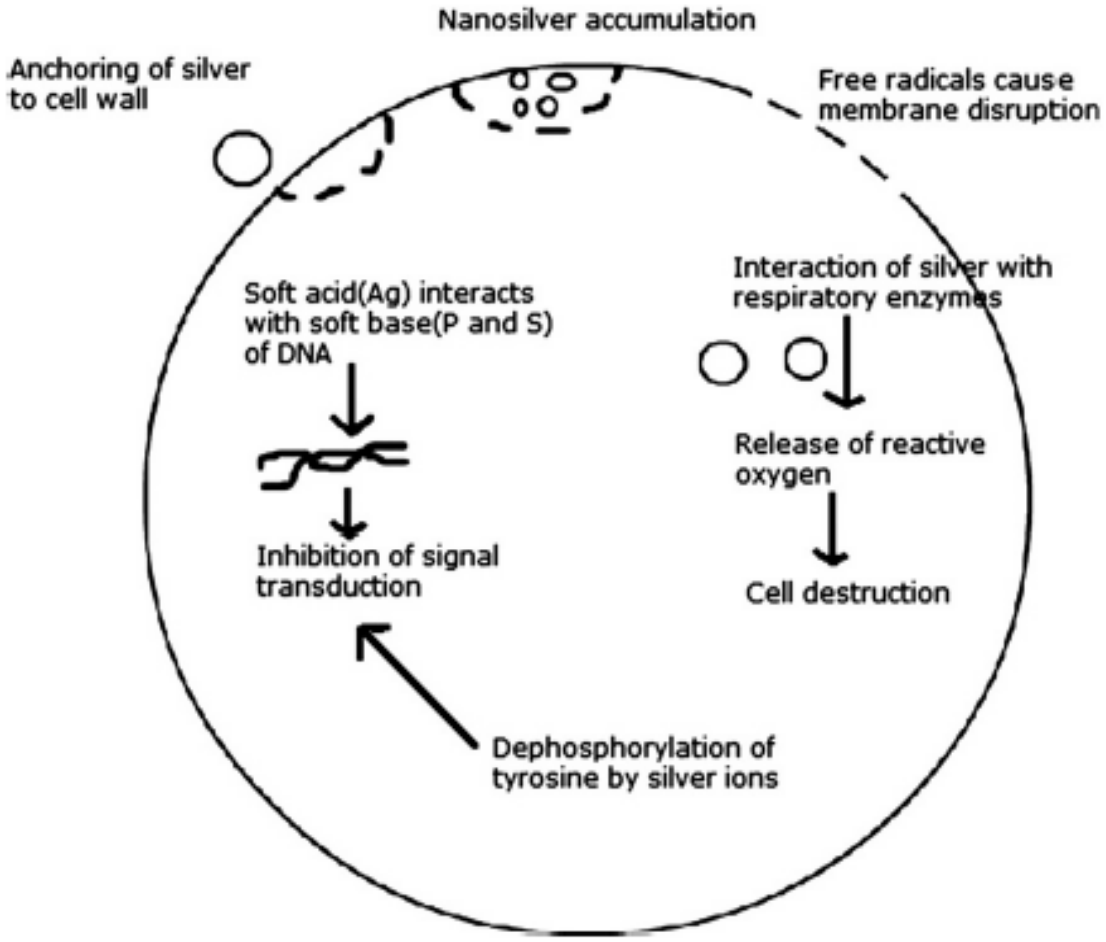
2.7.5 X- Ray diffraction

X-Ray diffraction is a technique to determine the phase composition of a sample, crystal structure, texture or orientation. The principle of XRD is that the X-rays are passed through a material and the pattern produced give information of size and shape of the unit cell. The atoms are crystal in structure arranged in a periodic array and thus can diffractedlight at different angle. When X-ray passing through a crystal it produces a diffraction pattern that diffract ion gives the information about the atomic arrangement within the crystals. In silver nanoparticles, the XRD gives nature and purity of the particles (Bindhu and Umadevi, 2013).

2.7 ANTIMICROBIAL ACTIVITY

Human beings are often infected by microorganisms such as bacterium, mold, yeast, virusin the living environment. Research has been intensive in antibacterial material containing various natural and inorganic substances (Cho *et al.,* 2005). The preparation of uniform nanosized drug particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products. Resistance of bacteria to bactericides and antibiotics has increased in recent years due to the development of resistant strains (Sondi *et al.,* 2004).

FIGURE 6



Various modes of action of silver nanoparticles on bacteria

(Prabhu and Poulose, 2012)

2.8.1 Nanoparticles as anti microbial agents

Silver nanoparticles possess strong antibacterial activity. Various researchers have reported the antimicrobial activity of AgNPs synthesised from plant based sources. The silver nanoparticles of Lycopersicone sculentum extract exhibited high bactericidal activity against Escherichia coli. The silver nanoparticles synthesized from stem bark extracts of Boswellia and Shorea showed activity against *Pseudomonas* and *Klebsiella* spp. Rajakannu et al. (2015) also reported the antibacterial activity of silver nanoparticles using Garcinia mangostana fruit extract against human pathogens such as Escherchia coli, Pseudomonas auroginosa and Staphylococcus aureus.

The silver nanoparticles interact with the virion surface and its core, which result in inactivation of virus. Khandelwal et al. (2014) documented that silver nanoparticles synthesized from the leaf extract of Argemonemaxicana exhibited strong antiviral efficacy against Pestedespetits ruminants virus. Tannic acid mediated silver nanoparticles inhibited Herpes simplex virus type 2, in both *in vitro* and *in vivo* conditions (Orlowski et al., 2014).

3.0 MATERIALS AND METHODS

The present study was udertaken to evaluate the **"*In vitro* antioxidative and antimicrobial activity of silver nanoparticles synthesised from *glycyrrhiza glabra* root extract"**

3.1 Collection of the plant materials

3.2 Preparation of root extract

3.3 Antioxidant activity

- 3.3.1 Enzymatic Antioxidant
- 3.3.2 Non – Enzymatic Antioxidant

3.4 Free radical scavenging activity

- 3.4.1 DPPH analysis
- 3.4.2 ABTS analysis
- 3.4.3 Superoxide generation
- 3.4.4 Hydroxyl radical scavenging activity
- 3.4.5 Hydrogen peroxide scavenging activity
- 3.4.6 Nitro oxide scavenging activity

3.5 Pytochemical analysis

- 3.5.1 Qualitative Analysis
- 3.5.2 Quantitative Analysis

3.6 Silver Naoparticals

- 3.6.1 SEM – EDX
- 3.6.2 UV – Visible
- 3.6.3 Zeta potential
- 3.6.4 FTIR
- 3.6.5 XRD

3.7 Antimicrobial activity

3.1 COLLECTION OF PLANT MATERIALS

The *glycyrrhiza glabra* root sample was collected by the ayurveda pharmacy in coimbatore. The root sample was indentified by the Scientist from Institute of Forest genetics, Coimbatore. The collected sample was grinded, stored by plastic bottles and use to the further experiments.

3.2 PREPARATION OF THE EXTRACT

10g root sample was taken in a conical flask and homogenised by the 100 ml solvents (Aqueous, Ethanol, Methanol, Petroleum ethar, Chloroform) and kept in overnight in shaker at room temperature, and filtered in whatman filter paper No.1. It was stored in blue cap bottles at 20℃.

The study was carried ouut in two phases

Phase 1: Evalution of *in vitro* antioxidant and free radicals scavenging activities of *Glcyrrhiza glabra* root extract

Phase 2: Synthesis and chharacterization of silver nanoparticles from *Glcyrrhiza glabra* and determination of its antimicroial activity

PHASE : 1

3.3 Estimation of antioxidant in *Glcyrrhiza glabra* root extarct

3.3.1 Enzymatic antioxidant activity

The activity of enzymatic antioxidant namely superoxide dismutase, catalase, peroxidase, glutathione peroxidase, glutathione-s-transferase were assessed by *Glcyrrhiza glabra*

3.3.1.1 Superoxide dismutase

The superoxide dismudase activity was estimated by the method of Misra *et al.,* (1972) and is explained in Appendix I.

3.3.1.2 Catalase

The activity of catalase in the root samples was estimated according to the Luck., (1974). The detailed procedure is given in Appendix II.

3.3.1.3 Peroxidase

The peroxidase activity was estimated the Raddy *et al,* (1995). Thus procedure was explained in Appendix III.

3.3.1.4 Glutathione peroxidase

The activity of enzymatic antioxidant in the *glycyrrhiza glabra* root sample was estimated in Rotruck *et al.,* (1977). The full procedure is given in Appendix VI.

3.3.1.5 Glutathione–s–tranferase

The actiity of glutathione-s-transferase in the root sample was estimated by the method Higbeg *et al.,* (1974). The full procedure given in Appendix V.

3.3.2 Non – enzymatic antioxidant activity

Non enzymatic antioxidant activity namely α – tocoperol, ascorbic acid, reduced glutathione assessed in *Glycyrrhiza glabbra* root sample

3.3.2.1 α – Tocoperol

α – tocoperol of root sample was estimated by the method Engel *et al.,* (1938). The procedure is given in Appendix VI.

3.3.2.2 Ascorbic acid

Ascorbic acid activity on root sample was determined by the Kuether *et al.,* (1953). The protocol was given in Appendix VII.

3.3.2.1 Reduced glutathione

Reduced glutathione activity on *glycyrrhiza glabra* root sample was estimated by Moron *et al.,* (1979) method. These procedure is given in Appendix VIII.

3.4 FREE RADICAL SCAVENGING ACTIVITY

Free radicals scavenging activity of *glycyrrhiza glabra* root sample was assessed by standard procedure

3.4.1 DPPH scavenging activity

DPPH scavenging radical activity was measured according to mensor *et al.,* (2001). The detailed procedure is given in Appendix IX.

3.4.2 ABTS radical cation scavenging activity

The 2,2 – azine – bis (3-ethabenzothiazloine-6- sulpfonic acid radical ection (ABTS+) decoloration test is a spectrophotometric method widely used assessment of the antioxidant activity of various substance. The ABTS+ scavenging activity was measured using the shirwaikar *et al.,* 2006. The detailed procedure given in Appendix X.

3.4.3 Superoxide radicals scavennging activity

The superoxide radical scavenging activity in root sample was measured using the method of Winterbourn *et al.,* (1975). The detailed procedure is given in Appendix XI.

3.4.4 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by the method Elizabath *et al.,* (1990). The detailed procedure is given in Appendix XII

3.4.5 Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity was measured by Ruch *et al.,* (1989). The detailed procedure given in Appendix XIII

3.4.6 Nitric oxide scavenging activity

The nitric oxide scavenging activity was assesed measured by Greenn and Hill (1984). detailed procedure is given in Appendix XI.

3.5 Phytochemical Analysis.

3.5.1 THE QUALITATIVE PHYTOCHEMICAL COMPOUNDS

The various extracts of *Glcyrrhiza glabra* roots were analysed for the presence of phytochemical such as alkaloids, flavannoids, phennols, saponin, tannin and terpenoids was standard procedure (Harbornne,1973) given in Appendix XV.

TABLE I

ASSESSMENT OF PRELIMINARY PHYTOCHEMICAL ANALYSIS

S.NO	PHYTOCONSTITUENTS	METHODS	APPENDICES
1.	Alkaloid	Raaman, 2006	XV
2.	Saponin	Siddiqui and Ali, 1997	XV
3.	Phytosterols	Siddiqui and Ali, 1997	XV
4.	Phenols	Raaman, 2006	XV
5.	Flavanoids	Raaman, 2006	XV
6.	Terpenoids	Siddiqui and Ali, 1997	XV
7.	Glycosides	Raaman, 2006	XV
8.	Tannin	Raaman, 2006	XV
9.	Hydrosible Tannin	Raaman, 2006	XV
10.	Sterols	Raaman, 2006	XV
11.	Catacol	Raaman, 2006	XV
12.	Volatile Oils	Siddiqui and Ali, 1997	XV

3.5.2 QUANTITATIVE ANALYSIS OF PHYTOCHHEMICAL COMPOUNDS

The level of total flavanoids, phenols and alkaloids were determined in *Glcyrrhiza glabra* root extract.

3.5.2.1 FLAVANOIDS

The amount of flavanoids present in the *glycyrriza glabra* root samples was extimated by the Zhishen *et al.*, (1999). That full procedure is given in Appendix XVI.

3.5.2.2 Phenols

The amount of phenol present in the root sample was estimated by the method of Malick and Singh, (1980). It is given in Appendix XVII.

3.5.2.3 ALKALOIDS

The amount of alkaloids present in the root sample was estimated by the method of Zhishen *et al.,* (1996). It is given in Appendix XVIII

PHASE II

3.6 Silver Naoparticals

10ml of plant extract was taken in conical flask and it was mixed with 100ml of silver nitrate solution (0.8g silver nitrate dissolved in 500ml of distilled water). Then this mixture was kept in sunlight for 2 to 3 hrs still brown colour was developed. Brown coloured solution was centrifuged at 10,000 rpm for 45 minutes. Then the supernatant was discarded. Pellet was taken and dried it for 60℃ for 30 minutes. Then powder was collected and taken for the further charcterisation.

3.6.1 SEM – EDAX

After 24h of storage in silver nitrate, the specimens were then rinsed thoroughly in distilled water and placed in photodeveloping solution for 8 hunder a fluorescent light. Final chemical drying was conducted according to the protocol of Perdigao *et al.,* using hexamamethyldisilazane. The dentin side of the fractured beams was sputter-coated with carbon for 10 s or non-sputter coated, and then analyzed in a field emission scanning electron microscope using secondary or backscattered electron mode and using energy dispersive X-ray spectrometry (Hashimoto *et al.,* 2004).

3.6.2 UV – Visible

The UV-Visible spectroscopy was used to confirm the synthesis of AgNPs based on their optical properties. The wavelength of green synthesised AgNPs based on light absorbance in the range of 300 to 600 nm with resolution of 1nm was measured by loading the sample of 2 ml in quartz cuvette in UV-Visible spectroscopy (Make: SPECORD 210 PLUS, Germany).

3.6.3 Zeta potential

zeta potential of the prepared hydrothermal nanoceria is positive while that of microemulsion-based nano ceria is negative. This difference in the zeta potential is due to the chemicals involved in the synthesis process. The pH buffers used were 1, 1.9, 3, 5, 7, 8, 11,

and 13. All of these buffers were hand made to ensure that each one had the same ions in solution. Each pH buffer had 25 ml of 0.2M KCl solution and 75 ml of nano pure water. HCl or NaOH solutions were added as necessary to adjust the pH. Acidic pH buffer treatment of ceria particles in theory will create a positive zeta potential because of the increase in the H+ ion concentration (Patil *et al.,* 2007).

3.6.4 Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR (Shimadzu, Japan) is a chemical analytical method for the measurement of infrared intensity, wavelength or wave number of light of the green synthesised AgNPs. Therefore for FTIR analysis a sample of 0.5 g of AgNPs was used and the spectra was scanned in the range of 4000–400 cm-1 at a resolution of 4 cm-1 for the characterization of chemical functional groups present over the surface of green synthesised AgNPs. The FTIR data measures the interaction between Ag and protein molecules. Therefore, when an infrared light interacts with the sample of AgNPs, the chemical bonds show stretching, contracting and bending nature of nanoparticles as indicated by peak in FTIR and the result was matched with the standard library search of Infrared charts.

