Antioxidant and antibacterial activity of *Quisqualis indica*

Dissertation II Report

Submitted

By

Parmjit kaur

Reg. No. 11510187

In partial fulfillment of M.Sc. in Botany

Under the guidance of

Dr. Anand Mohan

Associate Professor

Department of Bioengineering and Biosciences

Lovely Professional University, Punjab-14441, India

ABSTRACT

In the present study the methanolic leaf extract of *Quisqualis indica* was used for antioxidant and antibacterial activity. The Response Surface Methodology technique was used to optimize the extraction parameters for antioxidant activity. The antibacterial activity was evaluated with Disc diffusion method against Bacterial strain *(E.coli)*. The most significant variable for extraction parameters of antioxidant activity was solvent: solid ratio. The optimum values were found to be temperature (52.5), time (45 min), solvent: solid ratio (45ml/1g) and solvent composition(52.5) were optimum for antioxidant activity. At these optimum levels the value of antioxidant 98.95 which was close to the predicted value 98.92.The total phenolic content of plant extract, as determined by the Folin–Ciocalteu method, was 310mg/g (in GAE).The total flavonoid content was estimated with calorimetric method was 262 mg/g in (QAE). From the phytochemical tests for *Quisqualis indica* was found positive for alkaloids, anthraquinone, tannins, Glycosides, saponins and quinones and negative for cumarin and terpenoids. The methanolic extract possesses antibacterial activity against *E.coli* and the zone of inhibition was measured ranged from 12 to 18 mm.

DECLARATION

I Parmjit kaur, Student of M.sc botany (Honors), Department of Bioengineering and Biosciences, Lovely Professional University, hereby declare that all the information furnished in this dissertation report entitled "Antioxidant and antibacterial activity of *Quisqualis indica*" is based on my own intensive research and is genuine.

This report does not contain copying of any work for the award of any degree or diploma either of this university or any other University.

Parmjit kaur

11510187

CERTIFICATION

This is to certify that Parmjit kaur is undergoing her Project titled "**Antioxidant and antibacterial activity of *Quisqualis indica*"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of the project has ever been submitted for any other degree at any University.

The project is for the submission as part of the requirement necessary for the award of the degree M.Sc. Botany (honors).

Date:

Dr. Anand Mohan

Associate Professor

Department of Bioengineering & Biosciences

Lovely Professional university

ACKNOWLEDGEMENT

I am thankful to almighty God for his mercy and blessing. I take this opportunity to express my heartfelt regards to my beloved parents and my younger brother for their blessings, moral support and faith in me.

I feel great pleasure in expressing my profound indebtedness to Dr.Anand Mohan, Associate professor, Lovely Professional University, Phagwara (Punjab) for his skilled guidance, moral support and encouragement, all along my project work.

I owe my thanks to Dr. Neeta Raj Sharma, Head, School of Biotechnology and Biosciences, Lovely Professional University, Phagwara for her expert guidance and facility provided during the study.

I wish to express heartfelt gratitude to Dr. Joydeep Dutta the Head of Department.

I am thankful to Miss Sabreen Lab-technician, for her cooperation and encouragement she provided me during my lab work. It was always a pleasure to work in such friendly environment; she always provided guidance in the lab.

Thanks are due to all my classmates and to name a few Sarita Saroa, Pavneet kaur and Amandeep kaur, Lovely Professional University, Phagwara, for providing a friendly environment and lending help whenever required in the project lab**.**

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

DPPH	2, 2-diphenyl-1-picrylhydrazyl
DW	Dry Weight
FCR	Folin-Ciocalteau Reagent
GAE	Gallic Acid Equivalent
HCl	Hydrochloric acid
QE	Querecitin Equivalent
STDEV	Standard Deviation
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
RSM	Response surface methodology

Chapter 1

INTRODUCTION

India has a rich culture of Spices and medicinal plants that includes about more than 2000 species and also has huge geographical area that have potential features for, Unani, Ayurveda and Siddha traditional medicines (Sofowora *et al*, 2009). Out of these only few have been used for pharmacological studies. Human health and welfare is one of primary concerned in humankind. (Gupta *et al.*, 2005). There are different wide varieties of traditional procedures have been developed by different cultural groups to maintain and recover the health of large sectors of population (King, 1994). Two third part of the world population is still used herbal medicines to cure from different types of diseases. So the medicinal plants played an important role in the development of human culture.

Medicinal plants belong to big group of plants with a great interest due to its pharmaceutical, cosmetic and nutritional application. (Hill, 1989) . These plants are used for the production of large variety of chemical compounds. These compounds have ability to fight against many pathogens such as bacteria fungi, insect herbivorous and mammals. (Farnsworth and Soejarto, 1991). These plants possess many properties like antibacterial, antifungal, immune modulatory *etc.*, and they are act as pharmaceutical drugs. Any part of plants (roots, stem, leaves, fruits, flowers) is used to synthesize medicines. Medicinal plants are the sources of many novel drugs. In the early development of the medicinal plants uses were instinctive, as is the case with animals (Stojanoski *et al.*, 1999).

Sometimes fact that there was not adequate information concerning what are the reasons for the diseases or concerning which plant and how it could be used as a cure, everything was based on experience (Akerele,1992). Now these specific medicinal plants that are used for treatment of certain diseases caused by bacteria and other organisms were being discovered; thus, the medicinal plants usage gradually unrestricted the empiric framework and became founded on explicatory fact (Kelly, 2008). World health organization estimated that 80 percent population of the Asian and African countries are using the herbal medicine for some aspect of primary health care (WHO, 1993).

Historical background of medicinal plants:

The plants are use as medicines to cure from disease goes back to early man. According to the fossil records date the plants are use as medicines at least to the middle Paleolithic age (Hosseinzadeh *et al*, 2015). By the time of Ancient Egyptian civilization, great wealth information about medicinal plants already exited. The information about the hundreds of remedies of plants was preserved in papyrus about 3500 years ago (Nazim, 2012). Ancient china is great source of information about the medicinal plants (Xiao, 1988).Chinese used the first natural herbal preparation as medicines. Many hundred remedies of herbal found their way from china to Japanese system of traditional healing. These herbs are native to japan were classified in the first pharmacopoeia of Japanese traditional medicine in the 19th century (Saito, 2000). In 18th century the British doctor William withering discovered that foxglove from traditional European herbal medicine is used for treatment of dropsy (Nazim, 2012).

In 1949, the German chemists reported that roots of the Rauvolfia are used for the treatment of high blood pressure and he extracted the reserpine alkaloid from this plant (Balick, 1997). In 1972 the major biological active compound, is first isolated from wormwood (*Artemisia annua*) that have ability to fight against Malaria is reported by Chinese chemists (Mamedov and cracker, (2008). Researches on traditional medicinal plants were discovered alkaloids from Madascar periwinkle (*Catharanthus roseus)*, that is used in the chemotherapy of childhood leukemia and also used for treatment hodkings disease .More than 30 cardiac glycosides have been isolated from dried foxglove leaves including digitoxin and digoxin is reported in 20th century (Balick,1997) .

Significances of Medicinal Plants to Human Being

- The parts of Medicinal plants such as roots, leaves and flowers are normally used as raw materials for the extraction of active ingredients that are used in the synthesis of different types of drugs.
- Medicinal plants are indirectly used as the synthesis of modern medicines for example aspirin, paracetamol, codeine etc. in the pharmaceutical labs by different processes.
- Chemical studies of medicinal plants are also help to understand about the toxic nature of plants and to protect human and animals from natural poisons.

- The medicinal plants are playing an important role to protect biological diversity, for example metabolic engineering of plants by their cultivation and preservation.

Most of the Indians are using medicinal plants to cure them from different types of disease caused by pathogens. In the last era, the demand for the antimicrobial compounds is increasing due to developing clinical microbial strains resistant to one or several antibiotics (Diana, 2010). The objective of this study is to optimize extraction parameters for antioxidant activity and evaluate the antibacterial activity of plant from leaves of *Quisqualis indica* plant. Response research methodology was used to optimize the effects of extraction time, solvent solid ratio, and solvent composition for antioxidant activity of *Quisqualis indica*.

Antibacterial activity: antibacterial activity of medicinal plants is help to inhibit the growth of pathogenic bacteria's that causes diseases. There are many researches has explored that the leaves of many medicinal plants have antibacterial activity are *Ziziphus sativa, Calotropis procera, Ajuga bracteosa, Zingiber officinale, Quisqualis indica etc.*

Antioxidant activity: Antioxidants are the molecules that are used to inhibit the process of oxidation of other molecules. In the process of oxidation it donates electrons or hydrogen from a substance is to an oxidizing agent. After these oxidation reactions are completed free radicals are produced they damaged the cells or tissues. Antioxidants are used to stop these chain reactions by scavenging free radical intermediates, and inhibit different oxidation reactions that damage the cells. (Parkash and Gupta, 2005).

Today, new important antioxidant compounds are being discovered from plant sources and some medicinal plants that are already searched for their antioxidant properties are *Withania somnifera, Ocimum santum Piper nigrum, Quisqualis indica, Arentium lappa, Scutellaria barbata, Daucus carota etc.* **(**Hosseinzadeh *et al*., 2015).The present study is focused on the *Quisqualis indica* plant for evaluate its antioxidant and antibacterial activity.

Botanical classification (Plant taxonomy)

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Myrtales.

Family Combretaceae

Species Q.*indica*

Habitat and distribution

It is evergreen and large scandant shrub that is grown as ornamental climber.it is an excellent vine for outdoor gardens and does not require deep and anchoring roots. This plant is widely distributed all over the world especially on China, Phillipines, Bangladesh, Myanmar and Malaysia and now broadly grown throughout the India. It is produced best yield in tropical areas (Sahu and Dubey, 2012).

Cultivation and collection

The plant is grown and best blooms in full sunlight. It require regular water to keep the soil moist and needed more water in hot season and less in cold climate. The plant has ability to tolerant drought conditions also. For its growth and development it requires fertile soil that is mixed with sand that can easily retain.

