Phytochemical profiling and *in vitro* free radical scavenging assay activities of *Mussaenda frondosa* L. leaf extract

SHANMUGA PRIYA, S.

(16PZO010)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In Partial Fulfillment of the Requirements for the Degree of

Master of Science in Zoology

APRIL, 2018

Phytochemical profiling and *in vitro* free radical scavenging activities of *Mussaenda frondosa* L. leaf extract

SHANMUGA PRIYA, S.

(16PZO010)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education

for Women, Coimbatore – 641 043

In Partial Fulfillment of the Requirements for the Degree of

Master of Science in Zoology

APRIL, 2018

9.4.18

Signature of the

Head of the Department

Sandhya K.S.
9.4.2018

Signature of the

Supervisor

ACKNOWLEDGEMENT

I would like to express my sincere thanks to God Almighty, for His constant love and grace that he has showered upon me.

I am very grateful to **Padmashri Dr.P.R. Krishnakumar, Chancellor**, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for his support and encouragement during the curse of my study.

I heartily thank **Dr. (Mrs.) Premavathy Vijayan** M**.Sc., M.Ed., Dip.Spl.Edn., M.Phil., Ph.D., Vice Chancellor**, Avinashilingam Institute for Home Science and Higher Education for Women,Coimbatore, for extending all resources that facilitated the conduct of the present study.

I express my humble gratitude to **Dr. S. Kowsalya, Registrar,** Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing all facilities necessary for the study.

I am also thankful to **Dr. (Mrs.). A. Parvathi, M.Sc., D.H.Ed., M.Phil., Ph.D., Faulty of Science**, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for granting the facility required.

It is my pleasure to acknowledge the wholehearted support and encouragement extended to me by **Mrs.E.Shanthi, M.Sc., Dip.Ed., M.Phil., Associate professor & Head,** Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for her the constant support given during the period of investigation.

I wish to place on record my deep sense of gratitude to **Dr. (Mrs.). K.S. Santhy, M.Sc., M.Phil., Ph.D., MCA, PGDBI, Professor, Department of Zoology,** Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for imparting in the tremendous assistance and well timed support for triumph of my project.

I express my sincere thanks to all **Staff members and Lab assistants** in the Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for their help evinced throughout the course of this investigation.

I express my special thanks to my father **Mr. K. Selva kumar**, my mother **S. Selvi,** Brother **Mr. S. Arunagiri**, Sisters **Ms. Archana Das** and **Mrs. Mahalakshmi Iyyer** for their inspiration, unbounded affection, encouragement, support, prayers and loving care rendered throughout the study period.

Finally not but least, a warm thanks to my class mates and friends - **Ms. S. Dylin raja sheribha, Ms. C. Kalaiyarasi**, **Ms. K.P. Ranjitha Das** for their support rendered throughout the study period.

Shanmuga priya, S

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ALP	:	*Alkaline Phosphatase*
ALT	:	Alanine Amino transferase
AST	:	Aspartate Amino transferase
CAT	:	Catalase
DNA	:	Deoxyribo Nucleic Acid
DPPH	:	1, 1-diphenyl-2-picryl hydrazyl
EDTA	:	Ethylene Diamine Tetra Acetic acid
FDA	:	Food and Drug Administration
FRAP	:	Ferric Reducing Antioxidant Power
FTIR	:	Fourier Transform Infrared
GC-MS	:	Gas Chromatography – Mass Spectrometry
HDL	:	*High-density Lipoproteins*
HR	:	Hormone Receptor

MDA	:	*Malondialdehyde*
MIC	:	Minimum Inhibitory Concentration
PTEN	:	Phosphatase and Tensin homolog
ROS	:	Reactive Oxygen Species
SOD	:	Superoxide Dismutase
TCA	:	Trichloro acetic acid
UV	:	Ultra Violet Light
VLDL	:	Very-low-density lipoprotein
WHO	:	World Health Organization

INTRODUCTION

The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians (Samuelsson, 2011). Man's dependence on plants from his life since human race. No plant in this earth is completely worthless (Apata, 1979; Cragg, 2005). Medicinal plants are the "backbone" of traditional medicine (Hunt, 2000). Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values (Hassan, 2012). Nowadays, there is a huge use of plants for medicinal and therapeutic purpose for curing of diseases and improves human health. Plants have secondary metabolites called Phytochemical. These compounds protect plants against *microbial* infections or infestations by pests. Herbal medicine has been recognized by WHO as essential components for primary health care and about 11% of the 252 drugs are derived from plants (Martinez *et al*., 2008).

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies (Hassan, 2012). Medicinal plants not only provide relatively affordable drugs with marginal side effects, but they are also sources of other beneficial substances including phytochemicals and phytoalexins (Iriti *et al*., 2009.). Although scientists have estimated that over 250,000 species of angiosperms exist on earth, most of these plants have yet to be explored for their medicinal properties (Abruzzo, 2005).

The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996 (Singh, 2015). Globally, there has been an unparalleled growth in the plant – derived medicinally useful formulation, drugs and health care products. Medicinal plants have been encouraging because of their high content of potent antioxidants, accessibility, economic viability and next-to-no side effect (Auddy *et al*., 2003).

Among the human diseases treated with medicinal plants is cancer, which is probably the most important genetic disease (Kaur *et al*., 2011).Cancer is one of the most flourishing diseases of all over the world (Aslam *et al.*, 2014). Cancer is the second leading cause of deaths all over the world. Globally 7.6 million deaths are caused by cancer which represents 13% of all global deaths (Vijayapadma, 2015). In 1970, 15% of newly reported cancers were in developing countries, compared with 56% in 2008 (Farmer *et al.*, 2010). Cancer is the abnormal growth of cells in our bodies that can lead to death. Cancer cells usually invade and destroy normal cells (Kaur *et al*., 2011). Cancer is caused by both external factors (tobacco, chemicals, radiation, and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism) .

Breast cancer has increased universally and is considered as the second chief mortality cause in women (Anjum *et al*., 2017). It is not a single disease, which comprises of many biologically different entities with distinct pathological features and clinical implications (Dai *et al*., 2015). This cancer is principally a disease of older women and the risk increases with age. As the life expectancy has increased, this will become a frequent disease affecting the lives of older women, especially in more developed countries (Gamulin *et al*., 2010). In 2002, ~205,000 American women will be diagnosed with breast cancer, and 40,000 will die from the Disease (Smith *et al*., 2010).

The search for cancer drugs from natural sources started with discovery of Podophyllotoxin in late 1960s, further lead to discoveries of vincristine, vinblastine, campthothecin and taxol. Nature provides more than 1000 species of plant varieties which possess significant anti-cancer properties. Taxol, one of the most outstanding agents, has been found beneficial in treatment of refractory ovarian, breast and other cancers (Lakshmi Priya *et al*., 2015). Clinically, the choice of therapies for a particular patient is determined by the patient's age, health, stage of disease and prior treatment. Additionally, tumor size, nodal involvement, hormone receptor (HR) status, and Her2/ neu expression play very important roles in determining the use of chemotherapy, endocrine therapy and/or targeted therapies (Andreetta *et al*., 2010; Guarneri *et al*., 2004). Radiation is a physical agent, which is used to destroy cancer cells. This deposited energy can kill cancer cells or cause genetic changes resulting in cancer cell

death. High-energy radiation damages genetic material (deoxyribonucleic acid, DNA) of cells and thus blocking their ability to divide and proliferate further (Jackson *et al*., 2009).