3.6.5 X- Ray diffraction

The XRD (Make: Bruker D8 Advance Powder X-ray Diffractometer, Germany) analysis is \ helpful to know phase structure and purity of the synthesized AgNPs. It is generally used as common technique for studying the phase composition of AgNPs, its crystalline/amorphous structure and texture or orientation. For XRD analysis, one g of AgNPs was loaded in the sample holder of instrument and allowed to run at 40 kV voltage and 30 mA current with Cu K radiation for the identification of nature of the particles (Shankar *et al.*, 2004). Then the diffractogram was recorded with 2θ value ranging between 10-80o at a scanning speed of 0.080 for a step time of a second at room temperature (25ºC). The result obtained from the XRD pattern was interpreted with standard reference of Joint Committee on Powder Diffraction Standards for the characterization of AgNPs.

3.7 ANTIMICROBIAL ACTIVITY

10 g root powder was dissolved in 100ml of methanol and kept in shaker for overnight. After methanolic extract was filter in whatman filter paper NO.1. 1 ml of

methanolic extract was taken and diluted by 1 ml DMSO. It was used by well dillution method.

10 ml of methanol extract was taken and added 90 ml silver nitatre solution. These was allowed to developed into brown colour changed to sun light for few hours. 1ml of silver nanoparticles synthesis was taken and diluted by 1ml of DMSO.

The antimicrobial activity was evalueted by measurinng thhe diameter of zone of inhibition around by the well by agar. Well diffusion method (Nzlls, 1993) as given in Appendix XXI

4.0 RESULTS AND DISCUSSION

Plants have been used for long periods as a medicinal source which plays a central role in healthcare system around the world. It has been a good and cheaper source for the prevention and treatment of various diseases for thousands of years. Their uses grown more sophisticated with modern chemicals using compounds isolated from medical plants for the generating novel compounds with additional benefits like lower toxicity and potencial for combating drug resistant diseases. India is placed as the largest producer of medicinal plants, and many have been used traditionally for the treatment of various diseases (prasad and Tyagi, 2015)

The results of the present study entitled **"*In vitro* antioxidative and antimicrobial activity of silver nanoparticles synthesised from *Glycyrrhiza glabra* root extract"** are dicussed under the following headings

Phase 1: Evalution of *in vitro* antioxidant and free radicals scavenging activities of *Glcyrrhiza glabra* root extract

Phase 2: Synthesis and chharacterization of silver nanoparticles from *Glcyrrhiza glabra* and its antimicroial activity

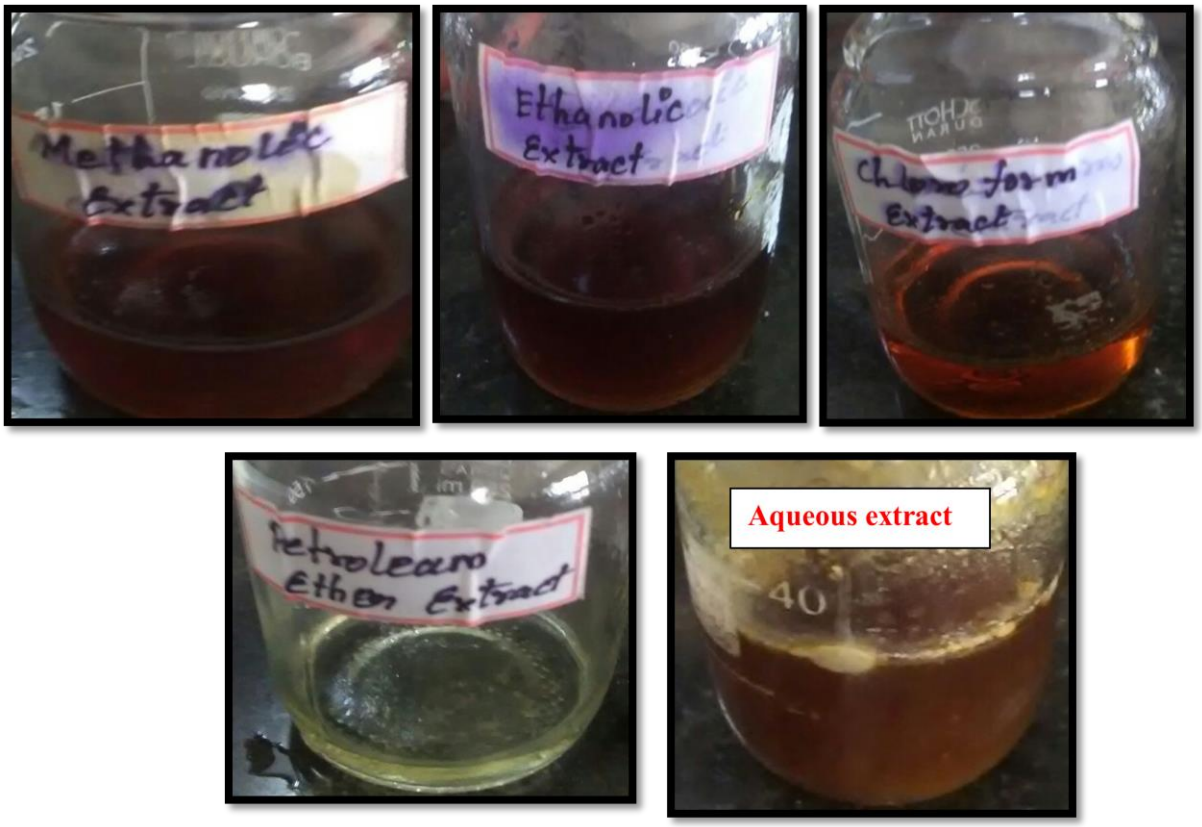
Preparetion of organic extract of *Glcyrrhiza glabra* root sample

In order to understand the active principle rendering te antioxidant activities the roots of *Glcyrrhiza glabra* were serilly extracted into solvents of increasing polarityy using sozlet apparatuus.after extraction the solvets were evaporated to dryness and stored at -20℃ unit u

FIGURE 7

ROOT SAMPLE AND POWDER SAMPLE

FIGURE 8



DIFFERENT EXTRACT OF *Glcyrrhiza glabra* ROOT SAMPLE

PHASE 1

PHYTOCHEMICAL SCREENING

Phytoconstituents are the natural bioactive compounds found in plants. This phytoconstituents work with utrients and fibres to form an integrated part of defense system against various diseases and stress condition. It is basically divided as primary and secondary costituents according to their functions in plant metabolism. The promissing pharmacological properties of medicinal plants are due to the presence of phytoconstituents such as alkaloids, phenols and proteins (thilagavathi *et al.*, 2015).

The root sample was analysed for the presence of phytochemical agents such as charbohydrates, pheols, flavanoids, alkaloids, tannin, sterols, hydrosible tannin, volitile oils, saponin.

TABLE II

QUALITATIVE PHYTOCHEMICAL ANALYSIS IN DIFFERENNT EXTRACT IN *glycyrriza glabbra*

S.No	Plant name / Parameters	*Glycyrrhiza glabra*				
		Methanolic Etract	Ethanolic Extract	Petrolium ethar Extract	Chloroform Extract	Aqueous Extract
1.	Alkaloids	+++	+++	+	+	+++
2.	Flavanoids	+++	+++	-	+	+++
3.	Phenols	+++	+++	++	+	+++
4.	Spanonin	+++	-	+	+	-
5.	Steroids	+	-	-	-	-
6.	Catachol	+++	-	-	-	-
7.	Terpenoids	+++	+++	+	++	++
8.	Volitile oils	-	-	+	-	-
9.	Tanin	+++	+++	-	-	+++
10	Hydrosible Tanin	-	-	+++	+++	-
11	Glycosides	-	-	-	-	-
12	Carbohydrate	+++	+++	-	++	+++

+ PRESENCES - ABSENCE

Table II represents the phytochemical screening of root extract of *glcyrriza glabra* showed positive results for alkaloids, phenols, flavaoids, tannins, glycosides, terpeoids and carboydrates in the methanolic, ethanolic, aqueous extract where as the valitile oils, hydrosible tannin in the only in the petroleum ether extract. The present study indicates that the ethanol and methanolic extracts of *Glycyrrhiza glabra* exhibited maximum number of phytochemicals than chloroform and petroleum ether.

Methanol and water being polar solvents would have resulted in the extraction of more phytochemical when compared to non-polar solvents namely petroleum ether and chloroform. A similar result was also observed by Mazumder *et al.,* (2012)

In a previous study by varsha *et al.,* (2013) also demonnstrated that the hydromethanolic extracts of *G. glabra* was found to be positive for the presence of saponin, flavanoids, alkaloids, steroids, terpennoids, tannins and glycosides. The constituents present in the extracts have significant antibacterial and hydroxyl radical scavennging activities.

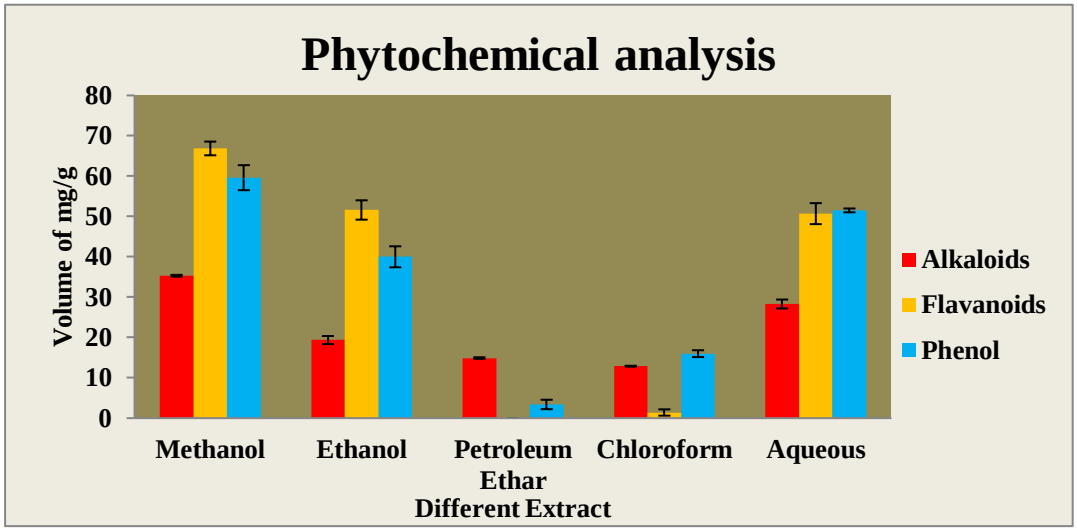
Patil *et al.,* (2009) determined the preliminary phytochemical screening of aqueous extract of *G. glabra* roots showed the presennce of saponin, tannins, flavanoids and triterpeniods. The main components are the triterpenoids, saponins, glycyrrhizin and glycyrrizic acid which are believed to be party responsible for anntioxidant properties (Lakshmi and Geetha, 2011)

The screening of phyochemical in the different extract of *albutilon indicum* showed the presence of alkaloids, steroids, tannins, and flavanoids (Yid *et al.,* 2011). The screening of ethannolic extract in *Cladophora glomerata* showed the presence of alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed (Mansuya *et al.,* 2010)

Phytochemicals found present in stem, bark and root extract of *deetia tripetala* indicates their potential source of principle that may supply novel medicines (Ugochukwu *et al.,* 2013). These findings are in accordance with results of current study.

QUNTITATIVE PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACT OF *glycyrriza glabbra* ROOT SAMPLS

FIGURE 9



QUANTITATIVE PHYTOCHEMICAL ANALYSIS

It is evident from thhe figure 9, the amont of flavanoids, alkaloids, phenols content were higher in methanolic extract in *Glcyrrhiza glabra* root sample.

Kaur and Arora, (2009), showed that majortity of the active priciples present in *Anethum graveolens* expressed a very high antioxidant activity which was attributed to flavanoids and phenolic acids.

The study carried out by Khan *et al.*,(2011), reported that the roots of *Taraxacum officinale* contains flavanoids, alkaloids, phenol, saponin and tannin. Flavanoids, phenols, alkaloids, β-carotinoids is present in the *Tirmania nivea* plant these is reported by (Hamza *et al.*, 2016).

***IN VITRO* ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACT IN *Glycyrrhiza glabra* ROOTEXTRACT**

Medicinal plants are the precursors or source of many bioactive compounts which acts as antioxidant (Saikai and Upadhyaya, 2011). Plant contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential. Antioxidant are a group of compounds that facilitate survival in plants and may promote the

health of humans that consume a variety of plant foods (Chanda *et al.,* 2011). Thus it is important to characterize the medicinal plants for their antioxidant potential (Farah *et al.,* 2015)

Antioxidant activity was done by

- Enzymatic antioxidant activity
- Non-Enzymatic antioxidannt activity

Enzymatic Antioxidant activity

TABLE III

Enzymatic antioxidant activity of *Glcyrrhiza glabra* root extract

S.No	Enzymes	Root extract
1.	**Catalase [@]**	**4.52±0.25**
2.	**Superoxide dismutase[$]**	**14.29±0.21**
3.	**Peroxidase[#]**	**1.89±0.12**
4.	**Gluutathione-s-transferase***	**0.56±0.02**
5.	**Glutathione Peroxidase[ϴ]**	**12.46±0.23**

Values are mean ± SD

Units

@ - Amount of enzyme required to decresed the absorbance by 0.05unit at 240nm

$ - Amount of ennzyme that cause 50% reductionn in NBT oxidant

- Change inn absorbance at 430nm/min

***** - nmol of CDNB connjucated/minutes

ϴ – μg in consumed/min/mg of proteins

The analysis of enzymatic antioxidannt reported *Glcyrrhiza glabra* root extract extibited considerble activity of all enzyamtic anntioxidant assessed.

The enzymatic antioxidant defense system is the nature protector against lipid peroxidation. Enzymatic antioxidant are important scavengers of superoxide ion and hydrogen peroxide. These antiocidants prevent the genetration of hydroxyl radical and protect the cellular constituent from oxidative damage in plants (khatun *et al.,* 2011).

Selium *et al.,* (2011), reported that the catalase activity of *coleus forskohil* was significantly highest roots, and in leaves.

Superoxide dismutase is the intracellular antioxidant, which is mainly present in cell cytoplasm and in superoxide anion. Its provides the primary defese against oxidative damage (Azeen *et al.* 2009). Visavadiya *et al.,* (2009) showed antioxidant property of *Glycyrrhiza glabra* root extracts using *in vitro* models.

Similarly in the previous study Singh *et al.*, (2012) reported that the catalase activity of aqueous extract of *Glycyrrhiza glabra* (16.080 units/mg proteins) was more than aqueous extract of *piper longuum* whose catalase activity was (14.05 units/mg proteins). It has been concluded that aqueous extract of *Glycyrrhiza glabra* is the good source of antioxidants.