Botanical Description

It is an impressive tropical vine and strong climber grown as ornamental plant. It is ligneous vine that can reach 2.5 to 8 m in height. It is well grown under favorable conditions with fresh green foliage and lush on branches (Munir, 1995). The plant bearing leaves are oblong to elliptic, 7-15 cm in length with round base and acuminate tip. They are arranged in simple and opposite manner. The stems are thorny in nature having pink and red colored flowers that exudes a strong fragrance at night (Murphy, 1988).These beautiful colored flowers are presence in the

form of clusters. The fruits are 2.5-3 cm long and narrowly ellipsoidal in shape. The taste of the fruits is like almonds when they are mature. Seeds are pentagonal, black color and 12-15mm long with five prominent wings.

Useful parts of the plant

As well as ornamental plant *Quisqualis indica* is used as traditional medicines due to the presence of phytoconstituents in the parts of the plant. These parts are leaves, Roots, flowers, seeds that contain active ingredients which are responsible for pharmaceutical property that act against many pathogens and free radicals (Joshi, 1992).

Importance of *Quisqualis indica*

- Roots, seeds and fruits decoction can be used as anthelmintic to expel parasitic worms and gargling. (Munir,1995) (Murphy, 1988).
- The methanolic extract of leaves of *Quisqualis indica* Linn plant was extensively investigated for its antipyretic activity against Brewer's yeast induced pyrexia model in rats (Nitu singh, 2010).
- The Quisqualis indica plant has anti-inflammatory and also PG synthesis inhibition property. (Gautam,2007) (yadav, 2011).
- The methanolic extract of *Quisqualis indica* leaves, flowers and Roots showed antioxidant activity because it have ability to scavange the free radicle and protect from the cell damage and also showed antibacterial activity (Kaisar,2010).

Chapter 2

REVIEW OF LITERATURE

There is lot of literature reporting about the medicinal values of *Quisqualis indica*. *Quisqualis indicia* Linn. Creeper belongs to the family, Combretaceae and genus Combretrum. This genus is represented by 370 species of trees and shrubs.

Studies on Phytoconstituents:

Sexena *et al*., (2013) conducted studies on Photochemistry of medicinal plants. He reported that the phytochemicals or phytoconstituents are present in all Medicinal plants that have play important role in preventing chronic diseases like diabetes, cancer and coronary heart diseases. These are the substance produced by all plants they have ability to fight against many pathogens and produced beautiful aroma in the plants (Hasler *et al*., 1999). These are the end product of metabolism process occurring in the plants. The phytoconstituents are present in *Quisqualis indica* are listed in Fig. 1 is reported in 1997 by Ta Chen Lin et al.

Table 2.1 List of Phytoconstituents present in the *Quisqualis indica*

Phytoconstituents	**Examples**
Alkaloids	trigonelline
Flavnoids	rutin
α-amino acids	L-proline and L-Asparagine
Enzymes	Isoenzyme A and isoenzyme B

Ishizaki *et al*., 1973 reported that Quisqualis acids and tannins are present in the seeds, leaves and flower of *Quisqualis indica.* sugary substance similar to levulose and organic acid similar to catharatic acid is present in it .The seeds of this plant contain fixed oil that contain palmitic, stearic, olieic , linoleic acid and arachidic acid .All thses hytoconstituents are responsible for pharmacological activity of *Quisqualis indica*.

Alkaloids:

(Mueller, 1992) reported that alkaloids are organic compounds that containing heterocyclic nitrogen atoms are bitter tasting in nature found in many vascular plants. These compounds are one of the largest classes of secondary metabolites derived from amino acids like lysine, aspartate, tyrosine, and tryptophan There are nearly 10,000 different types alkaloids are reported (Singh, 2009). Alkaloids are insoluble in water but their salts are soluble in water. The distribution of the alkaloids is restricted to some of angiosperms. The alkaloids are mostly found in Storage tissues, seeds, fruits and roots of the plants (Diana, 2010). In the early year of the 19th century the alkaloids were first obtained from the plant materials and it was found that these alkaloids were nitrogen containing bases which formed salts with acid.so they were known as the vegetable alkalis also and they are used as the local anesthetic and stimulant as cocaine (Krishna *et al*., 1983)

Examples of alkaloids are: **Nicotine**: It is tobacco alkaloid that is synthesized from nicotinic acid obtained from tobacco plant. **Caffeine**: It is obtained from coffee beans and tea leaves. **Morphine**: It is powerful narcotic drug used for relief of pain obtained from opium poppy plant. **Codeine**: it is methyl ether derivative of morphine. It is an excellent analgesic obtained from opium poppy plant

Trigonelline: Trigonelline is an alkaloid has been reported to utilize several pharmacological activities, including anti-hyperglycemic and anti-hyperlipidemic by Zhou *et al*., 2012 .It is a major active constituent of plants like coffee, fenugreek etc. so they are used as traditional medicines (Folwarczna, 2014) and in *Quisqualis indica* plant reported by Chen *et al*., 1997) .It is bitter in taste and next to caffeine most prevalent non protein nitrogenous compound. It is the product of methylation of the nitrogen atom of niacin i.e vitamin B_3 also known as nicotinic acid.Trigonelline is soluble in water or warm alcohol. Trigonelline is act as antidiabetic, anticancer, antibacterial agent.

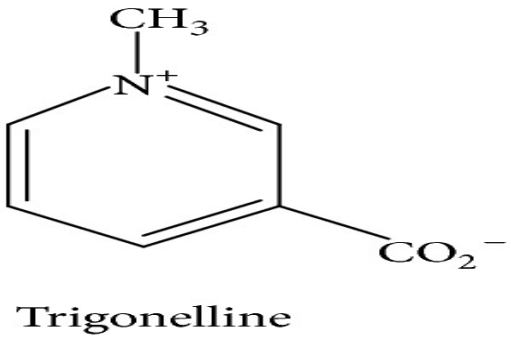

Fig 2.1 Structure of trigonelline

Terpenoids:

These are also largest class of secondary metabolites that occurs in all plants. They are natural products derived from five carbon isoprene units (Elbe in, 1999). It is the simplest terpenoid that emits volatile gas during the process of photosynthesis by leaves that protect the cell membranes of leaves damaged from high temperature. They are classified according to the numbers of isoprene units from they are construct. The terpenoids are the primary components of essential oil that produce fragrance of the plant. These essential oils also act against many fungal and bacterial pathogens. Harborne, 1999 reported that terpenoids are act as flavoring and fragrance agent in many cosmetics and foods so they are commercially interesting products. cosmetics examples are menthol and sclareol .

Phenolics :

Phenolics are another class of secondary metabolites produced by plants having ability to fight against pathogens (Singh *et al*., 2009). They are produced through shikimic acid and malonic acid pathway in plants and they include variety of chemical compounds are flavonoids, tannins, lignin etc. these toxic molecules disrupts the metabolism pathway of pathogens and also in their toxicity (Rojman et al., 2007). Tannins are water soluble in nature and they toxic to insects by inactivation of the proteins .Lignin are found in the secondary cell wall of the plants thus the walls become lignified and they are heterogeneous polymers. Lignin is formed physical barriers in cell walls that protect the plants from pathogen attacks (Singh, 2009). There are three main groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols (Sexena *et al*.,

2013).There were various Biological properties of phenolic acids reported .Phenolic acid have ability to act as antiulcer, anti- inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic, and antidepressant agent(Silva *et al*.,2013).

Flavonoids:

Flavonoids are polyphenol compounds that are ubiquitous in nature. Pridham *et al*., 1960 reported that more than 4,000 flavonoids have been known, many of flavonoids occur in vegetables, fruits and beverages like tea, coffee and fruit drinks are most exclusive studied compounds. Flavonoids are consisting of flavonoid nucleus in which of two benzene ring linked by close or C3 open structures. . More than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known. These are the most common group of the polyphenol compounds. The best known flavonoids are kaempferol and quercetin. The flavonoids have ability to consume free radicals and antiflammatory activity. Examples are flavnoles, flavones, flavones, rutin etc.

Tapas *et al*., 2008 have been reported about different biological properties of flavnoids including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities etc.but the flavanoids have ability to act as powerful antioxidants which helps to human body protect from cell damage

Rutin: Rutin is example of flavonoids that is present in fruits and vegetables. It is used to synthesize medicines. The flowers of *Quisqualis indica* contain rutin reported by Chen Lin *et al.,* 1997. It is present in many others plants include buckwheat, Eucalyptus macrorhyncha. rutin helps to strengthen the blood vessels. Rutin has chemicals that scavange free radicals and protect from various diseases. The flavonoids (rutin) have ablity to act as antibacterial, anti-allergic, anticarcinogenic agent, free radical scavengers. Rutin is important secondary metabolite have potent antioxidant power.

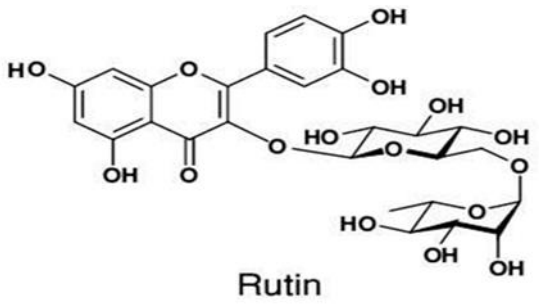

Fig 2.2 Structure of rutin

Studies on Antioxidant activity:

Extensive screening studies and researches were conducted for many medicinal plants with identification of their bioactive principle compounds responsible for their antioxidant properties and other physiological actions. These studies for antioxidant properties of medicinal and food plants have been completed increasingly for the last few eras in hope of discovery an efficient medication for several present-day diseases (Halliwell B., 2008). The recent study on the antioxidant activity, total phenolic content and toxicity of *Quisqualis indica* was done by Ahmed et al 2016. This study revealed that the antioxidant and cytotoxic activities of Q. *indica* fractions were determined by different antioxidant and cytotoxic assays. The Q. *indica* showed strong antioxidant activity by DPPH, phosphomolybdenum and reducing power activities, and it contain high amount of polyphenolic compounds. Furthermore, this study also showed a significant cytotoxic effect done via BSLT and MTT assays.