Of the new anticancer drugs approved by the U.S. Food and Drug Administration (FDA) since 2000, 15 have been targeted therapies. Targeted therapies have expanded the concept of individually tailored cancer treatment because some of these drugs may be effective in patients whose cancers have a specific molecular target, but they may not be effective in the absence of such a target (Calvo *et al*., 2006)

Rubiaceae is one of the larger families among dicotyledons and abundant in tropical and subtropical regions around the world. Plants of this family are mostly shrubs and trees, infrequently herbs consisting of about 660 genera and 13,200 species including some lianous forms (Nifio *et al.,* 2006). A large number of Rubiaceous plants are ethno medicinally important and also reported to have a wide variety of biological activities such as antimicrobial, antimalarial, hepatoprotective, antioxidant, and so many other interesting biological activities (Robbrecht, 2009). From Assam Rubiaceae is represented by 45 genera and approximately 140 species (Kanjilal *et al*., 1938).

The genus *Mussaenda* L. is one of the largest genera of rubiacea being represented by 200 species, when in India it is represented by 14 species (Kirtikarn *et al*., 1987; Huxley *et al*., 1999). The genus *Mussaenda* has been important in providing us with several natural products of interest to workers (scientists) in the field of pharmacology. The species of this genus have the further advantage of being easy to grow. They are pest and disease free and can withstand heavy pruning. Very few species have been explored for chemical and biological studies (Jayasinghe *et al*., 2002).

The *Mussaenda*'s are a group of highly ornamental shrubs suited to tropical and subtropical climates with a bright future, both as landscape plants and as potted floral decorations. Some species of *Mussaenda* have been used in Chinese and Fijian traditional medicine. There are *Mussaenda* species native to Africa, Madagascar, Asia and the Pacific. Commonly cultivated species include *Mussaenda philippica, M. erythrophylla, M. frondosa* and Pseudo *mussaenda flava* (also referred to as *Mussaenda flava, M. glabra, M. luteola, M. lutea* or *M. incana* in various publications).

Mussaenda frondosa is the commonly known as Vellaiilai in Tamil (Quriroga *et al*., 2010). Plant is known by various names in different languages as Bedina in Hindi, Bhutkasi in

Marathi, and Billothi in Kannada, (Basu et al.,1998) Nagavalli in Telungu, Sriparnah in Sanskrit (Vaidyaratnam, 1993). *Mussaenda frondosa* is a rambling shrub seen in Western ghats, Andamans, Konkan, Malabar and Tirunelvelly hills. *Mussaenda frondosa* is distributed in Central Nepal, India and Srilanka. The juice of the root is used in blemises on tongue and sepals are diuretics.

Leaves are broadly elliptic, shortly acuminate and more or less pubescent. Flowers are terminal open cymes berries 10-13cm, sub globose or obovoid, glabrous. Half a total of the root is given with cow'surine in leprosy. In jaundice two tolas of the whole leaves are given in milk. In Indochina the flowers considered pectoral and diuretic, they are given in asthma, intermittent fever and dropsy. Externally they are given as a deterrent to ulcers. It has been in Chinese folk medicine as a diuretic, anti phlogestic and antipyretic. It is also used to detoxify mushroom poisons and terminate early pregnancy (Kirtika and Basu, 1987; Huxley *et al*., 1999). Root shows antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Gunasekaran *et al*., 2015). The bark was found to be antibacterial (Jayasinghe *et al*., 2002).

Phytochemistry, evolved from natural products chemistry is confined to the study of the products elaborated by plants and it has developed as a distinct discipline between natural product organic chemistry and plant biochemistry in recent years. The prefix "phyto" is from a Greek word meaning plant (Miller, 1973; Loewus, 1965-1981). It deals with the study of chemical structures of plant constituents, their biosynthesis, metabolism, natural distribution and biological functions. The facts that only less than 10% of about 7.5% lakhs species of plants (Reinhold, *et al*., 1977; Harborne, 1988) on earth has been investigated indicates the opportunity provided and challenges thrown open to phytochemists.

Flavanoids and flavones are widely distributed secondary metabolite with antioxidant and antiradical properties. Plant based natural constituents can be derived from any part of plant's bark, leaves, flowers, roots, fruits and seeds, that is any part of the plant may contain active components (Siju *et al*., 2010). Iridoids, flavanoids and triterpenes are the common chemical ingredients distributed in *Mussaenda* species. Phytochemicals like astragalin, isoquercetin, kaempferol-3-o-beta drutinoside were previously isolated from leaves (Ranarivelo *et al*., 1990). A new compound sanzhi lactone along with mussaenside, barlerine lupeol and beta-d-glucose

has been obtained from the stem (Dinda *et al*., 2005). Quercetin, rutin, hyperin, ferulic acid, synaptic acid, beta sitosterol, saponins has also been isolated (WHO, 1998).

Antioxidants have evolved with protective roles against such damage (Halliwel, 1994). The negative cellular effects of ROS can be countered by enzymatic antioxidants such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD); non-enzymatic, metabolic and nutrient antioxidants including glutathione, vitamin C and vitamin E; metal binding proteins like ferritin, lactoferrin, albumin and ceruloplasmin; and well known phytochemicals such as quercetin, resveratrol and capsaicin (Jacob,1995).

The mechanisms of protective actions of antioxidants against ROS toxicity include prevention of the formation of ROS, interruption of ROS attack, scavenging of the reactive metabolites or their conversion to stable molecules or molecules of lower reactivity (Kaur *et al*., 2001). Antioxidant compounds like phenolic acids, polyphenols and flavanoids scavenge free radicals such as peroxide, hydro peroxide or lipidperoxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson's disease, mongolism, ageing process and perhaps dementias.

Antioxidant based drugs or formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer have appeared during the last three decades (Pourmorad *et al*., 2006). Since the phyto-constituents and volatiles of medicinal herbs have created renewed demand in their use by the public, explorations of health benefits and antioxidant potential of these metabolites in the prevention of problems raised due to oxidative stress is needed. Although a good number of natural agents have been developed from plants or their derived agents, development of a safe, economic and site-specific drug is still a challenge. In this scenario, the present investigation attempted to go forward a safe and economic natural derivative from *Mussaenda frondosa* leaf extracts, which can be developed as drug candidates for diseases rose due to oxidative stress.

With this background, we have formulated the following objectives

1. To analyze the main phytochemical components in the leaf extracts of *Mussaenda frondosa* L.
2. To estimate the free radical scavenging activity of the leaf extracts of *Mussaenda frondosa* L.
3. To identify the major functional group present in the leaf extracts of *Mussaenda frondosa* L.
4. To identify the major bioactive components in the leaf extracts of *Mussaenda frondosa* L.
5. To determine the efficacy of the ligands against the target protein, Phosphatase and Tensin homologue (PTEN)

I. REVIEW OF LITERATURE

The review of literature pertaining to the topic **"Phytochemical profiling and *in vitro* free radical scavenging activities of *Mussaenda frondosa L.* leaf extract"** is presented in the following headings:

2.1. Medicinal Plants

Since ancient times people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte, 1998). According to the World Health Organization "a medicinal plant is any plant which in one or more of its organ contains substances that can be used for therapeutic processes or which are precursors for the synthesis of useful drugs". The definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha (Sathyavathi *et al*., 1987). These systems contain a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India.

Scartezzini *et al*. (2000) investigated the medicinal plants used in ayurvedic Rasayana for their therapeutic action; some of these have been thoroughly investigated. Seven plants (*Emblica officinalis* L., *Curcuma longa* L., *Mangifera indica* L., *Momordica charantia* L., *Santalum album* L., *Swertia chirata* Buch-Ham, *Withania somnifera* (L.) Dunal are viewed for their historical, etymological, morphological, phytochemical and pharmacological aspects. The plants described contain antioxidant principles that can explain and justify their use in traditional medicine in the past as well as the present. In terms of the number of medicinal plant species, Piperaceae (8 species), Asteraceae (9 species) and Verbenaceae (6 species) are dominant families. In order to identify the plants with antioxidant activity in Ayurveda, a formulation of some rasayanas with well defined antioxidant properties has been examined. Among different plant parts used for the preparation of medicine, the leaves were most frequently used for the treatment of diseases followed by roots and whole plant.