Levels of antioxidant enzymes such as superoxide, gltathione peroxidase were due to hypoxia annd were restored to near normally by administrationnn of ethanol extract of *G.glabra* (Muralidharann *et al.,* 2009)

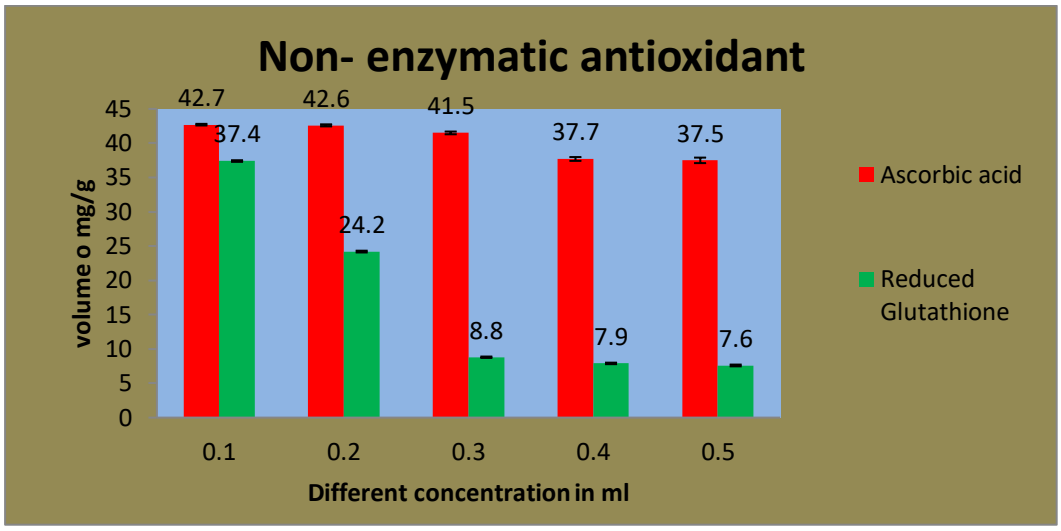
The antioxidant status *premma tomentosa* were analysed for ennzymatic anntioxidant present, where in significant activities of catalase, peroxidase, glutathione peroxidase, superoxide dismutase and glutathione-s-trannsferase were reported in Hennderson *et al.,* (2011).

NON-ENZYMATIC ANTIOXIDANT ACTIVITY

The level of non enzymatic antioxidant were assessed and the levels were dipicted in the figure

LEVEL OF NON ENZYMATIC ANTIOXIDANT IN *Glcyrrhiza glabra* ROOT EXTRACT

FIGURE 10



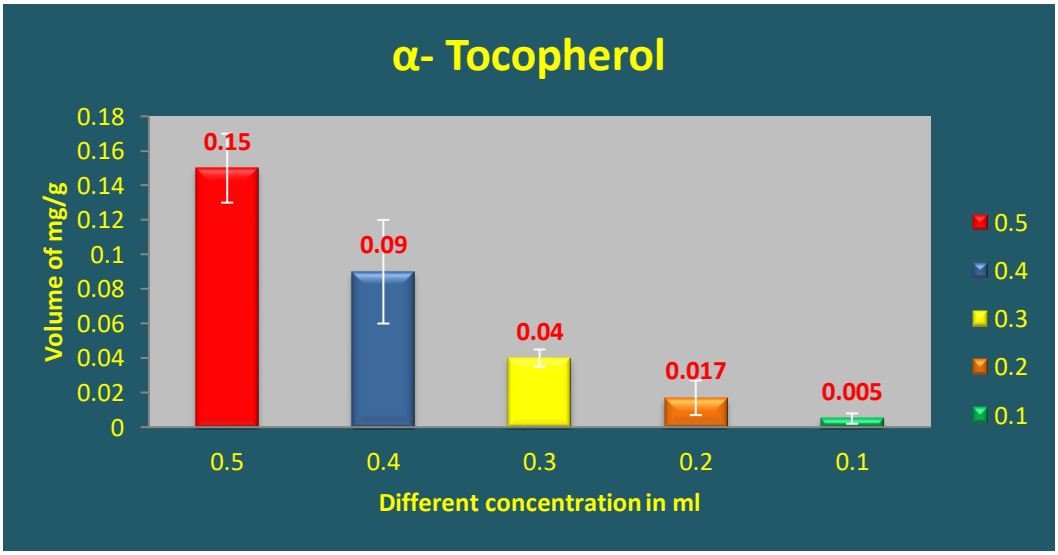
LEVEL OF ASCORBIC ACID, REDUCED GLUTATHIONE

Figure 10 acknowleges that the root conntainn significant amount of *Glcyrrhiza glabra* root extract inn ascorbic acid and reduced glutathione

High content of phenolic component in ethaolic extract of *Glycyrrhiza glabra* is resposible for its powerful antioxidant activity by means of signnificant free radicals scavenging. Antioxidant activity of licorice flavaoids was found to be over 100 times stronger than that of antioxidant activity of Vitamin E (Damle, 2014).

α-TOCOPHEROL

FIGURE 11



LEVELS OF α-TOCOPHEROL

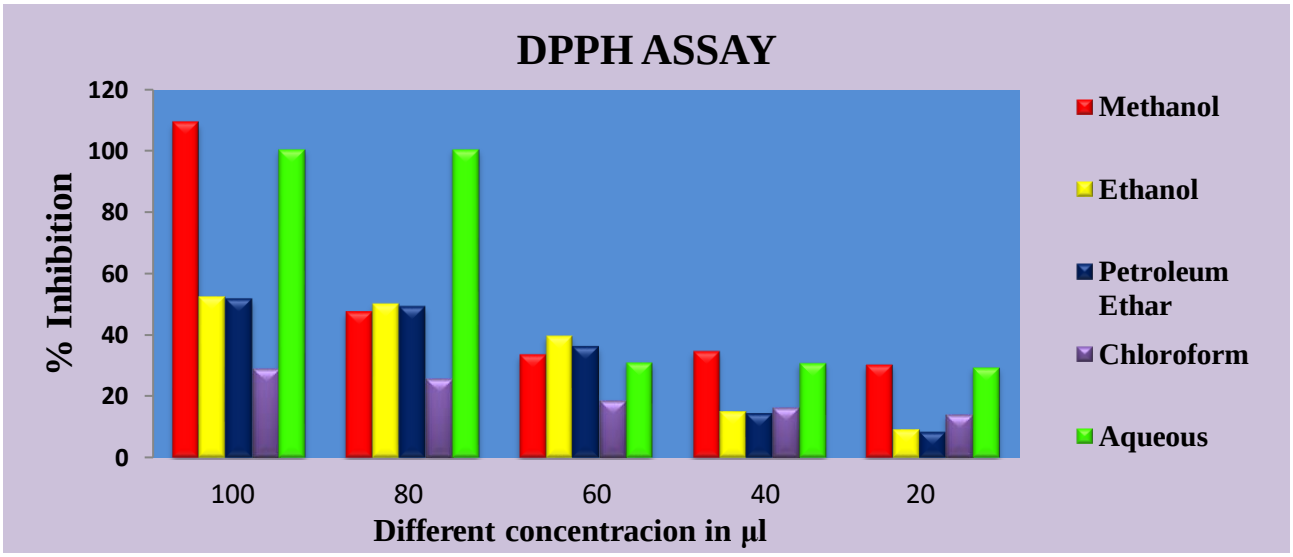
Vitamin E have beneficial effects in cardiovascular diseases both by inhibition LDL oxidation and by down regulating hydroxyl methylglutaric reductase a key enzyme of mevalonnate pathway (Hosseinian *et al.*, 2006)

Ju, *et al..*, (1989) reported thhat flavaoids from licorice are the strongest natural antioxidant known, similar results were obtained in the current study proved the presence of antioxidant activity in the root of *Glycyrrhiza glabra.*

FREE RADICALS SCAVENGING ACTIVITY OF ROOT EXTRACT IN *glycyrrhiza glabra*.

DPPH

FIGURE 12



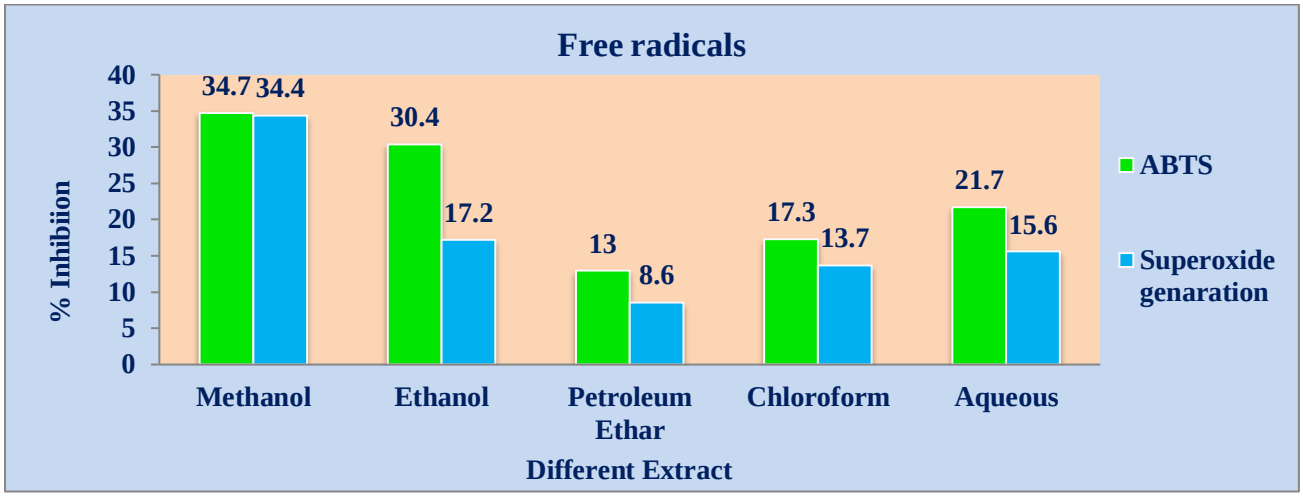
DPPH RADICAL SCAVENGING ACTIVITY

The result of figure 12 reported that the methannolic extract of root in *Glcyrrhiza glabra* exhibit highest activity when compared to other extract.

Cakmak *et al.*, (2012), reported that higest DPPH scavennginng activity was founnd in the ethannolic extract of fruits of *G. lepidota* was 49%.

ABTS AND SUPEROXIDE GENERATION

FIGURE 13



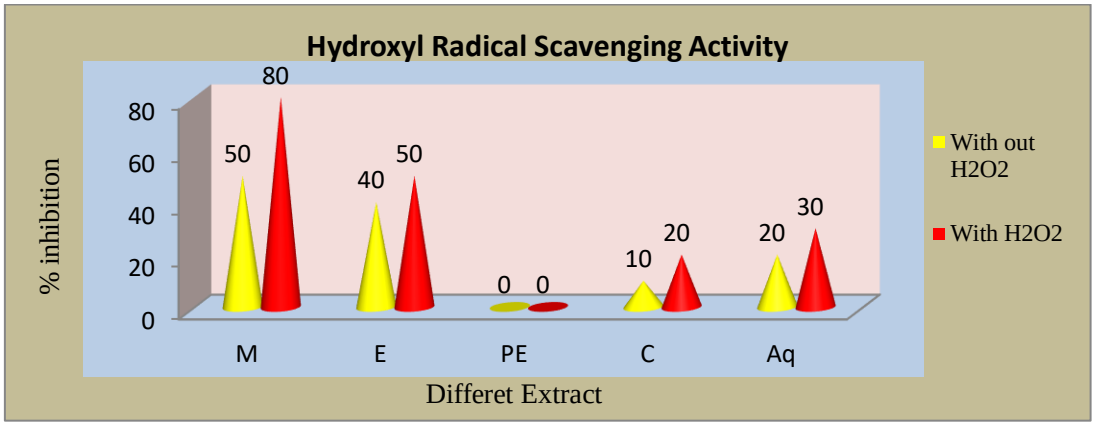
ABTS AND SUPEROXIDE RADICALS SCAVENGING ACTIVITY

Ani and Naidu, (2012), reported that ABTS radical was scavenged by the methanolic extract of *Ocineum sannctum* inn the concentration dependant manner. From the present findings it can be suggested that the costituents present in *Glycyrrhiza glabra* root extracts are capable of scavegig reactive species such as superoxide anion and may be able to prevent oxidative damage of major biomolecles.

Hence the present data suggest that the roots of *Glycyrrhiza glabra* are able to scavenging free radicals. Thus they are capable to innhibit the initiation and propagation of free radical mediated chain reactions by stabilizing reactive species through electron or hydrogen donation

HYDROXYL RADICAL ACTIVITY of *Glcyrrhiza glabra* ROOT EXTRACT

FIGURE 14



M-Methanol, E-Ethanol, PE-Petroleum Ethar, C-Chloroform, Aq- Aqueous

HYDROXYL RADICAL ACTIVITY

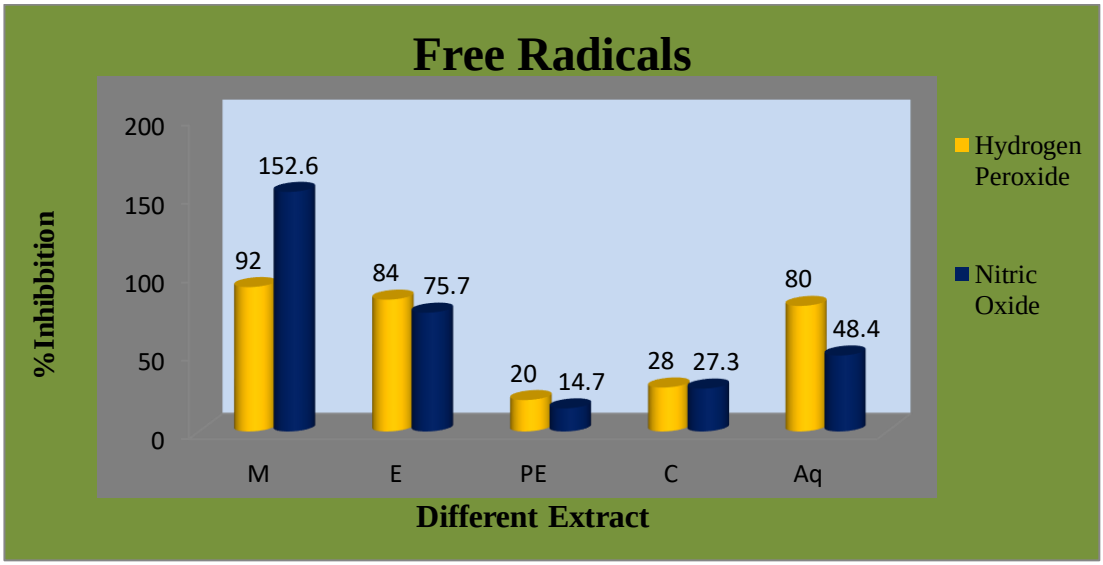
Unsaturated lipids in cell membrane or susceptibility to peroxidation consequently, these chain reactions inhibited by hydroxyl radical attacking lipids are extracded by the generation lipiid hydroperoxide free radical (Guo *et al.*, 2006).

Hydroxyl radical scavenging activities of the *Spondias pinnata* extract reported the IC10 = 112.18 ± 3.27 µg/ml. The IC50 value of the standard is 571.45 ± 20.12 µg/ml (Hazra *et al.*, 2008).