Mohammed and hajam, 2016 conducted study on Free radical scavenging activity of three different flowers-*Hibiscus rosa-sinensis, Quisqualis indica and Senna surattensis*. These flowers were taken to evaluate their antioxidant activity and estimation of total phenolic content with different solvents such as ethanol, water, and absolute ethanol. The results of this study showed that the highest total antioxidant capacity at concentration of 500 mg/L was found in S. *surattensis* as 0.479 ± 0.001. Scavenging activity of the flower extracts of H. *rosasinensis*, Q. *indica* and S. *surattensis* against 1, 1-diphenyl-2-picrylhydrazyl radical showed the highest activity of (90.20 ± 0.29)% with 500 mg/L.

One such study was done by (Anu *et. al*, 2014) on the assessment of antioxidant property of *Quisqualis indica* where methanol extract of flowers and leaves of Quisqualis indica were studied for antioxidant activity. The flowers and leaves have ability to scavange free radicals. The study showed that the antioxidant of the flower of *Quisqualis indica* involved three antioxidant assays based on different mechanisms namely DPPH, superoxide radicle and cation radicle.Another study was done by Milan *et.al* 2010 on the five different extracts from the whole of *Marrubium peregrinum* for antioxidant activity, Total phenolic content, Total flavonoid content. The study showed that this plant is act as antioxidant agent in terms of its ability to scavange free radicle so it is natural source of antioxidant with high importance. It is noticed that the methanolic extract of this plant showed high concentration of phenolic content.

The study on Free radical scavenging activity of *Quisqualis indica* was done by Bose *et al.,* 2009. The study showed that the leaves of this plant have ability to scavange free radicals such as superoxide ion, hydroxyl, and nitric oxide radicles. The ethyl acetate leaf extracts showed better activity than hydro alcoholic and aqueous. So the the leaves of quisqualis indica plant are effective against various free radicles.

Studies on Antibacterial activity:

Most of the medicinal plants having ability to inhibit the growth of the bacteria or kill the bacteria as shown by many work and studies. One such study conducted on Antibacterial activities of ethanol extracts of 12 Philippine medicinal plants against multidrug-resistant bacteria by (**Demetrio**, .2015). In this study the Crude ethanol extracts from 12 Philippine medicinal plants were evaluated for their antibacterial activity against multidrug resistant bacteria like methicillin-resistant, vancomycin-resistant, and carbapenem-resistant. Most of these plants leaf extract showed antibacterial activity against methicillin-resistant and vancomycin resistant bacteria.

Kumar *et.al* 2014 conducted study on the antibacterial activity of *Quisqualis indica* against two gram positive bacteria (M. *luteus* and B. *subtilis*) and one gram-negative (E. *coli)* were determined by agar well diffusion method. The study showed that the flower extracts of this plant with different types of solvents (methanol, ethanol, and Aqueous) have remarkable against the tested bacterial strains. The methanolic extract of flowers showed better activity than others.

Another such was study conducted by Mickymaray *et al*., 2015 on the screening and antibacterial activity of selected five indian medicinal plants such as *Acalypha indica*, *Aerva lanata, Juss. ex Schult. (A. lanata), Clerodendrum inerme (L.) Gaertn. Pergularia daemia (Forsk.) Chiov. and Solanum surattense Burm. f.* against the opportunistic bacterial pathogens that are isolated from HIV infected patients for the potential phytoconstituents in plant extracts. The study revealed that A. *indica* and A. *lanata* showed high antibacterial activity when compared with the other plant extracts tested.

Kusuma *et al*., 2014 conducted studies on some medicinal plants used by the Bentian tribe from Indonesia for evaluate the antibacterial activity against propionibacterium acnes and candida albicans were determined with agar well diffusion method. The study showed that ethanolic extracts of plants have good ability to kill or inhibit the growth of the bacteria. The potential of plants used by Bentian tribe from indonesia showed strong antimicrobial activity.

Evaluation of antibacterial activity of some selected Angiosperm flower extract in cludes *Quisqualis indica*, *Calotrophis gigantea* and *Polianthes tuberose* with methanol solvent *Klebsiella pneumoniae, Proteus mirabilis, Pseusdomonas aeruginosa, , Esherichia coli, Methicillin Resistant Staphylococcus aureus and Bacillus subtilis.* These flower extracts were prepared in both dry and wet forms. The study showed that the wet flower extracts of calotropis gigantean and polianthes tuberosa showed no any antibacterial activity but dry flower extracts of all three plants showed strong antimicrobial activity against bacterial strains (Kiruthika *et al* ., 2011).

Sanguri *et al*., 2012 conducted studies on the comparative screening of antibacterial and antifungal activities of some Weeds and medicinal plants leaf extracts. In this study medicinal plants were. *Calotropis procera Ait Quisqualis indica Linn*. *Achyranthes aspera Linn*. and *Ocimum sanctum Linn*. against ten microorganisms including of five bacteria *Escherichia coli Bacillus subtilis, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and five fungi *Aspergillus flavus, Alternaria porri, Aspergillus niger, Aspergillus oryzae , Penicillium chrysogenum)* using well diffusion method. The study showed that methanol extract was more effective than Aqueous extract. It is reported that leaf extract of *Quisqualis indica Linn*. and *Achyranthes aspera Linn*. was more effective on fungal species leaf extracts of *Calotropis procera Ait*. and *Ocimum sanctum Linn*. was more effective on bacterial species.

Agarwal *et.al* conducted studies on GC-MS Analysis and Antibacterial Activity of Aerial Parts of *Quisqualis indica* Plant Extracts. According to this study the three solvents like methanol, ethyl acetate and hexane used for extraction of dried aerial parts of plant for antibacterial activity was done by broth dilution method. This showed that 12, 15 and 18 compounds were confirmed by GC-MS qualitatively in methanol, ethylacetate and hexane extracts respectively and showed that all these extracts showed significant antibacterial activity against *Escherichia coli, Staphylococcus aureus Klebsiella pnemoniae,* and *Staphylococcus pneumonia* with ampicillin drug taken as positive control.

RATIONAL AND FUTURE SCOPE OF THE STUDY

- Pathogenic bacteria and free radicals is a problem that continues to challenge the healthcare sector in almost all parts of the world in both developing and developed countries. Therefore study on this plant will help to cure human health from diseases caused by bacteria and from cell damaged.
- Sometimes the synthetic antioxidants and antimicrobials are produced harmful effects on the human health and therefore, the food industry has interested to seek other natural alternatives i.e. medicinal plants having antioxidant and antibacterial activity.
- Free radicals damaging biomolecules such as lipids, proteins, RNA and DNA that induces numerous diseases this plant have ability to scavange these free radicals and protect biomolecules from damaging.
- This extract from this medicinal plant can be directly incorporated in the healthcare industry and can act as potent antibacterial agent for the reduction of implication of diseases

PROPOSED RESEARCH OBJECTIVES AND SCOPE

- To optimize extraction parameters for antioxidant activity of methanol extracts of leaves of *Quisqualis indica* by using Response surface methodology (RSM).
- To evaluate total phenol and flavonoid content from methanolic extract of leaves of *Quisqualis indica.*
- Evaluation of different extracts for the qualitative chemical tests for the identification of various phytoconstituents such as alkaloids, carbohydrates, protein, amino acids, steroids and sterols, glycosides, flavonoids, tannins, triterpinoids.
- To evaluate the susceptibility of *Esherichia coli* bacterial strain in vitro to the methanolic extract obtained from *Quisqualis indica*.

Chapter 3

Materials and Methods

3:1 Chemical and reagents

DPPH (2, 2- diphenyl-1-picrylhydrazyl), methanol, Gallic acid , Sodium acetate, acetic acid, hydrochloric acid (HCl), ferric chloride hexahydrate (FeCl3.6H2O), vitamin C, Sodium carbonate, methanol, Folin - Ciocalteu reagent and gallic acid, Aluminum chloride, ethanol, methanol and quercitin, sodium bicarbonate, sulphuric acid sodium hydroxide, chloroform, potassium hydroxide, Ferric chloride.

3:2 Spectrophotometer measurements

For quantitative analysis and measurements systronic spectrophotometer 106 was used to quantify the Total Phenolic content (TPC), Total Flavonoid content (TFC), and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) activity.

Table 3.1 List of instruments were used in this study.

S.no	Instrument
1	Weighing balance
2	Refrigerator
3	PH meter
4	Water bath
5	Autoclave
6	Laminar Air flow
7	Hot air oven
8	Incubator
9	Mixer Grinder

3:3 Collection of Plant material:

The leaves of the *Quisqualis indica* were procured from the campus of the Lovely Professional University, Jalandhar. These leaves were dried for 1 week at room temperature and then made into powder with Mixer grinder.

3.4 Extract Preparation:

The dried leaves of the *Quisqualis indica* were grinded with the help of Mixer Grinder. The methanolic leaf extract of the plant were obtained using Temperature control water bath. The extraction is carried by followed independent variables Temperature (35C◦-70◦C), time (30-60 min) Solvent composition(35%-70%) , and solvent solid ratio(30ml/1g -60ml/1g) and extraction steps(1-3).

3.5 Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Free radical scavenging assay:

The free radicle scavenging activity of plant extract against DPPH is evaluated by adopting the method of *Brand Williams et al* 1995 with slightly modifications. DPPH is stable free radical which reacts with plant extract (antioxidant compound) which donate hydrogen and then reduce DPPH. The color change from deep violet to light yellow was measured at 517nm on UV visible light spectrophotometer. The methanol solution of DPPH $6x10^5$ was prepared daily fresh for experiments. Then 100µg/ml concentration of individual plant extract (of different conditions) in triplicates mixed with 3 ml of DPPH solution. These samples were kept for 15 minutes in dark at room temperature and decreased in absorbance was measured.