Raja Kumar *et al.* (2010) reported an ethno-medico-botanical field survey during May 2006 to July 2007 to document the uses of medicinal plants by traditional herbal healers in Sagar taluk of Shimoga district, Karnataka state, India. 48 plant species belonging to 44 genera and 31 families used by folk practitioners to treat various common to chronic human and veterinary ailments were documented in the study.

Bose *et al.* (2015) studied an ethno botanical survey among tribals of Jalpaiguri district of West Bengal, India. People of all tribal villages mostly rely on ethno-medicinal practices; particularly in treating common physical problems like smaller injuries, cough and cold, skin diseases, stomachache and abdominal disorder, etc. A total of 115 plant species belonging to 103 genera and 62 families were reported to be used for treating 69 various physical ailments.

Hossein zadeh *et al.* (2015) reported that traditional medicine *Thymus vulgaris* is used to treat many diseases including inflammation-related ailments such as rheumatism, muscle swelling, insect bites and pains. In addition, the modern medicine in essential oil of thyme has demonstrated that the compounds have shown anti-inflammatory, antioxidant, antibacterial and antifungal properties. In a previous report, plant offers multiple health benefits because of presence of ingredients like proteins, alkaloids, saponins and tannins that help in improving fertility and vitality in women and men. Pharmacological activities of Asparagus include anticancer, antioxidant, antifungal, antibacterial, anti-dysenteric, anti-inflammatory, and anti-abortifacient, anti-oxytoxic, antiulcer, hypertensive and anticoagulant effects. Moreover, it is reported to reduce the risk of constipation, diarrhea, osteoporosis, obesity, cardiovascular disease, rheumatism and diabetes (Iqbal *et al*., 2017).

2.2 An over view of *Mussaenda frondosa L.*

Classification

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Rubiales
Family	Rubiaceae
Genus	Mussaenda L.
Species	Mussaendafrondosa

Vernacular name

General Name	Mussaenda Frondosa
English Name	White Flag Bush, Wild Mussenda, Bangkok Rose
Botanical Name	Mussaenda Frondosa
Tamil	velli-matantai
Hindi Name	Bebina, Bedina, Bebina, Bedina
Bengali	Nagaballi
Kannada	Bellotti
Konkani	Sarvadi
Malayalam	Vellila
Marathi	bhutakesha, sarvad
Sanskrit	Shrivati

Mussaenda frondosa L. is one of the medicinally important plants belonging to the family rubiaceae, commonly known as “Vellaiilai” in Tamil (Quriroga *et al*., 2010). Dhobi Tree-*Mussaenda frontosa* is a small shrub, erect, branching, commonly in Western ghats, plane areas, wastelands, forests, grows up to 2 meters tall. Leaves are evergreen, elliptic to ovate, opposite in arrangement, simple, 3 to 6 inches long, and light to medium green in color. It has prominent pinnate veins. The orange-red flowers are small with a large ovate, white sepal, starry funnel-

shaped, 5-lobed and are borne in terminal cymes. It will bloom periodically throughout the year with the heaviest bloom in the spring to fall just below flowers there is a white leaf among the green leaves. Fruit are ½ to ¾ inch long berries that are not often seen.

Traditionally the leaves are used in the treatment of jaundice, asthma, hyperacidity, ulcers, leprosy, diuretic, wounds, swelling, fever and cough. They are also used as antimicrobial, diuretic, hypolipidemic, hepatoprotective agents (Quriroga *et al*., 2010). The root is used as a treatment for leprosy. The juice of the roots, combined with about 10% by volume of cow's urine, is used in the treatment of jaundice. The juice of the bark is used in the treatment of body ache, diarrhoea and dysentery. The flowers are diuretic. They are used in the treatment of cough.

2.3. Medicinal uses of *Mussaenda frondosa* L.

Dilip *et al*. (2010) analyzed that mucilage extracted from the leaves of *Mussaenda frondosa* L. and studied for various physicochemical properties. The mucilage shows good physicochemical properties that assessed as an excipient in formulation of tablets. *Mussaenda frondosa* mucilage could be used as a good binding agent at very low concentrations. This can be used for sustaining the drug release from tablets, since the prepared tablets produced a sticky film of hydration on the surface, which ultimately reduces drug release rate and hence it can be evaluated for its efficacy to sustain the drug release.

In a previous report of wound healing activity in rats, after administration of the alcoholic extract of *Mussaenda frondosa* leaves, using excision, incision and dead space wound models. The antibacterial activity of the leaves extracts against four microorganisms was also assessed. The extract of *Mussaenda frondosa* leaves significantly increased the wound breaking strength in the incision wound model compared with controls. The extract treated wounds were found to epithelialize faster, and the rates of wound concentration was significantly increased in comparison to control wounds, Wet and Dry granulation tissue weights, and hydroxyl proline content in a dead space model increased significantly. *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus albus* have shown sensitivity to *Mussaenda frondosa*. Increased wound concentration and tensile strength, augmented hydroxyl proline content along with antibacterial activity support the use of *Mussaenda frondosa i*n the topical management of wound healing (Patil, 2010).

It has been suggest that the hypolipidemic effect of methanolic extract of *Mussaenda frondosa* may be due to the inhibition of hepatic cholesterol genesis and may have slight antioxidant activity. It is therefore concluded that the methanolic extract of *Mussaenda frondosa* possesses hypolipidemic activity in high fat diet fed rats (Wesley, 2009)

Earlier it has been described that the *in vitro* antioxidant effects of the ethyl alcohol and aqueous extracts of whole plant of *Mussaenda frondosa* were tested. BHA (Butylated Hydroxy Anisole) was used as standard antioxidant and positive control. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The reducing power of extract was carried out with ascorbic acid as a standard reducing agent (Siju *et al*., 2010).

In another study, it has been reported that ethanolic extract of *Mussaenda frondosa* has twenty chemical constituents such as Quinic acid, 4-(1E)-3-Hydroxy-1-propenyl) 2-methoxyphenol, Naphthalene, decahydro-2-methoxy and 1, 2,3Benzrenetriol (Gopalakrishnan *et al*., 2011).

It has been studied that both the plant extracts show a dose dependent increase from lower concentration to higher concentration. Ethanol extract of *Bauhiiato mentosa* L showed more cleavage activity than that of the *Mussaenda frondosa* L. The results have been compared with the standard DNA cleavage agent $FeSO_4$ (Gopala krishnan *et al*., 2011).

Earlier it has been studied that the plant contains hypercin, quercetin and β-sitosterol glucoside. Ethanolic and aqueous extracts of the plant showed the presence of flavanoids, phenolic compound, tannins and anthocyanins. The results obtained provide biochemical evidence for anti stress activity of the tested extract of *Mussaenda frondosa*. The inhibitory concentrations in DPPH, superoxide scavenging activity and hydroxide scavenging activity were found to be 51.3, 24.6 and 52.7μg/ml respectively. The ethanolic root extract of *Mussaenda frondosa* exhibits potential free radical scavenging effect that can reduce the oxidative stress (Koul, 2011).

Patilsuhas *et al*. (2011) reported that the alcoholic extract of leaves of *Mussaenda frondosa* L. and its isolated compound quercetin were evaluated for wound healing activity by using different types of wound healing models such as excision wound, incision wound and dead space wound. The results were obtained in terms of wound contraction, epithelialization time and tensile strength. All results were significant for different parameters in wound healing activity in quercetin and alcoholic extract treated animals compared with control groups. The isolated

compound quercetin was confirmed by preliminary phytochemical investigation, IR, NMR and Mass spectroscopic methods.