The hydroxyl radical scavengig activity of *Glycyrrhiza glabra* extract at a concentration of 100µg/ml was greater ta 75% where as, *G. uralensis* ad a scavenging activit greater tan 80% under the same conditions (Park *et al.*, 2014).

NITRIC OXIDE AND HYDROGEN PEROXIDE SCAVENGING ACTIVITY

FIGURE 15



M-Methanol, E-Ethanol, PE-Petroleum Ethar, C-Chloroform, Aq- Aqueous

NITRIC OXIDE AND HYDROGEN PEROXIDE SCAVENGING ACTIVITY

It was founnd that methanolic extract showed that highest inhibitory effect than the other extract. *Glycyrrhiza glabra* is found to be rich in flavaoids and exhibits good radical scavennginng activities (Dong *et al.*, 2014). Chidambaram *et al.*, (2013) explained that the root extract of the *Cordariocalyx motorium* showed potent nitric oxide scavenging activity at varing concentration of the extracts.

Pooja, *et al.*, (2010), stated that the methanolic root extracts of *Dalbergia sissoo* showed maximum scavenging of nitric oxide Sahriar *et al.*, (2013), demonstrated that the chloroform extract of *Withania somnifera* root has potent nitric oxide scavenging activity

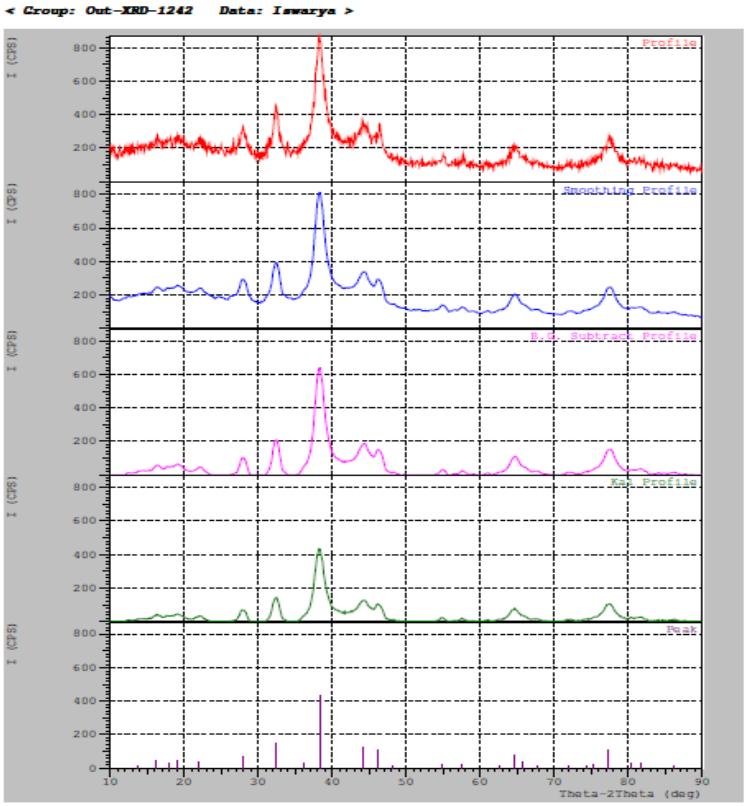
Kang *et al.*, (2004) demonstrated that glabridin, a polphenolic componnents present in *Glycyrrhizin* inhibits NO production.

PHASE II

CHARACTERIZATION OF SILVER NANOPARTICLES FROM *Glcyrrhiza glabra* ROOT EXTRACT

X-ray diffraction (XRD)

FIGURE 16



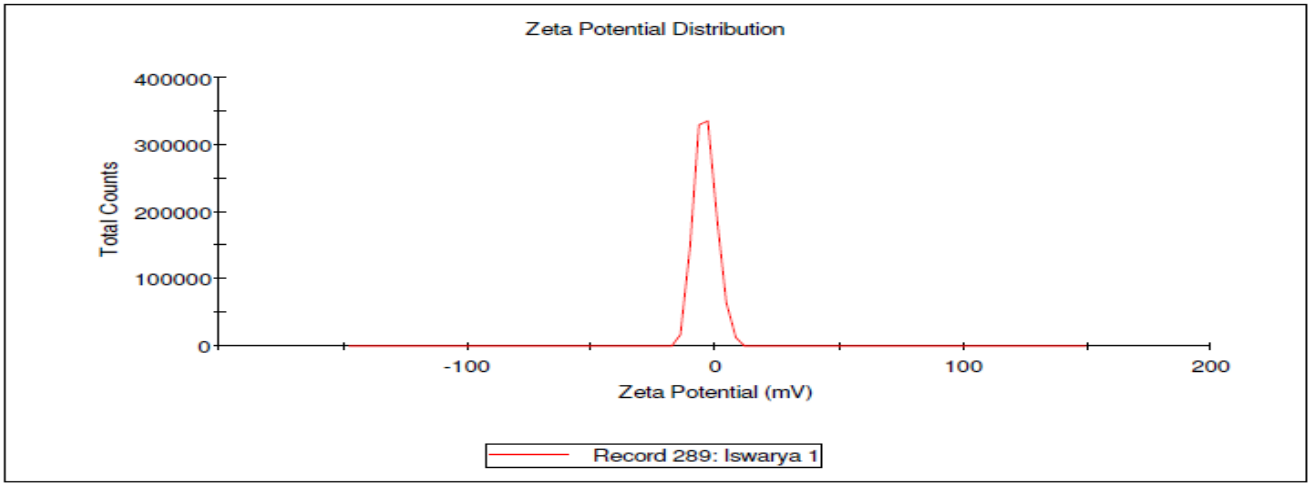
X-ray diffraction (XRD)

The X-ray diffraction profiles of the synthesized silver nanobioconjugates are depicted in figures 10. All the silver nanobioconjugate profiles showed typical peaks corresponding to the face intensities of standard silver nanoparticles of three different peaks of 2θ values at 32.42, 42.32 AND 45.20 that indexed to (1 0 0), (4 0 0), (2 0 0). Braggs reflections of the cubic structure of silver. This explore the presence of silver in the nanobioconjugates and the highly crystalline nature of the particles. The nanobioconjugates synthesised from *Glcyrrhiza glabra* root extracts showed some additional peaks, which indicated the presence of organic molecules present in the extract.

Zeta – potential

FIGURE 17

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -4.00	Peak 1:	-4.00	100.0	4.40
Zeta Deviation (mV): 4.40	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.297	Peak 3:	0.00	0.0	0.00
Result quality : Good				

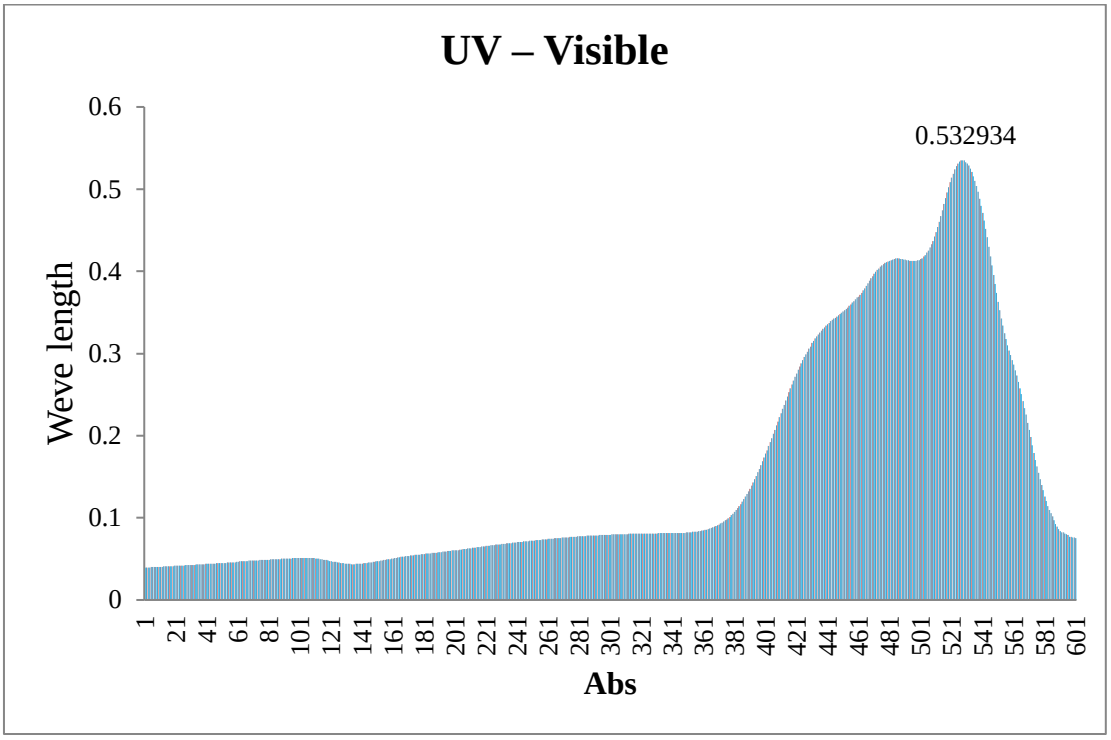


Zeta-Potential

The zeta potential values of the synthesised nanobioconjugates from *Glycyrrhiza glabra* root extract in the present study were well within the stable range (-20 to +20mV), with a shift to the negative side, indicating an overall negative charge on the nanoparticles. The purpose of zeta potential measurement is to indicate the repulsive force that is present and can be used to predict the long-term.stability of the product (Patil et al., 2012).

UV – Visible Spectroscopy

FIGURE 18

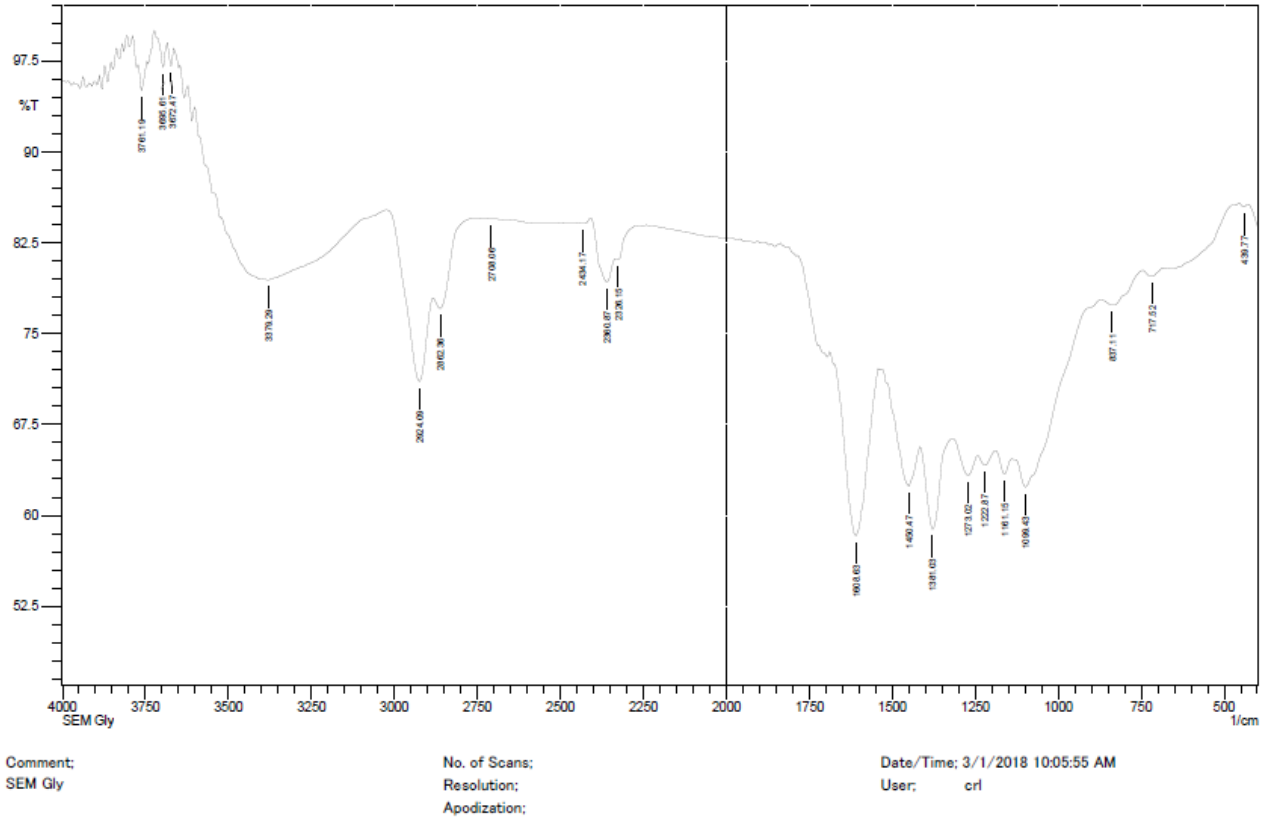


UV-Visible

The VU-Visible spectral analysis of the green synthesised nanoparticles was observed a sharp peak at 529 nm inndicates the 0.5329 wave length inn formation of nanoparticles in *Glycyrrhhiza glara* root extract in the methanolic extract. The UV-VIS Spectral analysis of the green synthesised nanoparticles was observed a sharp peak at 404 nm indicates the formation of silver nanoparticles

Fourier Transform Infrared (FTIR) Spectroscopy

FIGURE 19



FT-IR (Fourier Transform Infrared Spectroscopy)

FTIR measurement was carried out to identify the possible biomolecules in *Glycyrrhiza glabra* root responsible for capping leading to efficient stabilization of the silver nanoparticles. Prominent IR bands are observed at 2914, 2847, 1708, 1601, 1464, 1376, 1008, 880, 716 and 469 cm−1. Most of the IR bands are characteristic of flavonoids and tepenoids present in the root (Monshi *et al.,* 2012).

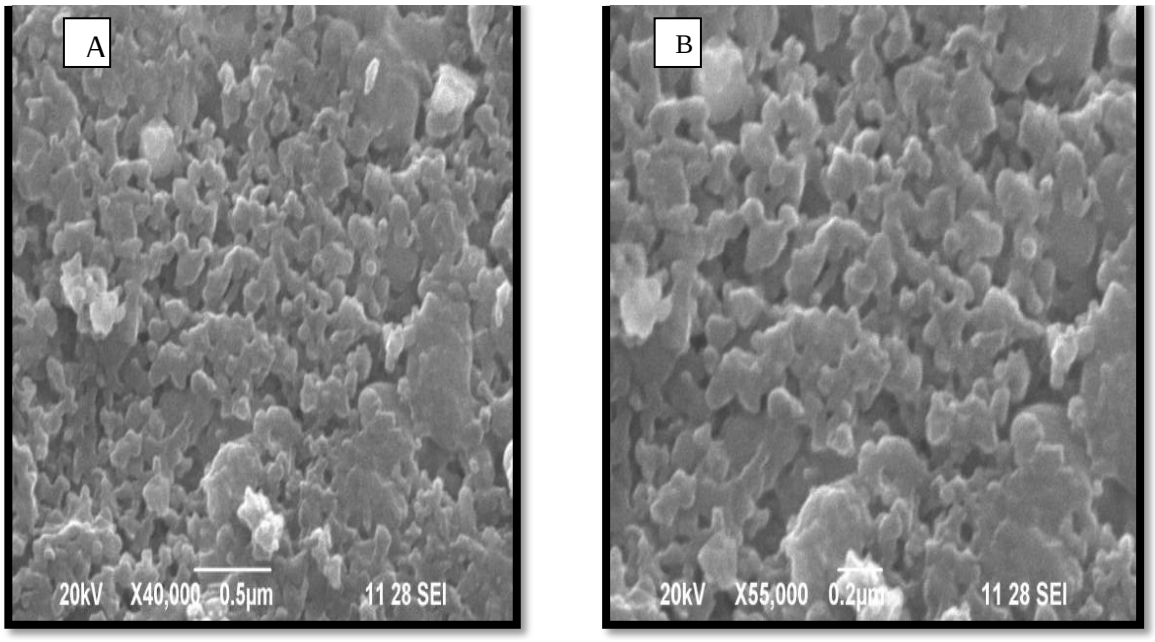
The FTIR spectrum of silver nanoparticles from leaf extract of Nigella sativa indicated the role of different functional groups in the synthetic process (Amooaghaie et al., 2015).