3.6 Estimation of total phenolic content

The estimation of total phenolic content is done by using the Folin-ciocalteu (FC) reagent method with some modifications given by Singleton and Rossi, 1965.According to this method Gallic acid used as standard and the results are reported at Gallic Acid Equivalent because the Gallic acid is small units of phenol present in plants. So from Gallic acid the presence of phenol is estimated. The calibration curve was prepared by adding 0, 2, 4, 6, 8, 10 ml of the Gallic acid stock solution into 100ml volumetric flasks and then dilutes this volume with distilled water. The

phenol concentration 100, 200, 300, 400, 500 µg/L gallic acid was prepared. From each callibration curve blank, solution and sample, 40µl was pipetted into separate test tubes and added 3.16 ml distilled water and then mixed 200µl of the Folin –ciocalteu reagent. After five minutes 600µl of the sodium carbonate was mixed. Then the prepared solution was kept at 40◦C for 30 minutes then absorbance taken at 715nm against blank.

3.7 Estimation of total flavonoid content

The total flavonoid content is estimated by used Aluminum chloride calorimetric method of chang *et al*., 2002 with slight modifications. Different concentrations quercetin like 20, 40, 60, 80, 100 µg in methanol from stock solution were prepared. Then pippeted 0.5 ml from each concentration mixed separately 1.5 ml of methanol, 0.1 ml of aluminium chloride (10%) and then potassium acetate (1M) and then add 2.8 ml of distilled water then the absorbance of this reaction mixture at 415nm and results were reported as mg/g of dry weight.

3.8 Phytochemical screening

Qualitative analysis of phytochemicals

Sample of methanolic extract *Quisqualis indica* was screened as per the protocols described by (Bhatnagar, S., et al, 2012), (Zohra, et al, 2012) and (Soni, A & Sosa, S., 2013). Stock solution of the crude extract was prepared with concentration 1mg/ml.

Test for Alkaloids: 1 ml of crude extract of methanol was mixed with 2ml Wagner's reagent. Precipitate of reddish brown shows the presence of alkaloids.

Test for Quinones: 1 ml of crude extract of methanol was added with 0.2 ml dilute NaOH. Presence of quinine is shown by blue green or red coloration.

Test for Steroids: 1ml of extract of methanol was added with 2 ml of acetic anhydride and 2 ml of sulphuric acid. It shows the change in color from violet to blue or green in samples, indicating the presence of steroids.

Test for Coumarin: 1ml of crude extract of methanol was added with 0.2 ml of 10% NaOH followed by 0.2 ml chloroform and yellow color was observed, indicating the presence of coumarin.

Test for Terpenoids: 1 ml of crude extract of methanol was added with 200µl of chloroform and 600µl of Conc. H2SO4, following Salkowski test with proportion of 5:2:3 of Extract: Chloroform: Conc. H2SO4. A reddish brown coloration was observed, showing the presence of terpenoids.

Test for Tannins: 1ml of extract was added with 200µl of 1% ferric chloride in drops. Precipitation of greenish black indicates the presence of tannin.

Test for Anthraquinone: 1ml of extract was added with 2 ml of 5 % KOH. Pink coloration shows the presence of anthraquinone.

Test for Saponins: 1ml of extract was treated with 2ml of 1% sodium bicarbonate shaked. Soapy bubbles are formed, indicating the presence of saponins.

3.9 Antimicrobial activity evaluation

Antimicrobial activity is the activity that is used to kill the bacteria and inhibit the growth of bacteria .This is employed by Disc diffusion method. This method was used to screen antimicrobial activities of the extracts.

Preparation of media for bacterial culture

Nutrient agar was used for culture of two bacteria, Escherichia coli and Staphylococcus aureus. pH was adjusted to 7.3 using 1 N NaOH or 1 N HCl. Then plug the conical flask with cotton cap and wrap it with brown paper. The media was autoclaved at 15 psi for 20 min at 121oC. The media were dispensed inside the Laminar Air Flow into autoclaved Petri plates as per requirement.

Disc diffusion method

Disc diffusion method was used to determine the antibacterial activity of the plant extract. Disc diffusion technique was used to determine the antibacterial activity of the methanolic plant

extract.The agar plates are inoculated with *E.coli* bacterial strain. Then, filter paper discs (about 6mm in diameter), containing the test compound at a different concentrations, are placed on the agar surface. Petri plate containing test organism and Nutrient agar media was kept as negative control and Petri plate containing media with test organism along with chloramphenicol (antibiotic) disc was kept as positive control. All the Petri plates were then left on the bench for 15 minutes for adequate diffusion of the extract and incubated at 37°C for 2 days. After incubation, the diameter of the zones of inhibition around each well was measured to the nearest millimeter.

Chapter 4

Result and discussion

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Free radical scavenging assay

In this present study, the antioxidant activity of the methanolic extract of the leaves of *Quisqualis indica* was investigated with DPPH scavenging assay and optimize the condition for antioxidant by using Response surface methodology (RSM). The stable free radical (DPPH) was used to evaluate the antioxidant property of the plant extract in terms of its ability to scavenge free radical.

4.1 Screening of extraction parameters using placket Burman design criterion

The total six variables were examined to optimize condition for antioxidant activity by using placket-Bur man design criterion shown in (Table 4.1).The placket-Bur man design pattern was selected for the screening of significant variables for the antioxidant activity of *Quisqualis indica* and corresponding responses are shown in (Table 4.2).The suitability of the model determined and the significant variables were analysis with regression analysis shown in (Table 4.3). Among the six extraction parameters (temperature, time, particle size, solvent solid ratio and solvent composition and extraction parameters) that were used for studied, only four were found significant parameters (Temperature, time, solvent solid ratio, solvent composition) that influence the antioxidant activity of *Quisqualis indica* because their P values (<0.05 at 5% level) that is obtained from Regression analysis.

4.2 Effect of Temperature, time, solvent solid ratio and solvent composition

To optimize the level of the significant extraction parameters obtained from the placket Burman design, the central composite design was applied by Response surface methodology (RSM). 27 experiments were obtained from the design and experimental values and predicted value from model were shown in (Table 4.4) all the experiments were done in duplicates. The effects of temperature, time, and solvent: solid ratio, solvent composition on the antioxidant activity was shown in (Table 4.4) .The fig from 4.2-4.7 shown the contour map of effects of solvent

composition, solvent solid ratio, temperature, time on the antioxidant activity. Among these parameters the solvent: solid was analysis having the highest impact on the antioxidant activity while the extraction steps and particle size have negative linear impact on the antioxidant activity. This was evident from the low p value obtained from the regression analysis. Out of these four parameters, solvent solid ratio was found to have highest impact on antioxidant activity by the highest linear coefficient followed by temperature solvent composition while particle size and extraction steps have negative linear effect on antioxidant shown in table 4.5. This table also shows the interaction between solvent solid ratio and time has significant effect on the antioxidant activity other interaction between other variables are insignificant. Analysis of variance for the antioxidant from this design was given table 4.6. The optimum values were found to be temperature (52.5), time (45 min),solvent:solid ratio(45ml/ig) and solvent composition(52.5) were optimum for antioxidant activity. At these optimum levels the value of antioxidant 98.95 which was close to the predicted value 98.92.

Table no. 4.1 Level of extraction parameters for antioxidant of *Quisqualis indica* by using placket Burman design criterion**.**

Extraction	Extraction condition	High level (+)	Low level(-)
X_1	Temperature	70◦C	35◦C
X_2	Time	60 min	30 min
X_3	Solvent: solid ratio	60:1 ml/g	30:1ml/g
X_4	Particle size	1.2mm	0.6mm
X_5	Solvent composition	70(v/v)	35(v/v)
X_6	Extraction steps	3	1

Table no.4.2 Antioxidant activity of *Quisqualis indica* using the different levels of extraction variables of Placket-Burman design criterion

Run	$\mathbf{X}_1$	X_2	X_3	X_4	X_5	X_6	Antioxidant activity
1	+	_	+	_	_	_	57.1200
2	+	+	_	+	_	_	62.8000
3	_	+	+	_	_	_	55.9517
4	+	_	+	+	_	+	55.8617
5	+	+	_	+	+	_	68.2600
6	+	+	+	_	+	+	59.8900
7	_	+	+	+	_	+	55.6317
8	_	_	+	+	+	_	55.7217
9	_	_	_	+	+	_	61.4850
10	+	_	_	_	+	+	61.9450
11	_	+	_	_	_	+	58.5283
12	_	_	_	_	_	+	55.4317

Table 4.3 Regression analysis for placket Burman design criterion for the prediction of the significant extraction.

Term	Effect	coefficient	S.E of coefficient	T	P
constant		59.052	0.4145	142.46	0.000
Temperature	3.854	1.927	0.4145	4.65	0.006
Time	2.249	1.125	0.4145	2.71	0.042
Solvent: solid	-4.712	-2.356	0.4145	-5.68	0.002
Particle size	1.816	0.908	0.4145	2.19	0.080
Sol.composition	2.980	1.490	0.4145	3.59	0.016
Extraction steps	-0.324	-0.162	0.4145	-0.39	0.712

Temperature, time, solvent solid ratio,solvent composition were significant (<0.05) T,T ratio= coefficient and p,Probability.