Sambrekarsudhir (2012) investigated the protective effect and possible mechanism of alcoholic (AlE) and aqueous extract (AqE) from *Mussaenda frondosa* L. on EtOH-induced hepatic injury in Wistar rat. Liv 52® was used as a reference hepatoprotective agent. AlE and AqE on oral administration decreased the level of AST, ALP, ALT, bilirubin, cholesterol**,** triglyceride, VLDL, MDA and increased the level of protein, HDL and antioxidants (SOD, GSH and CAT) in rats being treated with ethanol (EtOH). Histopathological observations confirmed the beneficial roles of *Mussaenda frondosa* against EtOH-induced liver injury in rats. Possible mechanism may involve their antioxidant activity.

In another study, Sambrekarsudhir (2014) reported the protective effect and possible mechanism of alcoholic (AlE) and aqueous extract (AqE) from *Mussaenda frondosa* L. leaf on Isoniazid (INH)-induced hepatic injury in Wistar rat Liv 52® was used as a reference hepato protective agent. AlE and AqE on oral administration decreased the level of AST, ALP, ALT, bilirubin, cholesterol**,** triglyceride, VLDL, MDA and increased the level of protein, HDL and antioxidants (SOD, GSH and CAT) in rats being treated with isoniazid (INH). Pentobarbitone - induced sleeping time study was carried out to verify the effect on microsomal enzymes. Histopathological observations confirmed the beneficial roles of *Mussaenda frondosa* against INH-induced liver injury in rats.

Sreelakshmi (2015) evaluated of diuretic activity of the plant *Mussaenda frondosa* L. Wistar albino rats was divided into 4 groups of 6 animals in each Frusemide used as a standard diuretics. Two doses of plant extract were used for the study. In urine the volume and concentration of electrolytes was measured at the end of 24 hrs. Result shows significant increase in diuretic activity.

Ashakesari (2015) evaluated the petroleum ether, chloroform and methanolic fractions of *Murraya koenigii* and *Mussaenda frondosa* significantly demonstrated the slowing down of the movement and caused death of the worms at a concentration of 20mg/ml as compared to the standard reference, Albendazole. MFC fractions showed the minimum time required for death. In conclusion, the traditional use of the *Murraya koenigii* and *Mussaenda frondosa* as an anthelmintic has been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

Mahesh (2017) analyzed phytochemical constituents of aqueous and methanolic leaf extracts of *Mussaenda frondosa* L. and anti-inflammatory activity of different leaf extracts. In this study presence of steroids, flavonoidal and glycosides were reported. The butanol extract showed marked anti-inflammatory activity when compared standard. The results clearly indicate that ethyl acetate and petroleum ether extracts of *Mussaenda frondosa* are effective against inflammatory diseases.

Earlier it has been described pale green, healthy, friable, and fast growing callus of *Mussaenda frondosa* were obtained on the medium enriched with NAA. Quantitative determination showed the highest concentration of total phenolics in the methanolic extract of *in vitro* grown callus, flavonoids in methanolic stem extract and alkaloids in methanolic extract of *Mussaenda frondosa* leaf. Also the methanolic leaf extract exhibited the highest free radical scavenging activity with an inhibitory concentration of 40.6μg/ml. The highest membrane stabilizing activity was shown by chloroform extract of the leaf (Manasa, 2017).

In a previous report of anti seizure activity of methanolic extract of aerial parts of *Mussaenda frondosa* in male Wistar albino rats by using Cobalt induced Epilepsy. The methanolic extracts at different doses inhibited Cobalt induced brain toxicity in Wistar albino rats as assessed by the biochemical changes and histopathological studies (Guggilla *et al*., 2017).

2.4. Phytochemical analysis

Edeoga*e et al*. (2005) analyzed Alkaloids, tannins, saponins, steroid, terpenoid, flavonoids, phlobatannin and cardic glycoside distribution in 10 medicinal plants belonging to different families were assessed and compared. The medicinal plants investigated were *Cleome nutidosperma, Emilia coccinea, Euphorbia heterophylla, Physalis angulata, Richardia bransitensis, Scopania dulcis, Sida acuta, Spigelia anthelmia, Stachytarpheta cayennensis* and *Tridax procumbens*. All the plants were found to contain alkaloids, tannins and flavonoids except for the absence of tannins in *S. acuta* and flavonoids in *S. cayennsis* respectively. The significance of the plants in traditional medicine and the importance of the distribution of these chemical constituents were discussed with respect to the role of these plants in ethno medicine in Nigeria.

In another study phytochemical constituents were analyzed by qualitative and quantitative methods in *Moringa concanensis* leaves, flowers and seeds using methanol extract. Alkaloids, flavonoids, terpenoids, carbohydrates, protein and amino acids were analyzed. Phenol

and saponin were present in only methanol extracts of leaves and flowers. Steroids, anthroquinone, tannin, oils and resins were absent in the extract. Quantitative analyses were also conducted to determine the amount of alkaloids, flavonoids, phenol and carbohydrate (Santhi *et al*., 2016).

It has been studied that the presence of phytochemical components and to determine the total phenolic and flavonoid contents of the seven medicinal plants .The Total phenolic contents obtained were 18.4mg/gm, 18.8mg/gm, 11.6mg/gm, 29.2mg/gm, 29.6mg/gm, 40.8mg/gm, 12.8mg/gm, 71.6mg/gm of the extract and total flavonoid contents obtained were 8.4mg/gm, 37.6mg/gm, 4.4mg/gm, 6mg/gm, 42.8mg/gm, 18mg/gm, 6mg/gm, 28.8mg/gm of the extract for the plants *Bryophyllum pinnatum* (Leaves), *Ipomea aquatica* (Leaves), *Oldenlandia corymbosa* (Whole plant), *Ricinus communis* (Roots), *Terminalia bellerica* (Leaves), *Tinospora cordifolia (*Leaves), *Tinospora cordifolia* (Stem), and *Xanthium strumarium* (Leaves) respectively. The above findings of the study, provided evidence that crude aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases (Yadav *et al*., 2011).

Earlier it has been described the qualitative phytochemical analysis in root tubers of six species of *Dioscorea* found in Meghalaya. The test confirms the presence of various phytochemicals like flavonoids, saponins, steroids, cardiac glycosides and terpenoids in two aqueous extracts of methanol and ethyl acetate. The results suggest that the methanolic extract shows the presence of maximum phytochemical compounds than ethyl acetate extract during screening (Sheikh *et al*., 2013).

Kumar *et al*. (2013) reported the phytochemical analysis of leaf extracts in aqueous, methanol, acetone, petroleum ether and chloroform extracts of indigenous medicinally important plants viz. *Holoptelea integrifolia*, *Celestruse marginata* and *Mammea suriga*. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xanthoproteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations.

Yusuf *et al*. (2013) performed medicinal activities of the leaf of the plant *Paullinia pinnata* and also to carry out phytochemical screening of the dried leaf of the plant, to extract and fractionate the leaf and finally to carry out thin layer chromatography of the different fractions. In the phytochemical investigation of the plant, the powdered leaves was tested

positive for steroids, triterpenes, alkaloids, saponins, tannins, anthraquinones and flavonoids. In East Africa, the leaves are used against snake bites, rabies, mental problems, blindness and eye troubles, together with the roots, against gonorrhoea, paralysis, wounds, threatened abortion, malaria, ancylostomiasis, and to expel placenta. Roots are applied against eczema, as a tonic and as a styptic medicine.

Mahesha *et al*. (2014) studied the extraction of phytochemical from the root bark of a well-known Indian traditional medicinal plant, with various solvents and evaluation of their *in vitro* antimicrobial and antioxidant activities using standard methods. The solvent extracts displayed promising anti- microbial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Cryptococcus neoformans* with inhibition zone in a range of 20-33mm. Then antioxidant screening revealed that aqueous extract and ethanolic extract were better antioxidants than standard ascorbic acid. Interestingly, FT-IR analysis of each extract established the presence of various biologically active functional groups in it.