The presence of different functional groups from alkane, methylene, alkene, amine and carboxylic acid present in Artemisia vulgaris leaves that may act as a stabilising agent in AgNPs (Murugan *et al.,* 2015).

SEM –EDX

SEM been used to idenntify the size, shape and morphogy of nanoparticles

FIGURE 20

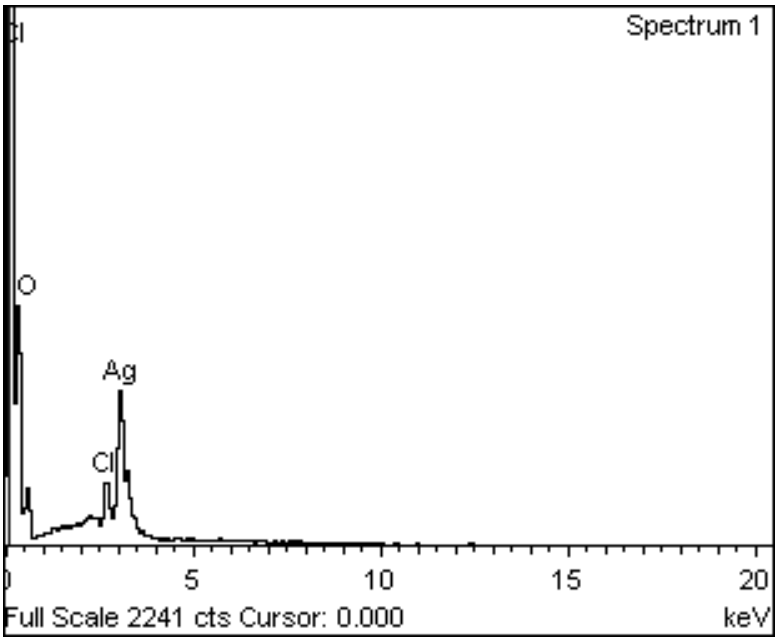


SEM A) 40,000 rpm B) 55,000 rpm

Scanning electron microscopy (SEM) micrographs for the films deposited at various bath temperatures from 55 ℃ to 75 ℃ are illustrated in the figure deposited at lower bath temperature (55 ℃), the films obtained present less compact morphology due to less materials deposited on the surface of the substrate (Kassim, *et al.*, 2011)

The SEM analyysis proved that there was successful conjucation between the organic phytocompounds and silver to form well dispersed spherical nanobioconjucation ranging in size between 0.5µm-2µm.

FIGURE 21



EDX (Eergy Dispersive X-ray analysis)

The EDX spectra of all the synthesized nanobioconjugates in our study showed the presence of carbon, oxygen and silver, which proved the conjugation of organic molecules in the synthesis AgNPs.

Similar results have been reported by several researchers. The presence of carbon and oxygen in AgNPs of *Ocimum tenuiflorum* (black tulsi) (Priya et al., 2014) The EDX spectrum of silver nanoparticles synthesized from Eichhorniacrassipes ascertained the presence silver and also indicated the presence of C, O, K, Cl, Na, Mg, P, Ca and S, which was attributed to the presence of peptides and enzymes present in the plant extract (Thombre et al., 2014).

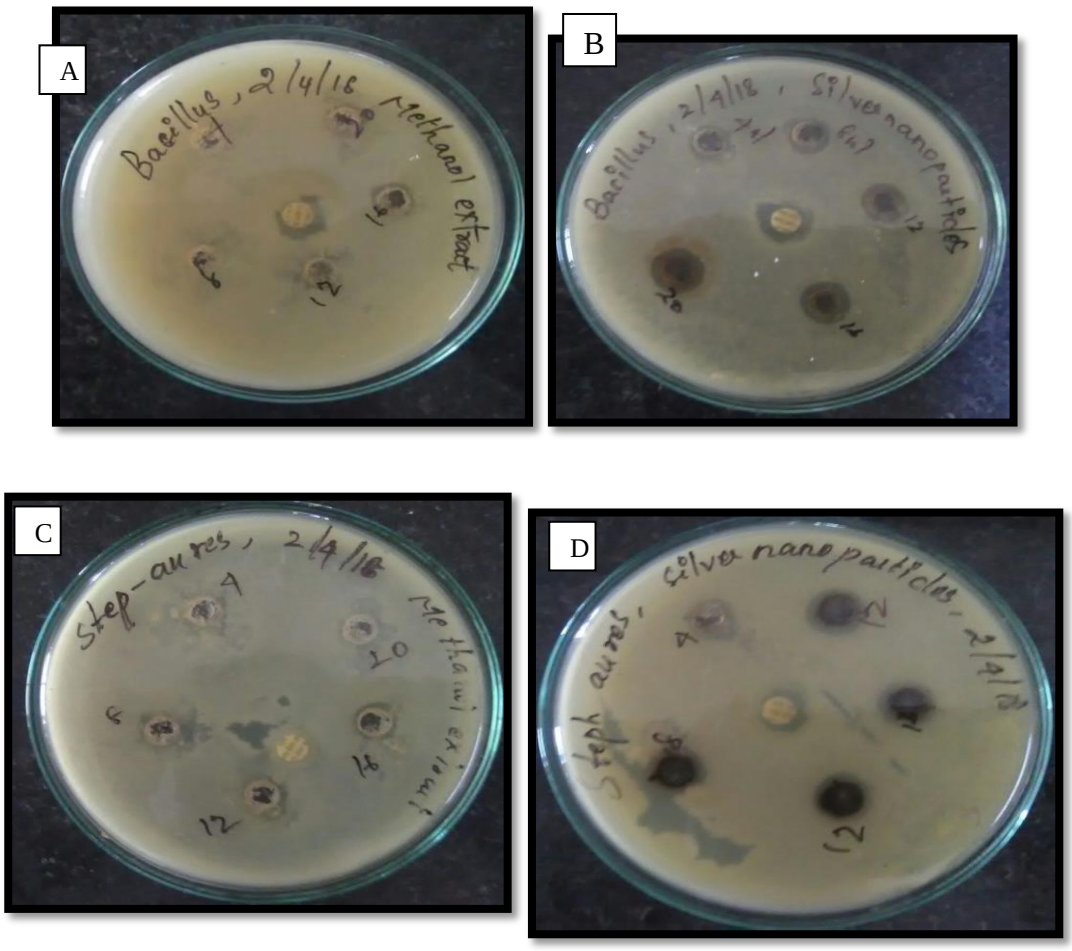
Te green synthesis of stable silver nanoparticles using *Glcyrrhiza glabra* root extarct at room temperature was reported in the study. Synthesis was founnd to be efficient in term of reaction time as well as stability of the synthesized nanoparticles.

ANTIMICROBIAL ACTIVITY

Silver nanoparticles due to their antimicrobial properties have been used most widely in the medicinne food storage, wound dressing and antisephtic creams

WELL DIFLUTION METHOD

FIGURE 22



Antimicrobial Activity

A) Methanol extract of *Glcyrrhiza glabra* on *Bacillus*

B) Silver nanoparticles of *Glcyrrhiza glabra* on *Bacillus*

C) Methanol extract of *Glcyrrhiza glabra* on *S-aureus*

D) Silver nanoparticles of *Glcyrrhiza glabra* on *S-aureus*

Silver nanoparticles of *Glycyrrhiza glabra* showed maximum zone of inhibition against the bacterial species *Bacillus* and *staphylococcus aureous*. Cho *et al.*, 2015 reported that aquoues extract of *Glycyrrhiza glabra* root extract exhibited higher degree of antibacterial activity

The antibacterial activity Ag-NPs and Pt-NPs solution stabilized with PVP for *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) was measured by cup diffusion method. Ag-NPs solution stabilized with PVPshows good antibacterial activity for both S. aureus and E. coli compared with Ag-NPs stabilized in the *Glycyrrhiza glabra* root extract in aqueous (Cho *et al.*, 2005)

5.0 SUMMARY AND CONCLUSION

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrated that many phytochemicals can protect humans against diseases. There are many phytochemicals in seeds, fruits and herbs and each works differently. Natural phytochemicals derived from medicinal plants have gained significant recognition in the potential management of several human clinical conditions, including cancer.

The present study entitled "***In vitro* antioxidative and antimicrobial activity of silver nanoparticles synthesised from *glycyrrhiza glabra* root extract**". The salient findings of current study are summarized below. The extract of *Glycyrrhiza glabra* root were screened qualitatively for the presence of phytochemical constituent such as alkaloids, polyphenols and flavonoids. Enzymic antioxidants (catalase, superoxide dismutase, peroxidase and glutathione-s-transferase) and Non enzymic (ascorbic acid, polyphenols, α tocopherol, reduced glutathione) were analysed in the root of *Glycyrrhiza glabra* to ascertain antioxidant studies.

The qualitative screening of phytochemicals revealed that the presence of phytochemicals namely alkaloids, polyphenols and flavonoids were in the root of *Glycyrrhiza glabra*. It was found that the methanolic extract shows maximum number of phytochemicals than chloroform and petroleum ether.

The root extract of *Glycyrrhiza glabra* showed strong enzymic antioxidant activity. The superoxide dismutase and glutathione-s-transferrase possesses maximum acitivity than catalase and peroxidase. Among the non enzymic antioxidants reduced glutathione and ascorbic acid showed maximum activity than polyphenols and α tocopherol.

Quantitative analysis of phytochemical constituents present in the methanolic root extract of *Glycyrrhiza glabra* showed flavonoids, alkaloids and phenols in higher concentration.

The effect of the methanolic extract of *Glycyrrhiza glabra* roots was found to be more in scavenging the free radicals such as DPPH, hydroxyl, hydrogen peroxide, superoxide, nitric oxide, ABTS when compared to other extracts.

Synthesis of silver nanobioconjugates, their bioactivity was determined as antibacterial effect against the isolates of *Bacillus* and *Staphylococcus aureus* organisms. The synthesized silver nanobioconjugates from Glycyrrhiiza glabra root extracts showed potent antimicrobial activity against both microorganisms. The silver bioconjugates were more effective in inhibiting the growth of the organisms, showing that the antimicrobial efficiency of the biomaterials used can be improved significantly by preparing nanobioconjugates from them.

The X-ray diffraction profiles confirmed the presence of silver in the nanobioconjugates and the highly crystalline nature of the particles. The XRD patterns of the nanobioconjugates synthesised from *Glycyrrhiza glabra* showed additional peaks, indicating the presence of other organic molecules in the extract.

The zeta potential values of the synthesised nano materilas from Glycyrrhiza glabra roots were -4.00 respectively. Conforming the stability of the nanoparticles synthesized. The FTIR spectra of the nanobioconjugates synthesized in the present study revealed the typical functional groups of phenolics, nitro, aliphatic groups, aldehyde, alkanes and alkenes, which are probably engaged in the synthesis of AgNPs from the extracts and compounds.

The EDX profile of all nanobioconjugates showed the presence of typical peaks of silver. Additional peaks were also observed which indicated the presence of carbon and oxygen, representing the existence of organic compounds in the silver nanobioconjugates.

A simple green synthesis of stable silver nanoparticles using *Glycyrrhiza glabra root extract* at room temperature was reported in this study. The synthesised silver nanoparticles showed efficient antimicrobial activities against both Basccillus and *S. aureus*. Benefits of using plant extract for synthesis is that it is energy efficient, cost effective, protecting human health and environment leading to lesser waste and safer products. The synthesised of silver nanoparticle has a potential to use in biomedical applications and will play an important role in near future.

BIBLIOGRAPHY

- Abdel-Aziz, M.S., Shaheen, M.S., El-Nekeety, A.A. and Abdel-Wahhab, M.A. (2014) Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using Chenopodiummurale leaf extract, J. Saudi. Chem. Soc., 18, 356-363
- Allah Ditta. 2012. How helpful is nanotechnology in agriculture -Advances in natural sciences: nanoscience and nanotechnology. Adv. Nat. Sci.: Nanosci. Nanotechnol., 3: 1-10.
- Altemimi.A, Lakhssassi.N, Baharlouei.A, Watson.D.G, and Lightfoot.D.A, (2017) Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts.
- Amooaghaie, R., Saeri, M.R. and Azizi, M. (2015) Synthesis, characterization and biocompatibility of silver nanoparticles synthesized from Nigella sativa leaf extract in comparison with chemical silver nanoparticles, Ecotoxicol Environ. Saf.,doi: 10.1016/j.ecoenv.2015.06.025.
- Anil.K, Jyotsha.D, (2012), Review on *Glcyrrhiza glabra* (Licorice), 1-4
- Arbona.V, Flors.V, Jacas.J, Agustín.P.G and Cadenas.A.G, (2003), Enzymatic and Non-enzymatic Antioxidant Responses of Carrizo citrange, a Salt-Sensitive Citrus Rootstock, to Different Levels of Salinity, 388-394
- Arora.R, Chawla.R, Marwah.R, Arora.P, Sharma.R.K, (2011), Potentialof complementary and Medicine in Preventive Management of novel H1N1 Flu (Swine Flu) Pandemic: Thwating Potential disasters in the Bud. Evidance based complementary and altrannative medicine, 1-16
- Ashokkumar, S., Ravi, S., Kathiravan, V. and Velmurugan, S. (2015) Synthesis of silver nanoparticles using A. indicum leaf extract and their antibacterial activity, Spectrochim. ActaA Mol. Biomol. Spectrosc., 134, 34-39.