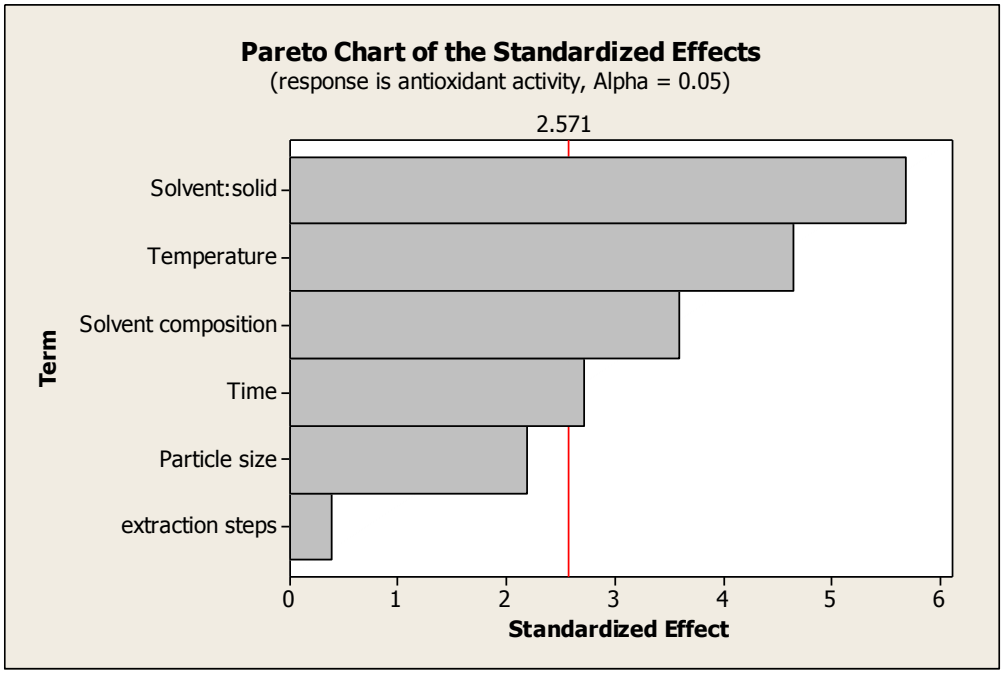

Fig.4.1 Factorial Fit: antioxidant activity versus Temperature, Time, solvent composition, solvent solid ratio particle size and extraction steps.

	Table 4.4.Central composite design criterion of Extraction parameters with corresponding experimental and predicted values.						
Run order		Extraction parameters level				Antioxidant activity	
	Temperature	Time	Solvent solid ratio	Solvent composition		Experimental values	Predicted values
1	35	30	45	52.5		97.0854	97.085
2	70	30	45	52.5		93.9887	93.989
3	35	60	45	52.5		95.8804	95.880
4	70	60	45	52.5		95.3638	95.364
5	52.5	45	30	35		94.9371	94.937
6	52.5	45	60	35		98.3000	101.119

7	52.5	45	30	70		96.1204	96.120
8	52.5	45	60	70		89.2521	89.252
9	35	45	45	35		94.4688	94.469
10	70	45	45	35		98.3000	101.062
11	35	45	45	70		97.5271	97.527
12	70	45	45	70		87.3204	87.320
13	52.5	30	30	52.5		96.0688	96.069
14	52.5	60	30	52.5		95.9588	95.959
15	52.5	30	60	52.5		95.5304	95.530
16	52.5	60	60	52.5		95.8104	95.810
17	35	45	30	52.5		95.2658	95.266
18	70	45	30	52.5		95.1392	95.139
19	35	45	60	52.5		96.6025	96.603
20	70	45	60	52.5		93.1158	93.116
21	52.5	30	45	35		98.2442	98.244
22	52.5	60	45	35		98.9092	98.909
23	52.5	30	45	70		93.4825	93.483
24	52.5	60	45	70		92.9875	92.988
25	52.5	45	45	52.5		98.7800	98.920
26	52.5	45	45	52.5		98.6700	98.920
27	52.5	45	45	52.5		98.9200	98.920

Table 4.5 Regression analysis for central composite design criterion data for antioxidant activity of Quisqualis indica

Model parameter	Regression coefficient	S.E.s coefficient	T	p
Constant	98.7900	0.3565	277.140	0.000
Temperature	-1.1335	0.1782	-6.360	0.000
Time	0.0425	0.1782	0.238	0.816
Solvent:solid	-0.4066	0.1782	-2.281	0.042
Solvent composition	-2.2058	0.1782	-12.376	0.000
Temperature*Temperature	-2.1236	0.2673	-7.943	0.000
Time*Time	-0.9670	0.2673	-3.617	0.004
Solvent:solid*Solvent:solid	-1.8681	0.2673	-6.988	0.000
Solvent composition* Solvent composition	-2.1497	0.2673	-8.041	0.000
Temperature*Time	0.6450	0.3087	2.089	0.059
Temperature*Solvent:solid	-0.8400	0.3087	-2.721	0.019
Temperature*Solvent composition	-3.5095	0.3087	-11.368	0.000
Time*Solvent:solid	0.0975	0.3087	0.316	0.758
Time*Solvent composition	-0.2900	0.3087	-0.939	0.366
Solvent:solid*Solvent composition	-2.5578	0.3087	-8.286	0.000

SE coefficient, standard error , T,T ratio = regression of coefficient /SE regression P probablity

Table 4.6 Analysis of varienec for antioxidant activity using central composite design criterion

Source	d.f.	sequential SS	Adjusted SS	Adjusted MS	P	P
Regression	14	197.177	197.1770	14.0841	36.95	0.000
Linear	4	75.808	75.8080	18.9520	49.72	0.000
Square	4	41.073	41.0727	10.2682	26.94	0.000
Interaction	6	80.296	80.2963	13.3827	35.11	0.000
Residual Error	12	4.574	4.5744	0.3812		
Lack-of-Fit	10	4.543	4.5430	0.4543	28.94	0.034
Pure Error	2	0.031	0.0314	0.0157		
Total	26	201.751				

Df degree of freedom, SS sequential sum of square, Adjusted sum of square, F statistics test to determine significance and P probability.

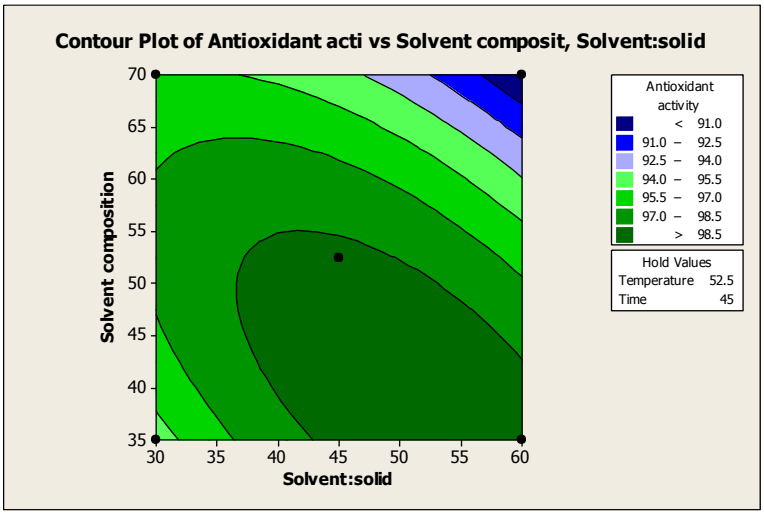

Fig 4.2 Contour plot of Antioxidant activity vs. solvent composition and solvent: solid ratio.

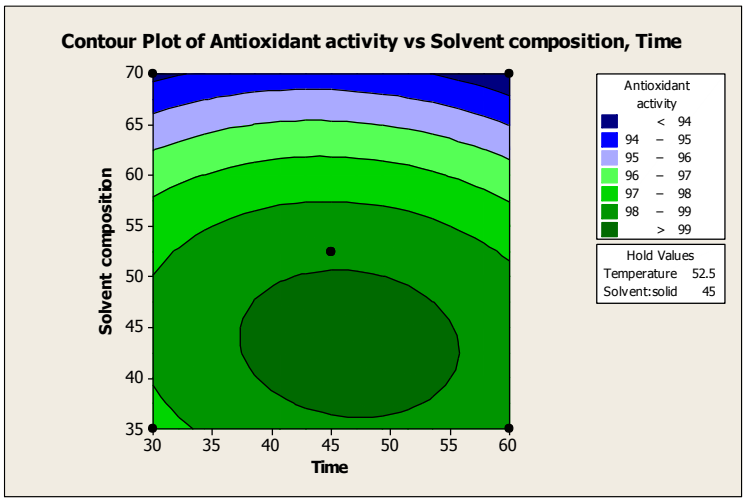

Fig4.3 Contour plot of Antioxidant activity vs. solvent composition and time.

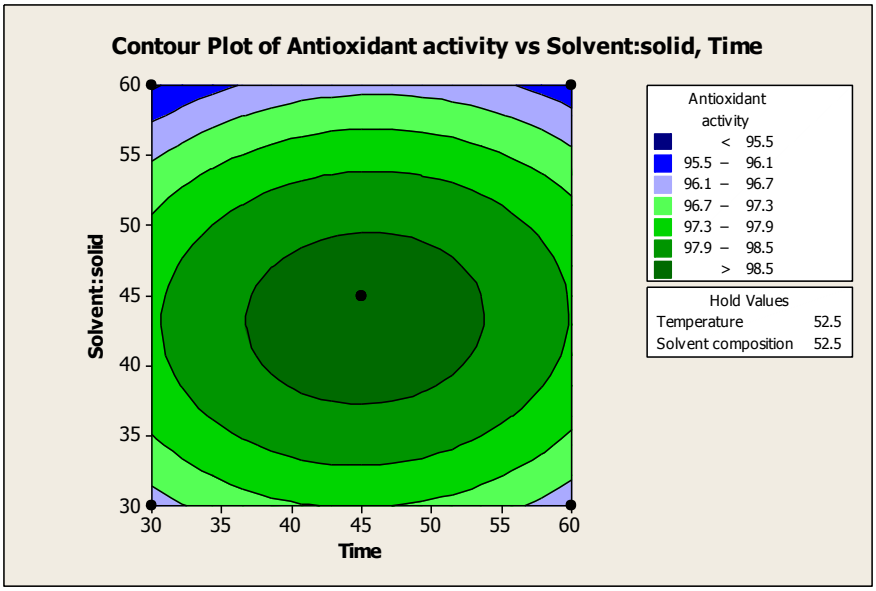

Fig 4.4 Contour plot of Antioxidant activity vs solvent: solid ratio and time.