Senguttuvan *et al*. (2014) performed qualitative and quantitative phytochemical analysis and evaluate *in vitro* antioxidant properties of various alcoholic and aqueous extracts of leaf and root parts of *Hypochaeris radicata*. The quantitative phytochemical analysis of this species exhibited the presence of alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid in considerable quantity. The *in vitro* antioxidant activity of the species, *Hypochaeris radicata* clearly demonstrated that both the leaf and root parts have prominent antioxidant properties. In a previous study, chief phytoconstituents of the six selected medicinal plants of different families were identified in order to relate their presence with bioactivities of the plants. All the selected medicinal plants were found to contain tannins and flavonoids. Moreover, terpenoids were also present in all the selected plants except *P. dactylifera*. On the other hand, saponins and steroids were absent in all plants except *S. chirata* and phlobatannins were absent in all plants except *R. sativus*. In addition, carbohydrates, glycosides and coumarins were present in all the selected plants except *P. dactylifera* and *R. sativus*.

Ram *et al*. (2015) investigated medicinal plants of Gujarat region (*Aerva lanata, Terminalia bellirica, Terminalia chebula, Terminalia catappa, Zea mays, Tribulus terrestris* and *Boerhaavia diffusa*) were selected for qualitative phytochemical screening and their reported biological activities. The screened plants were rich in flavonoids, tannins, steroids, cardiac

glycosides and alkaloids. Out of the seven plants screened, *Terminalia* species were the best possessing rich source of phytochemicals and justify their traditional use. Alkaloids were present in all the selected plants except *F. religiosa, P. dactylifera*and *R. sativus*. Proteins were present only in *F. religiosa*and *S. chirata*. Whereas emodins, anthraquinones, anthocyanins and leuco anthocyanins were absent in all the selected six plants (Yadav *et al*., 2014).

Bargah (2015) performed qualitative analysis for ethanol and aqueous extracts of leaves, stem bark and flowers of *Moringa pterygosperma* plant by various standard techniques available. Phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids, glycosides, steroids and phenols in all the extracts varying quantities. Since the plant contain high quantities of these new bioactive potential compounds, it is reliable to possess large number of pharmacological values like antioxidants, antifungal, antibacterial, anti-abortifacient, anti-inflammatory, antiulcer, diuretics activities and are being employed for the treatment of different ailments in the indigenous system of medicine.

Qadir *et al*. (2015) performed primary screening of the phytochemical contents of seven solvents (petroleum ether, benzene, chloroform, acetone, methanol, ethanol and a mixture of methanol and ethyl acetate (1:3) extracts of the seeds of *Anamirta cocculus* L. and to evaluate the antibacterial activity of these extracts against five species of pathogenic bacteria (*Staphylococcus aureus, Proteus vulgaris*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae*). The extracts of the seeds of *A. cocculus* contained various pharmaceutically active substances viz., aldehydes, alkaloids, phenolic compounds, flavonoids, saponins, carbohydrates, proteins, lipids, glycosides, phytosterols, volatile oils, gums and mucilage and other minor phytochemicals. All the extracts showed significant levels of antibacterial activity. Methanol extract was the most active one with remarkable antibacterial activity on the various species tested. MICs of the extracts revealed methanol extract as the most potent one with the lowest inhibitory concentration.

Earlier, it has been studied 10 medicinally important plant species were screened for their phytochemicals (quantitatively) by using 4 different solvent (water [AQ], Acetone [AE], Petroleum Ether [PE] and chloroform [CF]) extracted from their selected parts (leaves, stem, pericarp of the fruit and seeds cotyledons). All the plants which are selected for the study contains phytochemicals like alkaloids, flavonoids, steroids, phenols and saponins. The highest concentrations of alkaloids are observed in *Levisticum officinale* leaf and *Foeniculum vulgare*

stem extracts by using PE. The highest amounts of flavonoids are seen in AQ and PE extracts of *Garcinia indica, Dracaena loureiri, Sapindus saponaria.*

Prabhavathi *et al*. (2016) carried out phytochemical analysis of *Cissus quadrangularis*, proved to be one of the important medicines for treatment of bone fractures. The different parts of the plant extracted with methanol and ethanol solvents. The qualitative analysis showed that alkaloids were mainly seen in most of the samples except methanolic extract of stem and fruit. Tannins, proteins, carbohydrate and phenol were present in all the 4 samples extracted by both methanol and ethanol. Flavonoids were seen only in leaf samples where as cardiac glycosides were seen only in stem samples extracted with methanol. Saponins were mainly present in the samples extracted from methanolic solvent. The qualitative analysis carried out for the determination of phenols, carbohydrates, tannins, alkaloids and proteins. Tannins were seen mainly in leaf samples of both the extracts, ethanol extract of the fruit shown the maximum amount of phenol and proteins. The leaf samples extracted by the solvents possessed contained high carbohydrate content.

2.5. Antioxidant activity & GC-MS Analysis

Yizhong *et al*. (2003) reported antioxidant activity and phenolic compounds of traditional Chinese medicinal plants associated with anticancer, comprising 112 species from 50 plant families. The TEAC values and total phenolic content for methanolic extracts of herbs ranged from 46.7 to 17,323 Amol Trolox equivalent/100 g dry weights (DW) and from 0.22 to 50.3 g of Gallic acid equivalent/100 g DW, respectively. A positive, significant linear relationship between antioxidant activity and total phenolic content showed that phenolic compounds were the dominant antioxidant components in the tested medicinal herbs.

Almeida *et al*. (2011) compared contents of phenolics, vitamin C, anthocyanin and antioxidant activity of 11 fresh exotic fruits, cultivated in the northeastern part of Brazil. Results indicated that the above fruits, such as murici and mangaba, were good sources of antioxidants. The phenolic contents showed positive correlations with total antioxidant by ABTS and DPPH assays. However, this correlation was not noticed when examining vitamin C and anthocyanins contents. The 11 fruits studied had comparable antioxidant activity in both, ABTS and DPPH assays.

Arab *et al*. (2011) evaluated antioxidant activity of two Iranian rice bran varieties, Fajr and Tarem, extracted by three different solvents (methanol, ethanol and ethyl acetate). The

methanolic extract of Fajr rice bran showed a total phenolic content of 3.31 mg Gallic acid/gr rice bran and a DPPH free radical-scavenging activity of 93.91%, achieved at 50 mg/ml concentration, and a percent inhibition of linoleic acid peroxidation of 68.01% with reducing power.

In a previous report it has been showed that, an antioxidative activity of the ethanolic extract of *Desmodium gangeticum* was correlated with total phenolic content. The antioxidant activity of *D. gangeticum* ethanolic extract is comparable to a certain extent with that of butylated hydroxyl toluene, mannitol, EDTA and ascorbic acid (Venkatachalam *et al*., 2012).

Earlier it has been studied the hydro alcoholic extract of *Nardostachys jatamansi* (NJE) rhizomes was evaluated by various antioxidant assays, extract exhibited high reduction capability and powerful free radical scavenging, especially against DPPH and superoxide anions as well as a moderate effect on NO. The peroxidation inhibiting activity of NJE was demonstrated in the linoleic acid emulsion system (Sharma *et al*., 2012).

Somasundaram *et al*. (2012) studied the ethanolic and aqueous extracts of *Nymphaea stellata* flower extracts for antioxidant activity using 1, 1-diphenyl-2-picryl hydroxyl (DPPH) quenching assay, Hydroxyl Radical Scavenging Activity, Nitric Oxide Scavenging activity using established assay procedures. The extracts exhibited high antiradical activity against DPPH, nitric oxide and hydroxyl radicals. The FRP increased with increasing concentration of the sample. Ethanolic extract showed more scavenging activity than the aqueous extract.