- Badami.S & Channabasavaraj.K.P, (2017), In Vitro. Antioxidant Activity of Thirteen Medicinal Plants of India's Western Ghats
- Badkhane.Y, Yadav.A.S., Bajaj.A, Sharma.A.B, Raghuwanshi.D.K, (2015), *Glycyrrhiza glabra* L. A miracle medicinal herb
- Bajpai.V.K, Agrawal.P, (2015), Studies on Phytochemicals, Antioxidant, Free Radical Scavenging and Lipid Peroxidation Inhibitory effects of Trachyspermum ammi seeds.
- Banu.K.S, and Cathrine.L, (2015), General Techniques Involved in Phytochemical Analysis, 25-32
- Bar, H., D.K. Bhui, G.P. Sahoo, P. Sarkar, S.P. De and A. Misra. 2009. Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids Surf A: Physicochem. Eng. Asp., 339: 134–139.
- Bhardwaj.A, Singhal.N, Verma.S, Srivastava.S and Tripathi.K, (2012), Influence of ripening on antioxidant activity of cell wall polysaccharides in prunus armaniaca linn. 2082-2085
- Bindhu, M.R and M. Umadevi. 2013. Synthesis of monodispersed silver nanoparticles using Hibiscus cannabinus leaf extract and its antimicrobial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 101: 184-190.
- Carbone.M.C., Tatone.C, Delle Monache.S,Marci.R, Caserta.D, Colonna.R and Amicarelli.F, (2010), Antioxidant enzymatic defences in human follicular ¯uid: characterization and age-dependent changes, 639-641
- Chauhan.V.B, Kotadiya.C.R, Patel.U.D, Modi.C.M, Patel.H.B, Pandya.K.B, Bhatt.P.R, (2016). In-vitro free radical scavenging activity of different proportions of *glycyrrhiza glabra* and *tinospora cordifolia,* 1041-1045
- Choa.K.H, Park.J.E, Osaka.T, Park.S.G, (2005), The study of antimicrobial activity and preservative effects of nanosilver ingredient, 956-960

- Chodakowska.I.M, Witkowska.A.M, Zujiko.M.E, (2018), Endogenous Non-Enzymatic antioxidant in the human body
- Christine J. Weydert and Cullen.J.J, (2009), Measurement of superoxide dismutase, catalase, and glutathione peroxidase in cultured cells and tissue, 51-66 Conservation Reserve, Vol:1, 2321-2187
- Daisy.P, Celes.W.J, Nivedha.P.R, (2014), HPLC, GC-MS and n-silico analysis of Cucurbita maxima methanolic extract for its activit against Prostate Cancer, 6(2), 500-505
- Dinesh,S., Karthikeyan.S and P. Arumugam,(2012), Biosynthesis of silver nanoparticles from *Glycyrrhiza glabra* root extract. 178-187.
- Elibol, O.H., D.D. Morisette, J.P. Denton and R. Bashir. 2003. Integrated nanoscale silicon sensors using top-down fabrication. **Appl. Phys. Lett., 83**: 4613–4615.
- Gnanajobitha, G., Paulkumar, K., Vanaja, M., Rajeshkumar, S., Malarkodi, C., Annadurai, G. and Kannan, C. (2013) Fruit-mediated synthesis of silver nanoparticles using Vitisvinifera and evaluation of their antimicrobial efficacy, J. Nanostructure Chem., http://www.jnanochem.com/content/ 3/1/67
- Gurunathan, S., K. Kalishwaralal, R. Vaidyanathan, D. Venkataraman and S.R.K. Pandian. 2009. Biosynthesis, purification and characterization of silver nanoparticles usin *Escherichia coli*. **Colloids Surf. B: Biointerfaces, 74**: 328-335.
- Hadrup, N. and Lam, H.R. (2014) Oral toxicity of silver ions, silver nanoparticles and colloidal silver - A review, Regul.Toxicol.Pharmacol., 68, 1-7.
- Halliwell.B,Clement.V.M and Long.L.H,(2000), Hydrogen peroxidase in the human body,10-13
- Harwansh.R.K, Patra.K.C, Pareta.S.K, Singh.J, Biswas.R, Pharmacological Studies *Glycyrrhiza glabra*: A Review, 1032-1038

- Hashimotoa.H, Munck.J.D, Itoc.S, Sanod., Kaga.M, Oguchia.H, Meerbeek.B.V, Pashleye.D.H, (2004), In vitro effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM, 5565-5574
- Hassan.B.A.R, (2012), Medicinal Plants (Importance and Use,
- Heyland.D.K, Dhaliwal.R Suchner.U and Berger.M.M (2005), Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient, 327-337
- Iqbal.Z, Hai.Z, Ping.H.Y, Ghaffar.A, Mumtaz.M, Liaqat.L, (2017), Antioxidant and Antibacterial Activity of Organic Extracts of Roots of *Glycyrrhiza Glabra* Linn, 3(3): 33-37
- Isbrucker.R.A, Burdock.G.A, (2006),Regulatory toxicology and pharmacology, 4(6), 167-192
- Jabin.F and Nasreen.S, (2016), Phytochemical analysis of some medicinal plants, 293-295
- Javanmardia.J, Stushnoff.C, Locke.E, Vivanco.J.M Antioxidant activity and total phenolic content of Iranian *Ocimum accessions* 547-550
- Jenkins, R. and R.L. Snyder. 1996. Introduction to X-ray Powder Diffractiometry. John Wiley and Sons. New York. p. 544.
- Kalimuthu.K, Prabakaran.R, Preetha.V (2014) Antimicrobial and Antioxidant Activities of Ethanolic Crude Extracts of *Turnera ulmifolia* L. 329-333
- Kalsi.S, Dr.Verma.S.K, Neha, Kaur.A, Singh.N, (2016), A Review On *Glycyrrhiza Glabra* (Licorice) And Its Pharmacological Activities
- Kaur.R, Kaur, Dhhids,A., (2013), *Glyycrrhiza glabra:* A phtopharmocological review. 4(7)
- Kawazoe, Y and J.A. Meech. 2005. Nanotechnology: Do good things really come in small packages. Proceedings on 4th International Conference on IntelligentProcessing

and Manufacturing of Materials. J. Meech, Y. Kawazoe, V. Kumar,J.F. Maguire (Eds.). DSE Tech Publications, Inc. Lancaster, PA, USA, Pp. 3-11.

- Kawazoe, Y and J.A. Meech. 2005. Nanotechnology: Do good things really come in small packages. Proceedings on 4th International Conference on Intelligent Processing and Manufacturing of Materials. J. Meech, Y. Kawazoe, V. Kumar, J.F. Maguire (Eds.). DSE Tech Publications, Inc. Lancaster, PA, USA, Pp. 3-11.
- Khan.A.M, Qureshi.R.A, Ullah.F, Gilani.S.A, Nosheen.A, Sahreen.S, Laghari.M.K, Laghari.M.Y, Rehman.S.U, Hussain.I, and Murad.W, (2011), Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings, 6017-6023
- Khandelwal, N., Kaur, G., Chubey, K.K., Singh, P., Sharma, S., Tiwari, A., Singh, S.V. and Kumar, N. (2014) Silver nanoparticles impair Peste des petits ruminants virus replication, Virus Res., 190, 1-7.
- Kirthika, P., Dheeba, B., Sivakumar, R. and Sheik Abdulla, S. (2014) Plant mediated synthesis and characterization of silver nanoparticles, Int. J. Pharm. Pharm. Sci., 6, 304-310.
- Koruk.M, Taysi.S, Savas.M.C, Yilmaz.O, Akcay.F, and Karakok.M, (2004), Oxidative Stress and Enzymatic Antioxidant Status in Patients with Nonalcoholic Steatohepatitis
- Kumar.N, and Nautiyal.S (2011), An Inventory of Medicinal Wealth of Jhil-Mil Jheel
- Kumar.S, (2011), Free radicals and antioxidants, human and food system, 2(1), 129-135
- Kuppusamy, P., Yusoff, M.M., Maniam, G.P. and Govindan, N. (2015) Biosynthesize of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications an updated report, Saudi Pharm. J., http://dx.doi.org/10.1016/j.jsps.2014.11.013

- Lakshmi, P.T.V., Priyanka, D. and Annamalai, A. (2015) Reduction of silver ions by cell free extracts of Westiellopsis sp., Int. J. Biomater., http://dx.doi.org/10.1155/2015/539494.
- Lakshmi.T and Geetha.R.V, (2011), *Glcyrrhiza glabra* Linn Commonly known as Licorice, A therapeutic Review, vol:3
- Madhu.K, (2013), Phtochemical screening and antioxidant activity of *in vitro* grown plants *clitoria ternatea* sing DPPH assay, 6(2), 38-42
- Maheshwari, Abhimanyu A., Umesh A. and Jadhao.K.D, (2016), Quantitative estimation of phenolic compounds in the stem of *cissus quadrangularis l.* 4424-4431
- Moghaddam.M.G, Dabanlou.R.H, Khajeh.M, Rakhshanipour.M, and Shameli.K, (2014), Green synthesis of silver nanoparticles using plant extracts, 548-557 molecular weights, 527-530
- Mullar.FL, Lustgarten.MS, Jang Y, Richardson A, Remmen.V.H, (2007), Trends in oxidative aging theories, free radical Biology and Medicine, 43(4),477-503.
- Murugan, K., Priyanka, V., Dinesh, D., Madhiyazhagan, P., Panneerselvam, C., Subramaniam, J., Suresh, U., Chandramohan, B., Roni, M., Nicoletti, M., Alarfaj, A.A., Higuchi, A., Munusamy, M.A., Khater, H.F., Messing, R.H. and Benelli, G. (2015) Predation by Asian bullfrog tadpoles, Hoplobatrachustigerinus, against the dengue vector, Aedesaegypti, in an aquatic environment treated with mosquitocidal nanoparticles, Parasitol. Res., doi: 10.1007/s00436-015-4582-0
- Nakagawa.K, Kitano.M, Kishida., Hadaka.T, Nabae.K, Kawae.M, Hosoe.K, (2008), A 90-days repeated dose toxicity study of Licorice flavanoids oil (LFO) in rats. 2349-2357
- Nitalikar.M.M, Munde.K.C, Dhore.B.V, Shikalgar.S.N, (2010), Studies of Antibacterial Activities of *Glycyrrhiza glabra* Root Extract, 899-901

- Niture.N.J, Kendre.D.G, Kendre.S.B, Ghiware.N.B, (2015), Antioxidant, Antiinflammatory and Antiasthmatic Activity of *Cissus quadrangularis* Linn
- Ody.P, (2000), The complete Guide Medicinal Herbal, 2nd ed.,
- Orlowski, P.,Tomaszewska, E., Gniadek, M., Baska, P., Nowakowska, J., Sokolowska, J., Nowak, Z., Donten, M., Celichowski, G., Grobelny, J. and Krzyzowska, M. (2014) Tannic acid modified silver nanoparticles show antiviral activity in Herpes simplex virus type 2 infection, PLoS ONE, doi:10.1371/journal.pone.0104113
- Pal, S.L., Jana, U., Manna, P.K., Mohanata, G.P. and Manavalan, R. (2011) Nanoparticle: An overview of preparation and characterization, Int. J. Pharm. Sci., 1, 228-234
- Panche.A.N,Diwan.A.D and Chandra.S.R, (2016), Flavanoids, an overview
- Patil, C.D., Pathil, V.S., Borase, H.P., Salunke, B.K., Salunkhe, R.B. (2012) Larvicidal activity of silver nanoparticles synthesized using Plumeriarubra plant latex against Aedesaegypti and Anopheles stephensi, Parasitol Res., 110, 1815-1822
- Patil, D.C., V.S. Patil, P.H. Borase, K.B. Salunke and B.R. Salunkhe. 2012. Larvicidal activity of silver nanoparticles synthesized using Plumeria rubra plant latex against Aedes aegypti and Anopheles stephensi. Parasitol. Res., 110: 1815- 1822.
- Patolsky, F., G. Zheng and C.M. Lieber. 2006. Nanowire sensors for medicine and life sciences. Nanomedicine, 1: 51–65.
- Prabhavathi R. M, Prasad M. P, and Jayaramu M. (2016), Comparative Studies on Antioxidant Properties of *Cissus quadrangularis* Solvent Extracts.12-17
- Prabhu.S and Poulose.E.K, (2012) Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects

- Priya, B., Satapathy, M., Mukhopahayay, A. and Dap, P. (2014) Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis, Bioresour. Bioprocessing, http://www.bioresources bioprocessing.com/content/1/ 1/3.
- Puneetha,G.K, Mythrashree,S.R, Triveni,M.C, Murali.M, Jayaramu.S.C, and Amruthesh.K.N, (2016), Annalyse phytochemicals and evalution of phenolic contents and antioxidant activities of *Macrosolen parasiticus* L.4(2), 119-124
- Raffi, M., F. Hussain, T.M. Bhatti, J.I. Akhter, A. Hameed and M.M. Hasan. 2008. Antibacterial characterization of silver nanoparticles against *Escherichia coli* ATCC 15224. 24(2): 192-196
- Rahman.T, Hosen.I, Islam.M.M.T, Shekhar.H.U, (2012), Oxidative stress and human health, 997-1019
- Rai.M, Yadav.A, Gade.A, (2009), Silver nanoparticles as a new generation of antimicrobials 73-81
- Rajakannu, S., Shankar, S., Perumal, S., Subramanian, S. and Dhakshinamoorthy, G.P. (2015) Biosynthesis of silver nanoparticles using Garcinia mangostana fruit extract and their antibacterial, antioxidant activity, Int. J. Curr. Microbiol. App. Sci., 4, 944-952
- Rajan.P, Dr. Averal.H.I, (2017), In Vitro Anti Oxidant and Anti Lipid studies on Ethanolic Extract of *Pergularia Daemia* Leaves
- Reddy.K.P,Suhani.S.M, Khan.P.A, and Kumar.K.B, (1995), Effect of light and benzyl adenine on dark treated growing rice leaves, II changes in peroxidase activity, 24: 987-994
- Rice.O.F, (1962), Review of the Properties and Reactions of Some Inorganic Free Radicals

- Sathisha.A.D,Lingaraju.H.B and Prasad.K.S, (2011), Evaluation of Antioxidant Activity of Medicinal Plant Extracts Produced for Commercial Purpose, 882-886
- Saxena.S, (2005), *Glycyrrhiza glabra*: Medicine over the millennium.
- Scrinis, G and K. Lyons. 2007. The emerging nano corporate paradigm nanotechnology and the transformation of nature, food and agrifood systems. International Journal of Sociology Food Agriculture, 15(2): 22–44.
- Shang, L., Nienhaus, K. and Nienhaus, G.U. (2014) Engineered nanoparticles interacting with cells: Size matters, J. Nanobiotechnol., http://www.jnanobiotechnology.com /content/12/1/5.
- Shankar, S.S., A. Rai, B. Ankamwar, A. Singh, A. Ahmad and M. Sastry. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. Journal of Colloid and Interface Science, 275 (2): 496-502
- Shareef.M.I, Gopiat.S.M, Reddy.S.M, Dayananda.P.J.M, Mandal.K.S.S, and Purushotham.K.M, (2014), Antioxidant, free radicals scaveging activity of *Zingibero officinale*, 3(3), 10084,
- Sharma.V, Agrawal.R.C, Pandey.S, (2013), Phytochemical screening and determination of anti-bacterial and antioxidant potential of *Glcyrrhiza glabra* root extract, 7(4A): 1552-1558.
- Sindhu.S and Manorama, (2014), Ethnobotanical, Phytochemical and invitro antioxidant activity of medicinal plant Pimenta dioica (l.) Merr. (Myrtaceae) from Attappadi, Palakkad district, Kerala, 01-07
- Sofia.N.H, Walter.T.M, (2013), Review of *Glycyrrhiza glabra*, Linn.
- Sondi.I and Sondi.B.S, (2004), Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria, 1777-182