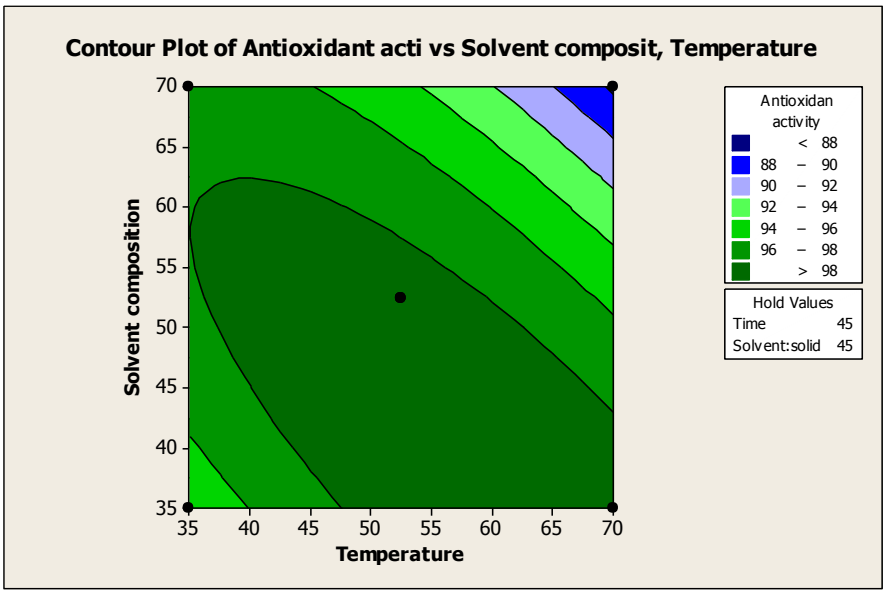

Fig 4.5 Contour plot of antioxidant activity vs solvent composition and temperature.

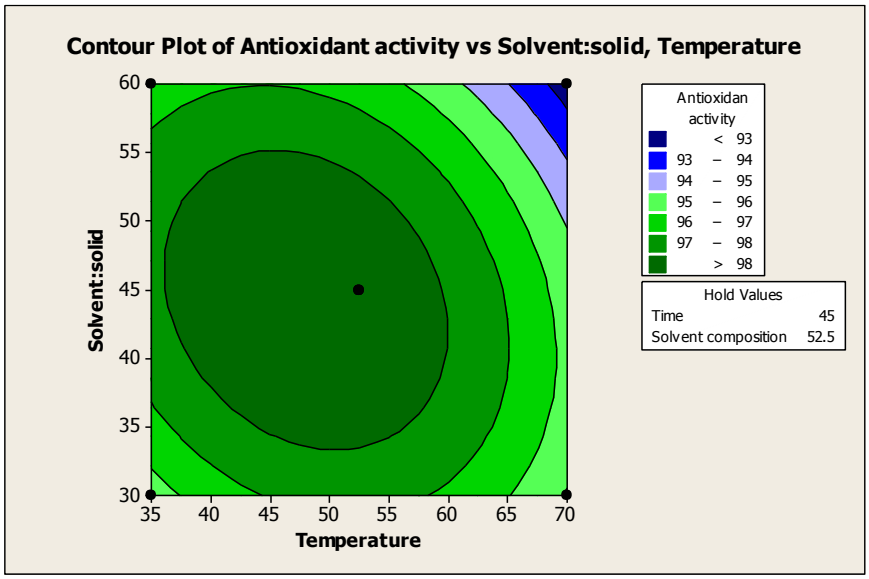

Fig 4.6 Contour plot of antioxidant activity vs. solvent: solid and temperature

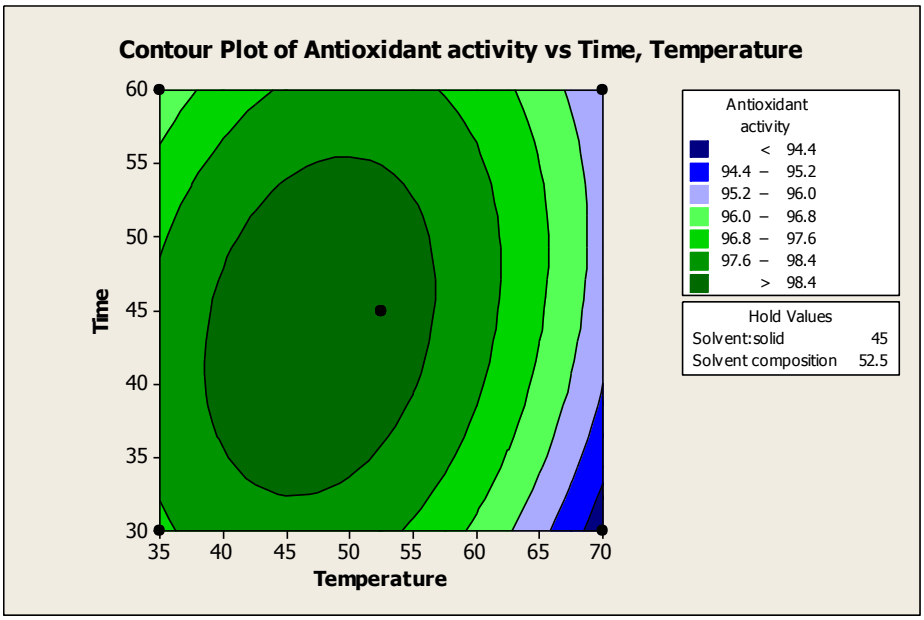

Fig 4.7 Contour plot of antioxidant activity vs time and temperature.

Total phenolic content: Total Phenolic Content (TPC) of the plant samples were determined spectrophotometric ally with Folin-Ciocalteu colorimetric method and expressed with calibration curve as Gallic Acid Equivalents (GAE) (Y= 0.0013x + 0.037, R2= 0.9982 shown in Fig 4.8.The methanolic extract of *Quisqualis indica* contain amount of total phenol is 310mg/g.

Table 4.7 Shows the absorbance of the Gallic acid at different concentration

Serial no.	Concentration of GA (µg/ml)	Absorbance at 765nm			Mean ± SD
		1st	2nd	3rd	
1	100	0.172	0.168	0.171	0.170 ±0.002
2	200	0.295	0.298	0.296	0.296± 0.001
3	300	0.404	0.454	0.428	0.428± 0.025
4	400	0.615	0.522	0.598	0.578 ±0.049
5	500	0.684	0.691	0.687	0.687± 0.003

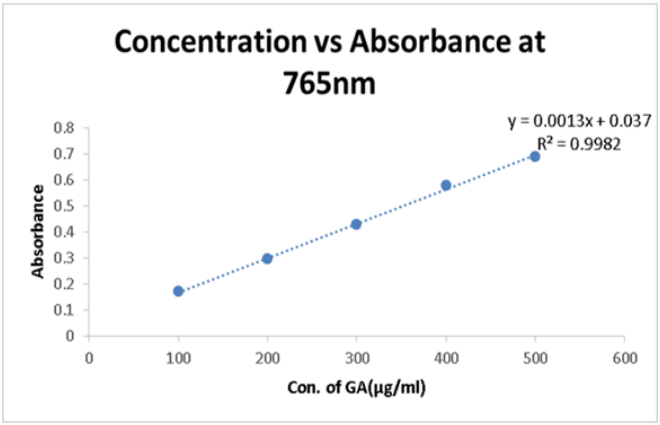

Fig 4.8 Gallic acid standard curve for Total phenolic content

Total flavonoid content: Flavonoids are important secondary metabolites present in plants, often used in the pharmacology for their ability to inhibit enzymes, anti-inflammatory and anti-microbial activity. They are also potent water – soluble antioxidants and free radical scavengers, which help to prevent the cells damages through oxidation and have strong anti-cancerous activity. Therefore with an interest to quantify the content of flavonoids in selected medicinal plants, TFC was determined and results expressed as mg/g of dry weight as Quericitin Equivalent curve (Y= 0.0014x+ 0.0029, R2 = 0.9924) shown in fig 4.9.The plant extract contain 262 mg/g of flavonoid content.

Table 4.8 Show the absorbance of Quercitin at different concentration.

Serial no.	Concentration of Quercitin (µg/ml)	Absorbance at 765nm			Mean ± SD
		1st	2nd	3rd	
1	20	0.035	0.030	0.025	0.03 ±0.005
2	40	0.066	0.067	0.065	0.066± 0.001
3	60	0.089	0.083	0.087	0.084± 0.003
4	80	0.119	0.112	0.117	0.116 ±0.003
5	100	0.151	0.150	0.145	0.148± 0.003

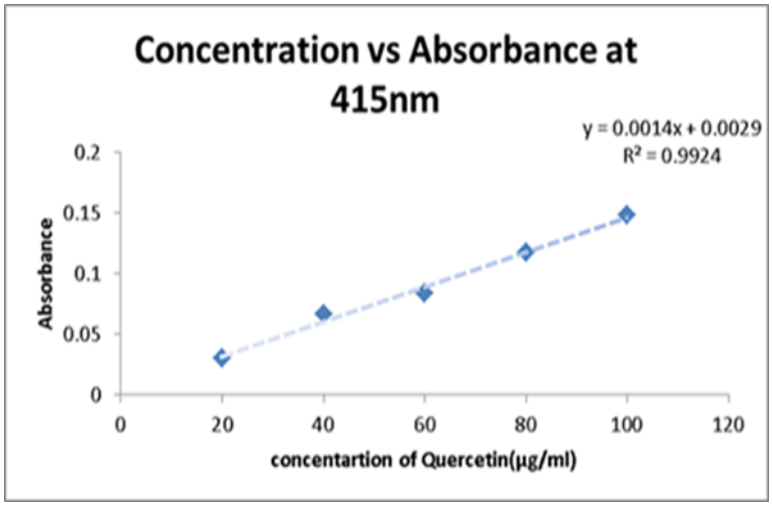

Fig 4.9 Quercitin standard curve for Total Flavonoid content.

Quantitative analysis of *Quisqualis indica*

Phytochemical screening:

Phytochemical screening of methanol extract of *Quisqualis indica* was carried out to check the potency of presence of bioactive compouds through screening of chemical reaction with change in color indicates the presence of chemical constituents shown in the below table. It is quantified as (+) indicate the presence of phytochemical and (-) indicate the absence of phytochemical constituents.

From the phytochemical tests that are conducted for *Quiqualis indica* was found positive for alkaloids anthraquinone, tannins, Glycosides, saponins and quinones and negative for cumarin and terpenoids.