Previously, Aksoy *et al*. (2013) evaluated free radical scavenging activity, total phenolic content, total oxidant status (TOS), and total antioxidant status (TAS) of methanol (TTM) and acetone (TTA) extracts of *Thermopsis turcica* were measured spectroscopically. Methanol and acetone extracts of *T. turcicawere* found to have a specific radical scavenging effect. This effect was found to be related to the total phenolic content of the extracts. Since the TTA had a higher phenolic content than the methanol extract, it had a stronger radical scavenging effect. In addition, the total antioxidant capacity of the methanol extracts was observed to be higher than that of its acetone counterpart.

Silva *et al*. (2013) investigated the *in vitro* and *in vivo* antioxidant potential of aqueous extract of *Passiflora edulis* leaves and identification of phenolic compounds by HPLC-PDA and ESI-MS/MS analysis. Antioxidant status was analyzed by FRAP, ORAC in serum and by SOD, GR and GPx activities, GSH contents and TBARS in liver, brain and kidneys. Vitexin, isovitexin

and isoorientin were analyzed in the extract of *P. edulis* leaves. The animals which received tea showed a decrease of 20% of TBARS in liver. GSH contents in kidneys increased 40% relative to control group. The GR activity was 2 times higher and GPx 3.2 times lower in liver than control group. Animals from TEA group showed a 45% reduction on SOD activities in liver and brain. Serum antioxidant potential was not altered. Tea intake also promoted colonic bacteria growth, although there was a decreasing in the SCFA production.

Malar *et al*. (2014) analyzed ethanolic extract of Cress (L*epidium sativum* L.) shoot, leaf, stem and seed for antioxidative activity against DPPH, total glutathione S - transferase assay, reduced glutathione activity, reducing power, and ascorbic acid is also estimated. Supreme scavenging activity was detected in shoot and least in stem. The activity of total glutathione S-transferase enzyme was found to be more in seed. The reduced glutathione content of the ethanolic extracts of *L. sativum* was found to be more in leaf. In the reducing power assay, ethanolic extracts gives the optical density in increasing concentration in all plant parts it shows that it has the reducing ability. Presence of vitamin C was tested. It was found that the shoot extract has highest amount of vitamin C.

Al-Owaisi *et al*. (2014) performed phytochemical screening, estimate total phenolics, flavonoids and to evaluate antioxidant potential of *Moringa peregrine* (*M. peregrina*) leaves. Gas chromatography-mass spectrometry revealed presence of 19 phytoconstituents in hexane extract, 6 in ethyl acetate and 7 compounds in methanolic extract. Methanol extract was found to contain the highest phenolic content and flavonoids. *In vitro* antioxidant activities of all crude extracts were significant and comparable with the standard ascorbic acid.

It has been studied maceration, Soxhlet and fractionation extraction from whole plant of *Osbeckia parvifolia* was studied for free radical scavenging and anti-inflammatory activities *in vitro*. Quantitative analysis showed that whole plant has high contents of total phenolic, tannin and flavonoid. Antioxidant assessment results registered higher anti-radical property for both macerated and soxhlet methanol extracts compared to other solvent extracts. Successively extracted methanol extract from Soxhlet apparatus protected protein denaturation and erythrocyte membrane lysis comparable to standard Diclofenac sodium (Murugan *et al*., 2014).

Diem Do *et al*. (2014) determined water and various concentrations of methanol, ethanol, and acetone in water were used as solvent in the extraction of *Limnophila aromatica*. The extract obtained by ethanol showed the highest total antioxidant activity, reducing power and DPPH

(2, 2- diphenyl-1-picrylhydrazyl) radical scavenging activity. The same extract also exhibited the highest phenolic content and the highest flavonoid content. The highest extraction yield was obtained by using aqueous acetone. The report indicates that *L. aromatica* can be used in dietary applications with a potential to reduce oxidative stress.

Abdel-Aziz *et al*. (2014) studied the plant extract for the biosynthesis of silver nanoparticles and to evaluate their antibacterial and antioxidant activity *in vitro*. The TEM analysis showed that the sizes of the synthesized AgNps ranged from 30 to 50 nm. The essential oil of *Chenopodium murale* leaf extract was formed mainly of a-Terpinene, (Z)-Ascaridole and cis-Ascaridole. The total phenolic compounds and total flavonides were higher in AgNPs-containing plant extract compared to the plant extract. AgNPs-containing leaf extract showed a higher antioxidant and antimicrobial activity compared to *C. murale* leaf extract alone or silver nitrate. It could be concluded that *C. murale* leaf extract can be used effectively in the production of potential antioxidant and antimicrobial AgNPs for commercial application.

Faten medina *et al*. (2014) report the antioxidant and antimicrobial activities *in vitro* of 10 extracts of the halophyte *Limonium delicatulum* harvested in two physiological stages, flowering and vegetative, and to determine their phenolic compounds by reverse-phase high-performance liquid chromatography. Ethanol extracts had the most total antioxidant activity, while the acetone extracts had the greatest radical scavenging capacity and the methanol extract the best inhibition of beta-carotene bleaching. The ethanol extract showed the highest total antioxidant activity and antibacterial activity, mainly against *Salmonella*. The main phenolic compounds were *p*-coumaric acid and chlorogenic acid.

Chaouche *et al*. (2014) evaluated the total phenolic, flavonoid, and tannin contents of three Algerian medicinal plants: *Echium pycnanthum* Pomel, *Haloxylon articulatum* Boiss, and *Solenostemma oleifolium* Bull. These medicinal plants exhibited significantly different total polyphenol contents varying from 27.3 -2.1 mg to120.3 -5.6 mg Gallic acid equivalents/g dry weight. The phenolic content in *H. articulatum* was superior to those in *E. pycnanthum* and *S.oleifolium*. The same tendency was observed for the relative amounts of flavonoids and condensed tannins in the three medicinal plants. The antioxidant activities varied greatly among the different plants used in this study. Indeed, *H. articulatum* shoots exhibited the strongest antioxidant activity, with the lowest IC_{50} and EC_{50} values for 1, 1-diphenyl-2-picrylhydrazyl and

iron reducing tests, respectively. In addition, the superiority of this plant was more marked as compared to positive controls.

Earlier it has been described in some selected medicinal plants used by Bentian tribe from Indonesia were evaluated for potential antimicrobial and antioxidant properties that *n*-hexane, ethyl acetate and ethanol extracts of *Cananga odorata*, ethanolic extract of *Chromolaena odorata*, and ethanolic extracts of *Hyptisca pitata* and *Ampelocissusc innamomeae* displayed good activity against *Propioni bacterium acnes* of the extracts tested. The *n*-hexane and ethanol extracts of *Chromolaena odorata*and *H. capitata*, and the *n*-hexane, ethylacetate and ethanol extracts of *Cananga odorata* displayed more activity against *Candida albicans* than other extracts tested. The most antioxidant activities against DPPH were displayed by the ethanol extracts of *Ficus variegate* stem bark, *Leucosyke quadrinerva* root and *Clausenae xcavata* leaves exhibiting 91%, 91% and 86% inhibition, respectively (Kusuma *et al*., 2014).

Foo *et al*. (2015) compare the *in vitro* antioxidant capacity of a diatom; *Chaetoceros calcitrans* (*C. calcitrans*) extracted using six types of solvents. The methanol extract exhibited the highest yield, total carotenoid, total phenolic and second highest fucoxanthin content as compared to other solvent extracts. Methanolic extract also exhibited significantly higher scavenging and iron chelating activities. Chahmi *et al*. (2015) investigated antioxidant activity, total phenolic and flavonoid content of ethanol and ethyl acetate extracts of *Inula viscosa* aerial parts selected from three regions of Morocco (Imouzzer, Sefrou and Taounate). All the extracts showed significant antioxidant activities and contained importantlevels of phenols. The ethanol extract from Sefrou showed the greatest antioxidant capacity in the three systems of assay, which was probably due to its high content of polyphenols. Total flavonoid content was found equal for all extract.