- Sumathi.S, and Sangavi.S, (2016), Phytochemical And Invitro Antioxidant Activity Of Aqueous Extract Of Nyctanthes Arbor-Tristis (L) Flower, 3(5), 412-419
- Syu, Y., Hung, J., Chen, J. and Chuang, H. (2014) Impacts of size and shape of silver nanoparticles on Arabidopsis plant growth and gene expression, Plant Physiol. Biochem., 83, 57-64
- Taylor, A.L and J.N. Sasser. 1978. Biology, Identification and control of root knot nematodes (Meloidogyne spp.) Croop. Publ. Deps. Plant Pathology, North Carolina State University and U.S. Agency for International Developement. Raliegh, North Carolina. p. 111.
- Thakur.D, Abhilasha.A, and Ghoshal.G, (2016), Evaluation of phytochemical, antioxidant and antimicrobial properties of Glycyyrhizin extracted from root of *Glycyyrhiza glabra*, 487-494
- Thombre, R., Chitnis, A., Kadam, V., Bogawat, Y., Colaco, R. and Kale, A. (2014) A facile method for synthesis of biostabilized silver nanoparticles using Eichhorniacrassipes (Mart.) Solms (water hyacinth), IJBT, 13, 337-341.
- Trivedi.R and Sharma.K, (2011), Extarct of *Glcyrrhiza glabra* linn. Attentuated Chronic Fatigue Stress Induced bahavourial Alteretions in Mice. 2(3): 996-1001
- Vidhu, V.K., S.A. Aromal and D. Philip. 2011. Green synthesis of silver nanoparticles using Macrotyloma uniflorum. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 83: 392-397.
- Vimala.K, Sivudu.K.S, Mohan.Y.M, Sreedhar.B, Raju.K.M, (2009) Controlled silver nanoparticles synthesis in semi-hydrogel networks of poly(acrylamide) and carbohydrates: A rational methodology for antibacterial application, 567-571

- Weissman.L Garty.J and Hochman.A, (2005), Characterization of Enzymatic Antioxidants in the Lichen Ramalina lacera and Their Response to Rehydration, 6508-6514
- Williams.W.B, Cuvelier.M.E and Berset.C (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity, 25-30
- Wua.H, Guo.J, Chen.S, Liu.X, Zhou.Y, Zhanga.X, and Xu.X, (2013), Recent developments in qualitative and quantitative analysis of phytochemical constituents and their metabolites using liquid chromatography–mass spectrometry, 267-291
- Yasodamma.N, Sree.K.S.S, Alekhya.C and Binny.A.J.R, (2013), in-vitro antioxidant activity and quantitative analysis of total phenolic and flavonoid compounds of sebastiania chamaelea muell. arg. leaf extracts, 623-629
- Yoshikawa.T and Naito .Y, (2002), What Is Oxidative Stress? 271-276
- Zaved, M.F., W.H. Eisa and A.A. Shabaka. 2012. Malva parviflora extract assisted green synthesis of silver nanoparticles. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 98: 423-428.
- Zheng.L.J, Zhu.J.F (2003) Study on antimicrobial activity of chitosan with different.

APPENDIX I

ESTIMATION OF SUPEROXIDE DISMUTASE

(Misra and Fridovich, 1972)

PRINCIPLE

The assay of SOD is based on the inhibition of formation of NADHphenazine methosulphate-nitroblue tetrazolium formazon, the extent of which can be assayed spectrophotometrically at 560nm.

REAGENTS

1. Sodium pyrophosphate buffer (0.025M, pH 8.3)
2. Phenazine methosulphate (PMS) (186µM)
3. Nitroblue tetrazolium (NBT)) (300µM)
4. NADH (700µM)
5. Glacial acetic acid
6. n-butanol

PROCEDURE

PREPARATION OF ENZYME EXTRACT

Plant sample (0.5g) were ground with 3.0ml of sodium pyrophosphate buffer, centrifuged at 2000g for 10 minutes and the supernatant was used for the assay.

ASSAY

The assay mixture contained in a total volume of 3.0ml, 1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS, 0.3ml of NBT, 0.2ml of enzyme preparations and 1.0ml of water. NADH (0.2ml) was added to start the reaction.

The assay mixture was incubated at 30ºC for 90 seconds and the reaction was stopped by the addition of 1.0ml of glacial acetic acid. n-butanol (4ml) was added to the above mixture, allowed to stand for 10 minutes and then centrifuged at 2000g for 5 minutes. The intensity of the chromogen in the butanol layer was measured at 560nm against butanol as blank. The system devoid of enzyme served as control. One unit of enzyme activity is defined as the amount of enzyme causing a 50% reduction in NBT oxidation/minute.

APPENDIX II

ESTIMATION OF CATALASE ACTIVITY

(Luck, 1974)

PRINCIPLE

The UV light absorption of hydrogen peroxide solution can be easily measured between 230 and 250nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. The enzyme activity could be arrived at from this decrease.

REAGENTS

1. Phosphate buffer (0.067M PH 7.0)

   Dissolved 3.522g of $KH_2PO_4$and 7.268g of $KHPO_4.2H_2O$ in distilled water and made up the volume to one litre.

2. Hydrogen peroxide – Phosphate buffer

   Dissolved 0.16 ml of H_2O_2 (10% W/V) to 100 ml phosphate buffer, prepared fresh. The absorbance of the solution should be about 0.5 at 240nm with 1 cm light path.

PROCEDURE

ENZYME EXTRACT

The sample was homogenized in a prechilled mortar and pestle with M/150 phosphate buffer (assay buffer diluted 10 times) at 1 - 4°C and centrifuged. Stirred the sediment with cold phosphate buffer, allowed to stand in the cold with occasional shaking and then repeated the extraction once or twice. The extraction should not take more than 24 hr. The combined supernatants were used for the assay. Used fresh extract for assay.

ASSAY

Read against a control cuvette 3ml of H_2O_2 containing the enzyme solution as in the phosphate buffer (M/15). Pipetted into the experimental cuvette 3ml of H_2O_2 phosphate buffer. Mixed in 0.01-0.04ml sample with the glass or plastic rod flattened at one end. Noted the time it required for a decrease in absorbance from 0.45-0.4. This value was used for calculations. If ‘t’ was more than 60 seconds, repeated the measurement with more concentrated solution of the sample.

APPENDIX III

ESTIMATION OF PEROXIDASE ACTIVITY

(Reddy *et al.*, 1995)

PRINCIPLE

In the presence of the hydrogen donor pyrogallol, peroxidase converts H2O2 to water and oxygen. The oxidation of pyrogallol to the coloured product purpurogalli can be quantified spectrophotometrically at 430nm. The formation of the product is proportional to the activity of the enzyme and can be used as a measure of the same.

$$\text{Pyrogallol} + H_2O_2 \longrightarrow \text{Oxidized pyrogallol} + H_2O$$

REAGENTS

1. Pyrogallol(0.05M); Phoshphate buffer (pH 6.5): 630 mg of pyrogallol in 100ml of 0.1M Phosphate buffer.
2. Hydrogen peroxide (1%)

PROCEDURE

Macerated one gram of the sample with 5 ml (w/v) 0.1M phosphate buffer (pH 6.5) in a homogenizer. Centrifuged the homogenate at 300 g for 15 min. Used the supernatant as the enzyme source. All procedure were carried out at 0-5° C.

Pipetted out 3ml of 0.05 M pyrogallol solution and 0.5 to 1.0 ml of enzyme extract in a test tube. Adjusted the spectrophotometer to read '0' at 400 nm. Added 0.5 ml of 1% hydrogen peroxide in the test cuvette. Recorded the change in the absorbance every 30 seconds upto 3 minutes.E

CALCULATION

Change in absorbance / min	= X
Weight of the plant material taken	= 300 mg
Volume of the extract taken for the assay	= 0.02 ml
Change in absorbance for 1.5 ml extract	= (X / 0.02) x 1.5 – Y
(i.e) Peroxidase activity in 300 mg plant tissue	= Y
Peroxidase activity / g plant tissue	= Yx (1000/300) Units

APPENDIX VI

ESTIMATION OD GLUTATHIONE PEROXIDASE ACTIVITY

(Rotruck *et al.*, 1977)

Principle

A known amount of enzyme preparation was used to react with hydrogen peroxide in the presence of GSH for a specified time pereiod when the screening was measured by the method of Ellman

$$2GSH + H_2O_2 \xrightarrow{Se\text{ - }GPx} GSSH + 2H_2O$$

Reagents

1. 0.4M Tris Buffer
2. 10µM sodium azide
3. 10% TCA
4. 0.4µM EDTA
5. 19µM hydrogen peroxide

Procedure

To 2ml of Tris buffer, 0.2ml of EDTA, 0.1ml of sodium azide and 0.5ml of plant extract were added following by 0.1ml hydrogen peroxide were added to the mixture, mixed well and incubated at 37℃ for 10 minutes along with the tube containing all the reagent expect sample. After 10 min the reaction was arrested by the addition of 0.5ml of 10% TCA centrifuged and supernatant was assayed for glutathione by the method of Ellman

The activities are expressed as µg GSH consumed/min/mg protein

APPENDIX V

ESTIMATION OF GLUTATHIONE-S-TRANSFERASE ACTIVITY

(Habig *et al.*, 1974)

PRINCIPLE

GST conjugates with GSH and CDNB and the extent of conjugation causes a proportionate change in the absorption at 340nm, which can be followed spectrophotometrically.

REAGENTS

1. 1mM – 1-chloro 2,4-dinitrobenzene (CDNB) in ethanol
2. 1mM - Glutathione
3. 0.1M Phosphate buffer
4. 50mM Tris –HCl buffer (pH 7.2)

PROCEDURE

ENZYME EXTRACT

The sample was homogenized with Tris–HCl buffer (pH7.2). The homogenated sample was centrifuged at 4℃ for 30 minutes at 8500rpm.The supernatant was used as the enzyme source.

The estimation was done at 25℃ under condition giving activities linear with respects to incubation time and protein concentration for at least 3 minutes.

The enzyme activity was determined by monitoring the change in absorbance at 340nm in a spectrophotometer. 0.1ml of both substrates GSH and CDNB was taken in 0.1M phosphate buffer (pH 6.5) at room temperature to make a volume of 2.9ml. The reaction was started by the addition of 0.1ml of sample to this mixture; the readings were recorded against distilled water blank for a minimum of three minutes. The complete assay mixture without the sample served as the control to monitor non-specific binding of the substrate. It was taken to ensure that final concentration of ethanol in the mixture was always less than 4%.

APPENDIX VI

ESTIMATION OF α-TOCOPHEROL

(Emmerie-Engel method, 1938 as described by Rosenberg, 1992)

PRINCIPLE

Tocopherol can be estimated using Emmerie – Engel reaction which based on the reduction of ferric to ferrous ions by tocopherols, which then forms a red colour with 2, 2'-dipyridyl. Tocopherol and carotenes are first extracted with xylene and the extraction read at 460nm to measure carotenes. A correlation is made for these after adding ferric chloride and reading at 520nm.

REAGENTS

1. Absolute alcohol
2. Xylene
3. 2, 2'- dipyridyl
4. Standard solution:

Dissolved 10mg/ 10ml of α-tocopherol in absolute alcohol 91mg of œ- tocopherol is equivalent to 100mg of tocopherol acetate.

EXTRACTION OF PLANT TISSUE

The sample was homogenized with water in a blender. Weighed accurately 2.5g of the homogenized sample into a conical flask. Added 50ml of 0.1N H_2SO_4 slowly without shaking. Stoppered and allowed to stand overnight. The next day contents of the flask were

shaken vigorously and filtered through whatman No.1 filter paper, discarding the initial 10-15ml of filtrate. Aliquots of the filtrate were used for the estimation.

PROCEDURE

Into 3 stoppered centrifuge tubes (test, standard and blank), pipetteed out 1.5ml of extract, 1.5ml of standard, 1.5ml of water respectively. To the test and blank added 1.5ml of ethanol and to the standard,added 1.5ml of water. Added 1.5ml xylene to all the test tubes, stoppered, mixed well and centrifuged. Transferred 1.0ml of xylene layer into another stoppered tube, taking care not to include any other ethanol or protein. Added 1.0ml of 2, 2'-dipyridyl reagent to each tube, stoppered and mixed. Pipetted out 1.5ml of the mixture into colorimeter cuvettes and read the extinction of the test and standard against the blank at 460nm. Then in turn beginning with the blank, added 0.33ml of ferric chloride solution.

The amount of vitamin E can be calculated using the formula,

$$\text{Amount of tocopherols in } \mu g = \frac{\text{Reading at 520nm} - \text{Reading at 460nm}}{\text{Reading of standard at 520nm} \times 0.24 \times 15}$$

APPENDIX VII

ESTIMATION OF ASCORBIC ACID

(Roe and Kuether, 1953)

PRINCIPLE

Ascorbate is converted to dehydroascorbate by treatment with activated charcoal and bromine. Dehydroascorbic acid then reacts with 2, 4- dinitrophenyl hydrazine to form osazones, which dissolves in sulpuric acid to give an orange coloured solution whose absorbance can be measured spectrophotomerically at 540nm.

REAGENTS

1. 4%TCA
2. 9N H_2SO_4
3. 2% 2, 4-dinitrophenyl hydrazine: Dissolved 2g of DNPH in 100ml of 9N H_2SO_4
4. 10% thiourea
5. 80% sulpuric acid
6. Stock standard solution: Dissolved 100mg of ascorbic acid in 100ml of 4%TCA
7. Working standard: Diluted 10ml of the stock solution to 100ml with 4%TCA

PROCEDURE

About 1g of the sample was homogenized in 4% TCA up to 10ml. Centrifuged at 2000rpm for 10 minutes. To the supernatant obtained, a pinch of activated charcoal was added, shaken well and kept for 10 minutes. Centrifuged once again and removed the charcoal residue. The volume of the clear supernatants was noted. 0.5 and 1.0 ml aliquots of this supernatant were taken for the assay.

The assay volumes were made up 2.0ml with 4%TCA. 0.2 to 1.0ml of the working standard solution containing 20-100 µg of ascorbate respectively were pipetted out into clean dry test tube, the volume of which were also made up to 2.0ml with 4%TCA. Added 0.5ml of DNPH reagent to all the test tubes, followed by 2 drops of 10% thiourea solution. Incubated at 37°C for 3 hours.

The osazones formed were dissolved in 2.5ml of 85% sulpuric acid, in cold, drop by drop, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of H_2SO_4. The tubes were incubated for 30 minutes at room temperature, and the absorbance was read spectrophotometrically at 540nm. Calculated the content of ascorbic acid in the sample using the standard graph.

APPENDIX VIII

ESTIMATION OF REDUCED GLUTATHIONE

Moron *et al*., (1979)

PRINCIPLE

Reduced glutathione (GSH) was measured by its reaction with 5,5'- dithiobis-2-nitrobenzoic acid (DTNB) (Ellman's reaction) to give a yellow coloured compound that absorbs at 412nm.

REAGENTS

1. TCA (5%)
2. TCA (25%)
3. Sodium phosphate buffer (0.2M, pH 8.0)
4. DTNB (0.6M in 0.2M sodium phosphate buffer)

PROCEDURE

PREPARATION OF PLANT EXTRACT

The leaves of *Gmelina arborea* (0.5g) were ground with 2.5ml of 5% TCA. The precipitated protein was centrifuged at 1000rpm for 10 minutes. The homogenate was cooled on ice and the supernatant (0.1ml) was taken for the estimation of GSH.