Table 4.9 Phytochemical analysis of methanolic extract of *Quisqualis indica* .

Phytochemicals	**Methanol extract of plant**
Alkaloids	+
Cumarin	–
Anthraquinone	+
Tannins	+
Glycosides	+
Terpenoids	–
Saponins	+
Quinones	+

Note: (+) presence of phytochemical and (-) absence of phytochemical.

Antibacterial activity:

The antibacterial activity of the leaf extract of *Quisqualis indica* were studied in different concentration (20, 40, 60, 80) against bacterial strain (*Esherichia coli*) .*E.coli* are gram negative, rod shaped bacteria mostly found in the environment, food, human intestine. The zone of growth inhibition of the methanolic extract of *Quisqualis indica* was measured ranged from 12 to 18 mm shown in table 4.10.The present study showed that the plant extract possess strong antibacterial activity against *E.coli* bacteria. The results of antibacterial activity of plant extract were compared with standard antibiotic drugs. (Chloramphenicol)

Table 4.10 Antibacterial activity of the flower extracts against - *Esherichia coli* zone of inhibition in diameter (mm).

Serial no.	Conc of the sample µg/ml	Zone of inhibition in mm			Mean±SD
		1st	2nd	3rd	
1	20	12	13	11	12±1
2	40	15	13	16	14.6±1.52
3	60	17	17	18	17.3±0.52
4	80	17	19	18	18±1

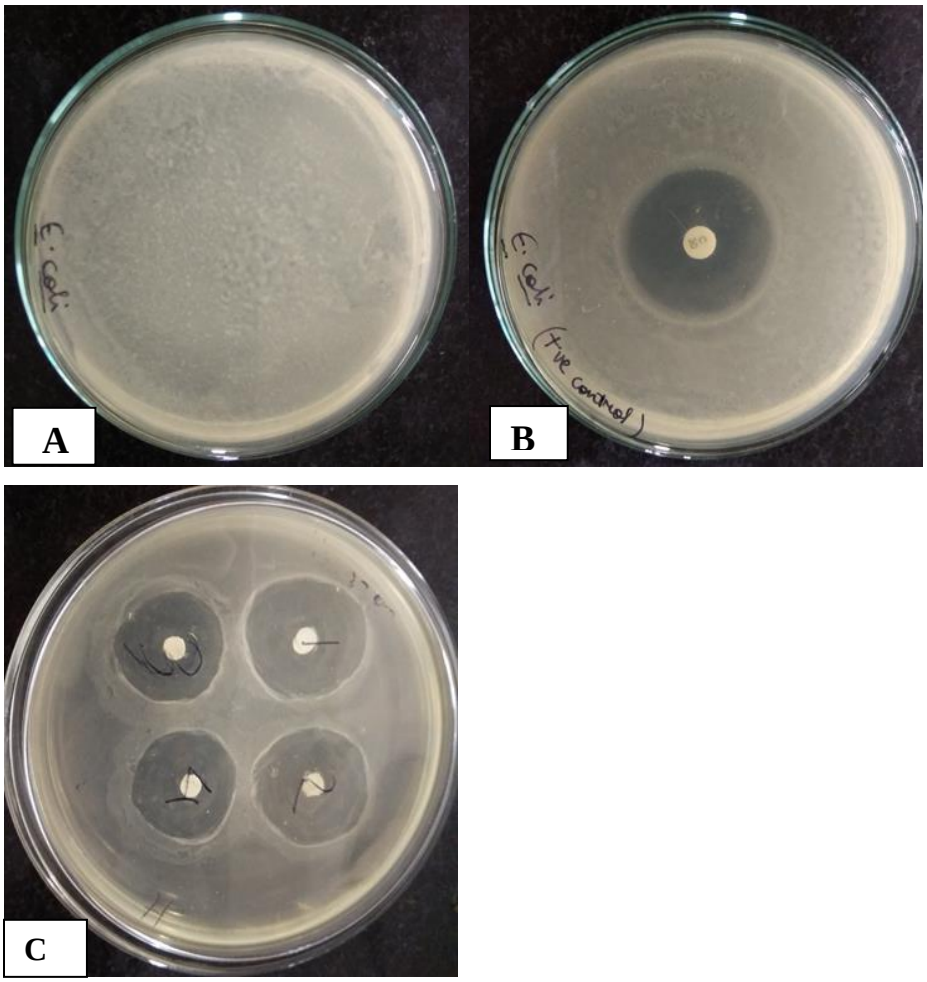

Fig 4.10 *E.coli*. (A) Negative control (B) Postive control (C) Plant extract test for antibacterial activity

Chapter 5

CONCLUSION AND FUTURE SCOPE

In conclusion, the results of the present studies indicated that the *Quisqualis indica* is a great source of bioactive compounds that possess antioxidant and antibacterial activity. The Response surface methodology was successfully used to optimize the extraction parameters for antioxidant activity. To optimize various parameters for for antioxidant (Temperature, Time, Solvent-solid ratio, Solvent composition, particle size, Extraction steps) by using Placket Barman criteria and four parameters temperature, Time, Solvent solid ratio and solvent composition showed significant effects on antioxidant activity was the significant variable for the for antioxidant activity. The optimum values were found to be temperature (52.5), time (45 min),solvent:solid ratio(45ml/ig) and solvent composition(52.5) were optimum for antioxidant activity. At these optimum levels the value of antioxidant 98.95 which was close to the predicted value 98.92.. Therefore the methanolic extract has ability of plant scavange free radical (DPPH) and act as strong antioxidant agent. The methanolic leaf extract of plant possess high amount of phenolic content 310 mg/g and flavonoid content 262mg/g. The zone of growth inhibition of the methanolic extract of *Quisqualis indica* was measured ranged from 12 to 18 mm that means the extract have ability to inhibit the growth of the bacteria or kill the bacteria. Phytochemical screening revealed that *Quiqualis indica* was found positive for alkaloids anthraquinone, tannins, Glycosides, saponins and quinones and negative for cumarin and terpenoids.so it is concluded that this plant is more effective in antioxidant and antibacterial activity.

REFERENCES

Afshari, A.T. (2007). Effect of Ginger on diabetic neuropathy, plasma antioxidant capacity and lipid peroxidation in rats. *Food chem* 101(1): 148–153

Agarwal, A., Prajapati, R., Raza, SK. (2017).Thakur LK. GC-MS Analysis and Antibacterial Activity of Aerial Parts of Quisqualis indica Plant Extracts. *Indian Journal of Pharmaceutical Education and Research.* 51(2):329-36.

Ahmed.,A., Ibrahim M., Laila, A.(2016).Total phenolic content, cytotoxic and antioxidant activities of *Quisqualis indica (Linn*.) growing in Egypt.,*Der Pharma Chemica*.8(3):53-59.

Akerele, O. (1992). Importance of medicinal plants: WHO's programme. In:Natural Resources and Human Health: plants of medicinal and nutritional value. *Elsevier, Amserdam, Netherlands*, 63-67.

Alam, M.N., Bristi, N.J. & Rafiquzzaman, M. (2012). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*. Vol. 21, 143-152.

Arsh, R., Koshy, P and sekaran, M.(2010) Antioxidant potential and phenol content of ethanol extract of selected Malaysian plants. *Research journal of Biotechnology*. (5):16-19.

Atmani, D., Nassima, C., Dina, A., Meriem, B., Nadjet, D., Hania, B. (2009) Flavonoids in Human Health: From Structure to Biological Activity.*Current Nutrition & Food Science*. (5): 225-237.

Balick, M.J., Cox PA (1997) plants, people, and culture. The science of ethonobotany, scientific American library.

Banick,R.M and Pandey, D.K (2007).Optimizing conditions for oleanic acid extraction from *Lantana camara* roots using response surface methodology.*Elsevier*.(27):241-248.

Bekro, JA., Konan, K.M., Bekro, YA., Bi, M.G.D., Bi, TJ., Mambo, V. & Boua, B.B. (2008). Phytocompounds of the extracts of four medicinal plants of cote Divoire and assessment of their

potential antioxidant by thin layer chromatography. *European Journal of Scientific Research*. Vol. 24(2), 219-228.

Bhatnagar, S., Sahoo, S., Mohapatra, A.K. and Behera, D.R. (2012). Phytochemical analysis, Antioxidant and Cytotoxic activity of medicinal plant *Combretum roxburghii* (Family: Combretaceae). *International Journal of Drug Development & Research.* 4(1), 193-202.

Bose, A., Bose, S., Maji, S., Chakrborty, P. (2009).Free Radical scavenging property of *Quisqualis indica.International journal of Biomedical and pharmaceutical sciences.*

Clarendon, Oxford, 1991.

Diana, S. (2010) .Medicinal plants with antioxidant properties from Banat region (Romania): A rich pool for the discovery of multi -target phytochemicals active in free radical related disorders, Analele Universităţii din Oradea Fascicula Biologie Tom. XVII / 1,14 22.

Elbein, A.D and Molyneux, R.J. (1999).Comprehensive Natural Products Chemistry.Barton D and Nakanishi K, ed. Amsterdam. 129.

Farnsworth, N.R, Soejarto, D.D. (1991) Global importance of medicinal plants. In: Conservation of Medicinal Plants. *Cambridge University Press*, UK 25-52.

Folwarczna, J., Zych, M., Nowi´ nska, B., Pytlik, M.., Janas, A.(2014). Unfavorable effect of trigonelline, an alkaloid present in coffee and fenugreek, on bone mechanical properties in estrogen-deficient rats. *Mol. Nutr. Food Res*.(58): 1457–1464.

Freeman, B.C and Beattie,G.C (2008). An overview of plant defenses against pathogens and herbivores, The Plant Health Instructor.

Gautam, R., Jachak, M.S.(2007) "Naturally occurring polyphenols with anti-inflammatory activity", CRlPS. 8(4): 20-32.

Gupta, M.P., Solis, P.N., Calderon, A. J., Guinneau – Sinclair, F.,Correa, M. Gladames, C., Guerra, C., Espinosa, A., Alvenda, G.L.,Robles G & Olampo, R. (2005). Medical ethnobotany of the tribes of Bocas del Toro, Panama. *Journal of Ethnopharmacology*(96):389-401.