Baba *et al*. (2015) evaluated the antioxidant and antimicrobial activity of a methanolic extract of the roots of *Arisaema jacquemontii.* The root extract prevented the growth of both Gram-positive and Gram-negative bacteria, at a minimum inhibitory concentration of 0.24–0.41 mg/ml. Antifungal activity, measured as inhibition of mycelium growth, was 28.32–36.50%. The antimicrobial and antioxidant activities of the extracts were positively associated with the total phenolic and flavonoid contents of the extract. Hossain *et al*. (2015) studied the antioxidant activity and total phenols content of the essential oil and different solvent extracts of the endemic plant *Merremia borneensis*. In aqueous ethanol extract exhibited a higher activity potential than

that of other extracts (hexane, chloroform, ethyl acetate and butanol) and the essential oil the total phenolics was very high in this extract. Chloroform extract has been found to be rich in flavonoids. A positive result was observed between the antioxidant activity potential and total flavonoid levels of the extracts.

Wong-paz *et al*. (2015) determine the extraction suitable conditions of total phenolic content by heat-reflux system, antioxidant activities and HPLC characterization of the aqueous-ethanolic extracts of *Jatropha dioica*, *Flourensia cemua*, *Eucalyptus camaldulensis* and *Tumera diffusa*. TPC in the plant extracts ranged from 2.3 to 14.12 mg Gallic acid equivalents/g for *J. dioica* and *E. camaldulensis*, respectively. The plant extracts of *F. cemua*, *E. camaldulensis* and *T. diffusa* showed similar strong antioxidant activities on scavenging of DPPH and lipid oxidation inhibition. In contrast, *J. dioica* extracts had lowest potential antioxidant in three assays used. HPLC assay showed the presence of several phenolic compounds in the extracts used.

Krishnaiah *et al*. (2015) investigated antioxidant activity and total phenolic content of an isolated *Morinda citrifolia* L. methanolic extract. The extract of *M. citrifolia* L. fruit by methanol was separated into permeate and retentate by Poly-ether sulphone (PES). The effect of temperature in the range of 30–70 C, and pressure in the range of 0.5–1.5 bar on the antioxidant activity and total phenolic content was studied. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity exhibited a gradual increase in permeates' collection from membrane separation. The total phenolic content was also found to follow the same trend. The optimum magnitudes of DPPH radical scavenging activity and total phenolic content were found to be 55.60% and43.18 mg GAE/10 gm of sample respectively.

Upadhya *et al*. (2015) studied the effect of extraction method with respect to time of exposure on total phenolic content and antioxidant potential of methanolic extracts (95%) from *Achyranthes aspera* leaves, stem and roots. The antioxidant assays as that of TPC, indicating phenolics to be the major contributor in the antioxidant potential of the plant. In conclusion it can be said that the yield of phenolic compounds depends on parameters viz. age of plant, part used for extraction, method of extraction and time required for the same.

In the previous report, surface methodology based on a central composite rotatable design was used to determine optimum conditions for the extraction of antioxidant compounds from *Origanum vulgare* leaves. Analysis of variance (ANOVA) was applied and the significant effect

of the factors and their interactions were tested at 95% confidence interval. The antioxidant extract (AE) yield was significantly influenced by solvent composition, solute to solvent ratio, and time. The maximum AE was obtained at methanol, liquid solid ratio, time (16 h), and particle size (20 micron). Predicted values thus obtained were closer to the experimental value indicating suitability of the model. Run 25 (methanol: water 70:30; solute: solvent 1:20 (Majeed *et al*., 2016).

In another report, designed to assess the *in vitro* antioxidant activity and free radical scavenging capacity of ten medicinal plants, which are extensively used in the Ayurvedic treatment systems in Sri Lanka showed that DPPH and ABTS radical scavenging activities were higher for the Nelli (*Phyllanthus emblica*) extract while the least activity was observed in Venivel (*Coscinium fenestratum*) extract. The FRAP activity of the extracts was well proved with the DPPH and ABTS radical scavenging activities. A positive, significant linear relationship between antioxidant activity and TPC and TFC content showed that phenolic compounds and flavonoids were the dominant antioxidant components in the medicinal herbs studied (Jayathilake *et al*., 2016).

Boonsong *et al*. (2016) performed the estimation of total phenolic and flavonoid contents and antioxidant properties of five edible mushroom samples *Lentinus edodes*, *Volvariella volvacea*, *Pleurotus eous*, *Pleurotus sajor-caju* and *Auricularia auriculard* using three different extractants. The ethanolic extract showed a lower reducing power compared to BHA and a-tocopherol. Moreover, the *L. edodes* ethanolic extract also had the highest chelating ability which was lower than for ethylene diamine tetra acetic acid and showed the strongest superoxide radical scavenging activity compared to BHA and a-tocopherol. Therefore, the ethanolic extract of *L. edodes* could be used as a potential natural antioxidative source or as an ingredient in the fish and fishery product industries.

Abootalebian *et al*. (2016) investigated total phenolic and antioxidant activity of five Iranian mint accessions from two different species, *Mentha spicata* L. (Mzin1, Mzin3 and Mzin8) and *M. longifolia* L. (Mzin5 and Mzin6). The content of total phenolics (mg tannic acid equivalent per g dry weight of the sample) differed from 50.1 in Mzin3 to 67.2 in Mzin6. The highest percent radical scavenging activity was observed with Mzin6 at all concentrations studied. Peroxide value of sunflower oil containing Mzin5 and Mzin6 was the lowest among the mint accessions and almost equivalent to that of butylated hydroxyl toluene. In overall, *M.*

longifolia was superior to *M. spicata,* as determined by two model systems, indicating its potential use as a natural source of dietary antioxidant.

It has been studied antioxidant, anti-hemolytic and antimicrobial activities of six Moroccan date fruit varieties. Estimation of total phenolic and flavonoid contents revealed that, Bousrdoun and Jihl had the highest phenolic and flavonoid contents, respectively. That date fruit extract, especially Jihl and Bousrdon extract, is not only an important source of antioxidants, which possess a high protective effect of membrane against free radical, but also a potential source of antibacterial components (Bouhlali *et al*., 2016).

Joshy *et al*. (2016) evaluated the hepatoprotective, anti-inflammatory and antioxidant activities of *Flacourtia montana* methanolic extract. The hepatoprotective effect of *F. Montana* was evaluated against paracetamol induced hepatotoxicity in Wistar rats. The anti-inflammatory activity of *F. Montana* was evaluated by carrageenan-induced paw edema and cotton pellet-induced granuloma models. *F. Montana* showed a significant reduction in rat paw edema with 76.39% and 80.32%, respectively induced by carrageenan against the reference anti-inflammatory drug ibuprofen. Oral administration of *F. Montana* also significantly reduced the granuloma mass formation in cotton pellet granuloma method. The reducing power and hydrogen peroxide radical scavenging were increased at increasing doses of *F. Montana* in this study demonstrate that the methanolic extract of *F. Montana* possess hepatoprotective, anti-inflammatory and antioxidant activities.

Nemanjastankovic *et al*. (2016) evaluated the antibacterial activities and antioxidant capacity of eight aromatic plants, indigenous to the flora of the Balkan Peninsula, which are used as medicinal plants in traditional medicine. The plants studied were *Hyssopus officinalis*, *Angelica pancicii*, *Angelica sylvestris*, *Laserpitium latifolium*, *Achillea grandifolia*, *Achillea crithmi-folia*, *Artemisia absinthium* and *Tanacetum parthenium*. Minimal inhibitory concentration (MIC) of the extracts ranged from 6.3 to 100 mg mL-1, and minimal bactericidal concen-tration (MBC) ranged from 12.5 to 100 mg mL-1. Then antioxidative activities from the 4 methods demonstrated similar sequence of activity: *A. crithmifolia* > *A. grandifolia* > *H. officinalis* > *A. absinthium* > *T. parthenium* > *L. latifolium* > *A. pan-cicii* > *A. sylvestris*. The total content of polyphenols and flavonoids in the methanol extracts of the studied species positively correlated with their antioxidant properties, confirming their major role in anti oxidant activity of these species.