ESTIMATION

The volume of different aliquots (0.2 to 1.0ml) was made up to 1ml with phosphate buffer. Freshly prepared DTNB (2ml) was added to the tubes and the intensity of the yellow colour was read at 412nm in a spectrophotometer after 10 minutes. A standard curve of GSH was prepared using concentrations ranging from 2 to 10nmoles of GSH. The concentration of GSH in the samples was calculated from this and the result were expressed as nmoles GSH/g leaf.

APPENDIX XI

DPPH RADICAL SCAVENGING ACTIVITY

(Mensor *et al.*, 2001)

PRINCIPLE

Antioxidants react with DPPH and convert it to diphenyl-picryl hydrazine by donating its OH group. The degree of discoloration from purple to yellow colour can be measured at 519nm, which is a measure of the radical scavenging potential of the extracts.

REAGENTS

1. DPPH (0.3mM in methanol)
2. Methanol

PROCEDURE

The different solvent extracts and crude aqueous extract (5µl) was added with 0.5ml of methanolic solution of DPPH and 0.495ml of methanol. The mixture was then allowed to stand at room temperature for 30 minutes. DPPH methanol solution was used as positive control and methanol alone acted as blank. After incubation, the conversion of purple colour to yellow colour was read at 518nm in a spectrophotometer. The per cent inhibition was calculated using the following formula;

$$\text{Scavenging activity (\%)} = 100 - \frac{A\,(\text{Control})}{A(\text{Control}) - A(\text{Sample})} \times 100$$

APPENDIX X

ABTS RADICAL SCAVENGING EFFECTS

(Shirwaikar *et al.*, 2006)

PRINCIPLE

ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation de-colourisation assay was employed to assess the radical-scavenging effect of the leaf extracts

of the candidate plant. ABTS is a chromogen, which changes into a coloured mono-cation radical form (ABTS+) in the presence of oxidative agent and the ABTS+ has an absorption peak at 750nm. Antioxidants will reduce ABTS+ into its colourless form and the extent of decolourisation corresponds to the per cent reduction of ABTS+.

REAGENTS

1. Ethanol
2. ABTS solution (7mM ABTS with 2.45mM ammonium persulfate).

The solution was incubated at room temperature for 12-16 hours before use.

PROCEDURE

The six different extracts (100µl each) were added to ABTS solution (300µl) and the final volume of each was made up to 1ml with ethanol. The absorbance was read at 745nm and the percentage inhibition was calculated using the formula,

$$\text{Inhibition (\%)} = \frac{\text{A(Control) - A(Sample)}}{\text{A(Control)}} \times 100$$

APPENDIX XI

SUPEROXIDE GENERATION SCAVENGING ACTIVITY

(Winterbourn *et al*., 1975)

PRINCIPLE

The extent of superoxide generation was studied on the basis of inhibition of the production of nitroblue tetrazolium formazon of the superoxide ion by the plant extracts and is measured spectrophotometrically at 560nm.

REAGENTS

1. EDTA (0.1M containing 1.5mg of sodium cyanide/10 ml)
2. Nitroblue tetrazolium (NBT) (1.5mM)
3. Riboflavin (0.12mM)
4. Phosphate buffer (0.067M, pH 7.6)
5. Dimethyl sulfoxide (DMSO)

PROCEDURE

The assay mixture contained 0.02ml of plant (solvent and crude aqueous) extracts with 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.63ml of phosphate buffer.

DMSO, instead of plant extract, was considered as control. All tubes were vortexed and the initial absorbance was read at 560nm. The tubes were illuminated uniformly using a fluorescent lamp for 30 minutes. The absorbance was read again at 560nm. The difference in optical density before and after illumination is the measure of superoxide generation and the percentage inhibition was calculated using the formula,

$$\% \text{ Superoxide Scavenging} = \frac{A \text{ (After illumination)} - A \text{ (Reference)}}{A \text{ (Control)}} \times 100$$

APPENDIX XII

HYDROXYL RADICAL SCAVENGING EFFECTS

(Elizabeth and Rao 1990).

PRINCIPLE

Hydroxyl radicals are generated from a Fe_2+/ascorbate/EDTA/H_2O_2 system, which attacks deoxyribose and eventually produces thiobarbituric acid reactive substances (TBARS). The ability of the plant extracts to inhibit TBARS formation is measured spectrophotometrically at 532nm.

REAGENT

1. Deoxyribose (28mM)
2. $FeCl_3$ (1mM)
3. EDTA (1mM)
4. H_2O_2 (10mM)
5. Ascorbate (1mM)
6. KH_2PO_4-KOH buffer (20mM, pH 7.4)
7. Thiobarbituric acid (1%)
8. HCl (25%)

PROCEDURE

The reaction mixture contained deoxyribose (0.1ml), $FeCl_3$ (0.1ml), H_2O_2 (0.1ml), ascorbate (0.1ml), buffer (0.1ml) and 20µl of leaf extracts which corresponded to 10mg concentration. The total volume was made up to 1ml with water. The tubes were capped tightly and incubated in a water bath at 37ºC for one hour. The reaction was terminated by the addition of TBA (0.5ml) and HCl (0.5ml). The tubes were heated in a boiling water bath for 20 minutes for colour development. The intensity of the pink colour formed, as the indication of TBARS formation, was measured at 532nm. The per cent TBARS produced for positive

control (H_2O_2) was fixed as 100% and the relative per cent TBARS was calculated for the extract treated groups.

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{\text{A (Control)} - \text{A (Sample)}}{\text{A (Control)}} \times 100$$

APPENDIX XIII

HYDROGEN PEROXIDE SCAVENGING EFFECTS

(Ruch *et al.*, 1989)

PRINCIPLE

H_2O_2 scavenging activity was measured in terms of a decrease in the absorbance at 230nm spectrophotometrically.

REAGENTS

1. H_2O_2 (40mM in 0.1M phosphate buffer)
2. Phosphate buffer (0.1M, pH 7.4)

PROCEDURE

The plant extract was diluted to a concentration of 10mg in 10µl. This extract (10µl corresponding to 10mg) was added to 0.6ml of H_2O_2 solution and the final volume was made up to 3ml with the same buffer. After 10 minutes, the absorbance values at 230nm of the reaction mixtures were recorded against a blank containing phosphate buffer without H2O2 for each sample. The percent inhibition was calculated using the formula,

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{\text{A (Control)} - \text{A (Sample)}}{\text{A (Control)}} \times 100$$

APPENDIX XIV

NITRIC OXIDE SCAVENGING ACTIVITY

(Green and Hill, 1984)

PRINCIPLE

An aqueous solution of sodium nitroprusside spontaneously generates nitric oxide at physiological pH, which interacts with oxygen to produce nitrite ions, which is measured at 546nm.

REAGENTS

1. Sodium nitroprusside (100mM)

2. Phosphate buffered saline (PBS) (pH 7.4)

3. Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride)

PROCEDURE

Sodium nitroprusside (2.0ml), phosphate buffered saline (0.5ml) and each of the six different plant extracts (0.5ml) were mixed and incubated at 25ºC for 30 minutes. Griess reagent (0.5ml) was added and allowed to stand for 30 minutes. The control tube was prepared without leaf extracts. The absorbance of the pink coloured chromogen was read at 546nm against a reagent blank.

$$\text{Nitric oxide scavenging activity (\%)} = \frac{A\ (\text{Sample})}{A\ (\text{Control})} \times 100$$

APPENDIX XV

QUALITATIVE PHYTOCHEMICAL ANALYSIS

DETECTION OF ALKALOIDS (Raaman, 2006)

Mayer's test

A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100ml of distilled water) and observed for the formation of crean coloured precipitate.

Dragenodorff's test

An aliquot of the extract was tested with Dragendorff's reagent (solution A: Bismuth nitrate (0.7g) in glacial aceticacid (2ml) and distilled water (8ml) Solution B: potassium iodide (4g) in glacial aceticacid (10ml) in water (20ml), mix Solution A and B dilute to 100ml with distilled water) and observed for the formation of rediish orange precipitate

Wagner's test

A fration of the extract was treated with Wagner;s reagent (1.2g of iodine and 2g potassium iodide in 100ml of distiied water) and observed for the formation of reddish brown coloured precipitate.

Hager's test

To afew ml of filtrate 1 or 2 ml of hager's reagent (Saturated aqueous solution of picric acid) were added. A prominent yellow precipitate indicated the test as positive

DETECTION OF PHENOLS (Raaman, 2006)

Ferric chloride test

To a fraction of theextract, 5% $Fecl_3$ solution was added and obseved for the formation of deep blue colour

Lead acetate test

A fraction of the extract was treated with 10% lead acetate soution and observed for the formation of white precipitate

DETECTION OF FLAVANOIDS

Aqueous NaOH test

To a fraction of the extract, a drop of 1N aqueous NaOH solution was added and observed for the formation of yellow orange colouration

Alkaline reagent test

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavanoids.

DETECTION OF TANNIN

For detection of treated with a few drops of lead solution. The formation of whit precipitate indicates the presence of tannin

IDENTIFICATION OF GLYCOSIDES (Raaman, 2006)

For detection of glycosides, 50mg of extract was hydrolysed with concentrated hydrochloric acid for 2hrs on water both, filtrated and the hydrolysete was subjected to the following tests.

Borntrager's test

To 2ml of filtrated hydrolysate, 3ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it pink colour indicated the presence of glycosides

DETECTION FOR SAPONIN (Raaman, 2006)

Sodium bicarbonate test

In a teat tube,he presence of about 5ml of extract was added and a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. The formation of a honey comb like froth showed of saponins

DETECTION FOR TERPENOIDS (Siddiqui and Ali, 1997)

5ml of extract was mixed with 2ml of chloroform and conc.H_2SO_4 (3m) was carefully added to form of layer. A reddish brown colouration of the interface formed to show positive for the presence of terpenoids.

DETECTION FOR STEROIDS (Siddiqui and Ali, 1997)

Liebermann Burchard reaction

A portion of the extract was treated with 10ml chloroform and filtrated. To 2m of fitrate was treate with 2ml of acetic anhydride and conc. Sulpuric acid. Blue, green ring indicates presence of steroids

TEST FOR PHYTOSTEROLS

To 1ml of plant extract, equal volume of choloroform and 3 drops of concentrated sulpuric acid were added. Formation of rown ring indicates the presence of steroids and formation of bluish green colour indicated the presence of phytosterols

TEST FOR PROTEINS:

To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.

TEST FOR CARBOHYDRATES*:*

Take 1 ml of extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the sample extract.

APPENDIX XVI

ESTIMATION OF FLAVONOIDS

(Zhishen *et al.*, 1999)

PRINCIPEL

Flavonoids react with vanillin to produce a coloured product that can be measured spectrophotometrically at 360nm.

REAGENTS

1. Vanillin reagent (1% vanillin in 70% sulphuric acid)
2. Sulphuric acid (70%)
3. Methanol : water (2:1)
4. Methanol : water (1:1)

5. Hexane
6. Catechin standard (110µg/ 1ml)

PROCEDURE

PREPARATION OF PLANT EXTRACT

The leaves were weighed (0.5g) and divided equally into two parts. One part was extracted with methanol : water (2:1) and other part was extracted with methanol : water in the ratio of 1:1. These homogenates were allowed to stand over night. The next day, the two extracts were combined and evaporated to 1/3rd the original volume. The resultant aqueous extract was cleared of low polarity contaminants by extracting with hexane. The solvent-extracted aqueous layer containing the bulk of the flavonoids was then concentrated.

ESTIMATION

An aliquot of the leaf extract was pipetted out and evaporated to dryness. Aliquots of the standard (0.2 to 1.0ml) were taken in test tubes and made up to 1.0ml with distilled water. Vanillin reagent (4ml) was added to all the test tubes and heated in a boiling water bath for 15 minutes. The absorbance was measured at 340nm after cooling. The values are expressed as mg flavonoids/g leaf.

APPENDIX XVII

ESTIMATION OF TOTAL PHENOL

(Malick and Singh, 1980)

Principle

Phenol react with phosphomolybdic acid in Folin – ciocalteau reagent alkaline medium and produce blue coloured comples (molybdenum blue), which is read in a spectrometer at 650nm.

Reagent

1. 80% ethanol
2. Diluted Folin – ciocalteau reagent
3. 20% Sodium carbonate
4. Stock solution

   100mg of catechol was made up with 100ml of distilled water
5. Working standard

   10ml of stock solution was diluted in 100ml of distilled water. 1.0ml of this contains 100µg of catechol

Procedure

1g of sample was homogenized using 20ml of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20 minutes. The supernatant was saved. The residue was reextracted with 10ml 0f 80% ethanol, centrifuged and collected the supernatant and evaporated to dryness. The residue was dissolved in known volume of distilled water (50ml) and 2.0m was taken in the experiment. A working standard of 0.5 to 2.5ml catechol solution was correspomding to 50-250µg of catechol were pipetted out into a series of test tubes. The volume was made upto 2.5ml with water. To all the tubes added 0.5ml of dilted Folin – ciocalteau reagent. After 3 minutes, added 2.0m of 20% Na_2CO_3 soution to each tube and mixed thoroughly.

The tubes were placed in a boiling water oth for exactly one minute. Cooled and measured at 650nm against of ca reagent blank. Calculate the amount of polyphenol present in the sample used to regression value.

APPENDIX XVIII

ESTIMATION OF TOTAL ALKALOIDS

Aim

To extimate the amount of alkaloids content in the given samples

Materials

1. **Standard**

   Weight 10mg of pure caffeine and dissolved inn 25ml of 20% acetic acid A.R dilute as diquotes. Further 10 times with 20% acetic acid. Thus solution contains 40µg/ml
2. 20% acetic acid
3. Chloroform
4. Anhydrous Na_2SO_4
5. Acetate buffer pH 4.8

Preparation

5.4g of sodium acetate and 2.66ml of glacial acetic acid in 100ml double distilled water

Procedure

1. Into 5 test tubes pipette out 1,2,3,4,5 ml of 40µg/ml solution.
2. The volume of each was made upto 5ml with acetic acid
3. To each test tube 5ml of acetic buffer and 1 ml of methyl orange were added
4. After shaking for 10 secs 5ml of chloroform was added
5. The separated were stopped and shaken for 3 minutes

6. After standing for a few minutes chloroform layer were with drawn into dry test tubes, dried with small amount anhydrous Na_2SO_4
7. Absorbance read on a spectrophotometer at 420nm using 10mm cells
8. From the reading standard curve was constracted.

Calculation

Express the amount in mg/g or 100ml of sample

APPENDIX XXI

WELL DIFFUSION METHOD

Nutrient broth was prepare by 1.3g in 100ml distilled water added 1 g of Agar Agar type 1. Autocleved the petriplate and medium. Medium was poured in after cool the petriplate and medim. Solitify the medium overnight broth was separate the medium plate. Anti – disc was placed in centrol in the plate. Cut the medium by small five wells in sorrounded by disc. Added byy different (4, 8, 12, 16, 20µl) concentration. And kept in to the 37℃ in over night. Also follwed by methanolic extract to allowed by 37℃ in over night. After incubition measure, the zone of inhibition in the compare to methhanolic extract and silver nanoparticle synthesis.