Halliwell, B. (2008) Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? Arch. Biochem. Biophys, 476: 107-112.

Hammer K.A., Carson, C.F., Riley, and T.V. (1996) Susceptibility of transient and commensal skin flora to the essential oil of Melaleuca alternifolia (tea tree oil). *American Journal of Infection Control*. (24):186 189.

Hammer, k., Carson, C.F., Relay T.V.(1999). Antimicrobial activity of essential oils and other plant extracts.*National library of medicine National institute of health*.86(6):985-90

Harborne, J.B., Tomas-Barberan, F.A.(1999). Ecological Chemistry and Biochemistryof Plant Terpenoids,Hasler CM, Blumberg JB. Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition.(129): 756S-757S

Hill AF. Economic Botany: A Text Book of Useful Plants and Plant Products. 2nd Edn. New York: McGraw Hill Book Company, Inc.; 1989.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. and Armand, R. (2015) The Application of Medicinal Plants in Traditional and Modern Medicine: *A Review of Thymus vulgaris. International Journal of Clinical Medicine*. (6):635-642

http://.cals.ncsu.edu/.../chapter1-1.

Ishizaki, T., Kato, k and Kumara, M.1973. (1973). Effect of quisqualis acid upon *Ascaris sunn in vitro* comparison with those of alphar- allokainik acid pyrantel palmote Jpn J parasitol, 22:81-1986.

Jaydeep,M. (2012).In *vitro* regeneration of Rangoon creeper (Quisqualis indica linn). *indian journal of biotechnology*.v(12): 415-419

Joshi SG," Medicinal plants", Mohan Primlani for Oxford and IBH publishing Co.pvt. Ltd, New Delhi, 1992, 141.

Kaisar ,M.D., Abul, R.(2009). Total Phenolic Content, Free Radical Scavenging Activity and Reducing Power of Quisqualis indica Linn. *Dhaka Univ. J.Pharm. Sci.* 8(2): 173-175. l

Kelly, K (2008). History of medicine. *New York: Facts on file*. pp. 29–50

Kinghorn, A.D. (1994). The discovery of drugs from higher plants. In The Discovery of Natural Products with Therapeutic Potential. 81-108.

Kiruthika,A and Sornaraj R.(2014).ASSESSMENT OF ANTIOXIDANT PROPERTY OF QUISQUALIS INDICA. *Asian Journal of Pharmaceutical Science* & *Technology*.(4): 34-36.

Krishnan,R., Chandravadana, M.V., Ramachander, P.R., Bharathkumar, H. (1983).Inter-relationships between growth and alkaloid production in Catharanthus roseus G. Don. Herba Hungarica. (22): 47-54.

Kumar,M., Gitika1., Sharma.A.(2014). In vitro antibacterial activity of flower extracts of *Quisqualis indica* Linn. against gram-positive and gram-negative bacteria. *International Journal of advances in Pharmacy,Biology and chemistry*.3(3): 2277 – 4688.

Kusuma,I., Murdiyanto., Arung. E., Syafrizal, kim yong. (2014) Antimacrobial and antioxidant properties of medicinal plants used by the Bentian Tribe From Indonesia. *Food science and human wellness*.(3)191-196.

Mamedov, N. (2012). Medicinal plants studies: History, challenges and prospective.medicinal aromatic plants. 1:e133. doi:10.4172/2167-0412.1000e133.

Mamedov, N., Craker, L. (2008). Endemic species of Artemisia L. (Asteraceae) from Caspian Sea region as alternative sources for malaria treatment. Proceedings of the First International Medicinal and Aromatic Plants Conference on Culinary Herbs, Antalya, Turkey. Acta Horticulturae(826): 277-280.

Mickymaray, S.,Al, Aboody.M., Kumar R.P., Annamalai. P., Nooruddin.T. (2016) Screening and antibacterial efficacy of selected Indian medicinal plants. *Asian Pacific Journal of Tropical Biomedicine*.6 (3): 185–191.

Mihoubi, M., Micale, N., Scala, A., Jarraya, R.M., Bouazir, A., Schirmeister, T., Risitano, F., Piperno, A. & Grassi, G. (2015). Synthesis of C3/C1- Substituted Tetrahydroisoquinolines. Molecules.(20):14902-14914.

Munir M. et al 1995 “Excitotoxic cell death and delayed rescue in human neurons derived from NT2 cells”, Journal of Neuroscience. (15):7847–7860.

Murphy, T.H., Schnaar R.L, et al.(1988) “Glutamate cytotoxicity in a neuronal cell line is blocked by membrane depolarization, Brain Research”. 510(1): 155–160.

Padmanabhan, P and jangle, S.N (2012). Evaluation of DPPH radical scavenging activity and reducing power of four selected medicinal plants and their combinations.*International journal of pharmaceutical sciences and drug research.*.4 (2):143-146.

Pharm. Biol. 2014, 52, 243–254. [CrossRef] [PubMed]

Prakash, P and Gupta, N. (2005) Therapeutic Uses of Ocimum sanctum Linn (Tulsi) with a Note on Eugenol and Its Pharmacological Actions: *A Review. Indian Journal of Physiology and Pharmacology,* 49, 125-131.

Qazi, P.H., Rashid, A. & Shawl, S.A. (2011). Podophyllum hexandrum- a versatile medicinal plant. *International Journal of Pharmacy and Pharmaceutical Sciences.* 3(5), 261-268.

Radwan.A.E.A Afify and Hassan.H.M.M (2016): Free radical scavenging activity of three different flowers-*Hibiscus rosa-sinensis, Quisqualis indica and Senna surattensis*. *Asian Pacific Journal of Tropical Biomedicine.* 6(9): 771–777.

Ramamoorthy, P. K & Bono, A. (2007). Antioxidant activity, total Phenolic and Flavonoid content of *Quisqualis indica* extracts from various extraction processes. Vol. 2(1), 70-80.

Rozman,V., Kalinovic and Korunic,Z (2007).Toxicity of naturally occurring compounds of lamiaceae and lauraceae to three stored-product insects, *journal Stored Products* Res., (43): 349-355. .

Sahu, K. (2014). How Antioxidant work?[Succeedwith Monavie.com] Retrieved from: https://www.youtube.com/watch?v=lG3OOXIXvxw.

Sahu,J.,Kumar and Dubey,B.(2012). Quisqualis indica Linn : A Review of its Medicinal Properties. *International Journa l of Pharmaceutical and Phytopharmacological Research.* 1(5): 313-321.

Saito,H.(2000) Regulation of herbal medicines in japan. *Pharmacological Research,* 41,515-519.

Sandhu, D. S. & Heinrich, M. (2005). The use of health foods, spices and other botanicals in the sikh community in London, Phytotherapy Research (19):633-642.

Sanguri,S., Kapil,S., Gopinathan,P, Pandey,FK and Bhatnagar.T(2012). Comparative screening of antibacterial and antifungal activities of some Weeds and medicinal plants leaf extracts: An in-vitro study. *Elixir International Journal* .(47) 8903-8905

Saxena, M.,Saxena1, J., Neema,R., Singh, D.,& Gupta,A.(2013) Phytochemistry of Medicinal plant. *Journal of Pharmacognosy and Phytochemistry.* 1(6): 168-182..

Sharma, O.P. & Khat, T.K. (2009). DPPH antioxidant assay revisited. *Food Chemistry.* Vol.113 (4), 1202-1205.

Singh Nitu et al.(2010). Antipyretic activity of methanolic extract of leaves of *Quisqualic indica* linn. IJPRD. (2):122-126.

Singh, N.,Mohan,G., Kumar,R and Ganeshwan,(.2017). Evaluation of Anti-diarrhoeal activity of *quisqualis indica. indian jornal of natural products and resource.*4(2):.155-160

Singh.G. , (2009). plant systematics third edition.

Singleton, V.L., Rossi, J.A.,(1965) Calorimetery of total phenolics with Phosphomolybdic-phosphotungstic reagents.Am J E nol Vitic16:144-158.

Sofowora, A. (1993). Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicinal in Africa, second ed., Spectrum Books Ltd., Sunshine House, Ibadan, Nigeria, pp. 134–156.

Sofowora, A.(1982).Medicinal Plants and Traditional Medicinal in Africa. New York: John Wiley and Sons.

Soni, A. & Sosa,S. (2013). Phytochemical Analysis and Free Radical Scvenging Potential of Herbal and Medicinal Plant Extracts. *Journal of Pharmacognosy and Phytochemistry.* 2(4), 22-29

Stojanoski, N. (1999). Development of health culture in Veles and its region from the past to the end of the 20th century. *Veles: Society of science and art*.(13):13–34.

Ta Chen Lin et al, “Tannin and related compounds from Quisqualis indica”, Journal

Tapas, A.R., Sakarkar,D.M., Kakde, R.B(.2008). Flavonoids as Nutraceuticals: A Review.Tropical *Journal of Pharmaceutical Research*, (7): 1089-1099.

WHO (1993) Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines Manila.

Williams,B., Cuvelier,M.E.,Berset,C.(1995).Use of Free Radical method to evaluate antioxidant activity.*Lebensm Wiss technolog*y.(28): 25-30.

Xiao,P. (1988) pictorial Encyclopedia of chinese medicine. *commercial press.Hong kong* v(1-10)88-90

Yadav Yashraj, Mohanty PK, et al, “Antiinflammatory activity of hydroalcoholic extract of Quisqualis indica Linn. flower in rats” International Journal of Pharmacy and Life Sciences, 2011, 2 :977-981.

Yadav, y., and Mohanty PK, et al, (2011)“Evaluation of immunomodulatory activity of hydroalcoholic extract of *Quisqualis indica* Linn. flower in wistar rats*”, international journal of pharmaceutical and life sciences.*(2):689-686.

Zhou, J., Chan, L., Zhou, S. (2012). Trigonelline. A plant alkaloid with therapeutic potential for diabetes and central nervous system disease. *Curr. Med. Chem*. 19: 3523–3531.