Saha *et al*. (2016) investigated the *in vitro* antioxidant activities of polyphenolic extract of *Terminalia chebula* Retzius (Combretaceae) fruits. The phytochemical characterisation of the extract was also measured by determining the total phenolic, flavonoid, tannin and ascorbic acid contents. Characterisation of the extract was also performed by HPLC profiling with the standard Gallic acid. The results demonstrated that the extract had significant reducing capacity and nitric oxide scavenging activity. It also scavenges hydrogen peroxide-induced radicals. The activity of the extract may be due to the total polyphenolic content. The antioxidant activity of the extract is significantly higher than the standard ascorbic acid, and its activity is concentration-dependent.

Chigayo *et al*. (2016) determined phytochemicals, total phenol and flavonoid contents and the antioxidant activities of different solvent extracts *of Kirkia wilmsii* (*K. wilmsii*), an ethno medicine in South Africa. Phytochemical screening confirmed the presence of phenolics, flavonoids, terpenoids, tannins, cardenolidedeoxy sugars and reducing sugars. Of the 12 solvent extracts used, six gave yields higher than 5%, while the other six gave yields less than 1%. The flavonoid content for methanol was significantly higher than all the other extracts. The scavenging profiles of *K. wilmsii* extracts were significantly lower than that of ascorbic acid and IC_{50} values ranged from 129.94 mg/ml for methanol to 225.04 mg/ml for water. An IC_{50} value of 56.52 mg/mlwas obtained with ascorbic acid.

Boutennoun *et al*. (2017) studied the methanol extract from *Achillea odorata* was evaluated for its phenolic contents and antioxidant activity. The preliminary screening was concluded in the presence of substances with large therapeutic values. The total phenolic content confirmed the presence of total phenolics in the extract and showed strong association with antioxidant activity. An important content of flavonoids and flavonolswas also detected. The results of the antioxidant activities obtained indicate that *A. odorata* recorded a good capacity. For the cytotoxic activity, the results showed the plant extract significantly inhibited tumor cell growth and colony formation at various concentrations.

Hidayat *et al*. (2017) reported chromogenic radical, DPPH solution was immobilized on the micro well plate as dry reagent to construct a simple antioxidant sensor. The red, green, and blue (RGB) value when used as sensor response, it is possible to determine antioxidant capacity in the range 1–25 ppm as Gallic acid equivalent (GAE) with the response time of 9min. There reducibility of sensor was good with recovery at 93% - 96%. The antioxidant sensor was applied

to the plant extracts, such as Sapp and wood and Turmeric Rhizome. The results are good when compared to the same procedure using a UV/V is spectrophotometer.

Adebiyi *et al*. (2017) evaluated the free radical scavenging activity of ethanol extract of leaf and stem of *Grewia carpinifolia* using various *in vitro* models. *Grewia carpinifolia* has a high radical scavenging activity in the various radical systems. The total phenolic content was 19.08 ± 1.21 mg gallic acid equivalent (GAE)/g extract and 14.85 ± 1.09 mg GAE/g extract for the leaf and stem respectively while the flavonoid content was 9.00 ± 0.13 and 13.22 ± 1.53 mg quercetin/g extract. The antioxidant activity of *Grewia carpinifolia* extract may be due to the high level of flavonoids and phenols in the plant.

II. MATERIALS AND MEHODS

The materials and methods adopted in the present study entitled **"Phytochemical profiling and *in vitro* free radical scavenging activities of *Mussaenda frondosa* L. leaf extract"** is presented as follows:

3.1. Collection of the plant

Mussaenda frondosa (Plate.1) the whole plant were collected from Agali, Palakkad district in the state of Kerala, India.

Plate 1: *Mussaenda frondosa* L.

3.2. Preparation of *Mussaenda frondosa* leaf

The *Mussaenda frondosa* leaf was washed in running tap water to remove soil and adhered debris. The plant was then washed using sterile distilled water dried at room temperature. The dried plant material was ground into a fine powder in an electric grinder. The powdered samples were stored in screw cap bottles until further analysis (Plate. 2)

Plate 2: *Mussaenda frondosa* leaf powder

3.3. Preparation of extract

Ten grams of dried *Mussaenda frondosa* leaf powder were taken and dissolved in 50 ml of different solvents (Chloroform, Ethyl acetate, Hydro ethanol and Methanol), mixed, and incubated for ten hours. The contents were periodically shaken using an electric shaker in room temperature (37˚C) at 200rpm, then the contents were filtered through a Buchner funnel in a conical flask and it was further concentrated by evaporation by keeping the filtrate in a round bottomed flask, till the solvent completely evaporated and the extract settled down to the bottom.

3.4. Preliminary phytochemical screening

The powder of *Mussaenda frondosa* extracted using different organic solvents to ensure obtaining polar and non- polar constituents. Qualitative study of the plant was done according to the standard procedure for following alkaloids, flavonoids, glycosides, polyphenols, saponins, steroids, tannins, carbohydrates and reducing sugar (Deepti *et al*., 2012; Majumder *et al*, 2013).

3.4.1. Test for Flavonoids

3.4.1. 1.Shinoda Test:

In a few mg of sample 1ml of ethanol was added and heated in boiling water both. To the ethanolic solution added 1 drop of concentrated HCL followed by few pieces of magnesium fillings and kept at room temperature for 10-15 minutes. Red color formation indicated the presence of Flavonoids.

3.4.2. Test for Alkaloids

3.4.2.1. Dragendroff's Test

To 1ml of test extract, 1ml of Dragendroff's reagent was added. Formation of orange precipitate indicated the presence of alkaloids.

3.4.3. Test for Steroids/ Triterpenoid

3.4.3.1. Libermann Buchard Test

To the test extracts added 2ml of chloroform followed by 10 drops of acetic anhydride and 2 drops of concentrated H_2SO_4.RosyRed color developed which quickly changed through blue (sterol) to green (phenol)indicated the presence of cholesterol.

3.4.4. Test for Polyphenol

1 drop of extract dissolved in alcohol and mixed well followed by addition of 1 drop of neutral ferric chloride solution. Green color (or) any dark color indicated presence of polyphenol.

3.4.5.Test for Glycosides

Few ml of test extract was dissolved in a few drops of pyridine. To this added a drop of 2% w/v sodium nitro prusside solution and a drop of 20% sodium hydroxide solution. Appearance of pink/ deep red color will indicate the presence of glycosides.

3.4.6. Test for Tannins:

To the test extract added 10 ml of water, boiled and filtered. To the filtrate few drops of 10% ferric chloride was added. A dark green, blue (or) brown color indicated the presence of tannins.

3.4.7. Test for Saponins:

3.4.7.1 Sodium bicarbonate Test:

In 1ml of test extract, three drops of sodium bicarbonate was added and shaken well. Formation of honey comb indicated the presence of saponins.

3.4.8 Test for Carbohydrates:

3.4.8.1 Benedict's test:

To 0.5 ml of the test extract, 2ml of Benedict's solution was added. Formation of Reddish brown precipitate indicates the presence of carbohydrates.

3.5. Free Radical scavenging assays:

3.5.1. DPPH free radical scavenging activity:

This assay was carried out following (Mensor *et al*., 2001) standard protocols. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. The diluted working solutions of the test extracts were prepared in methanol. About 3ml of graded concentration (20 - 100µg/ml) of extracts were taken in different test tubes. 1 ml of 0.3mM DPPH methanol solution was added to these test tubes and shaken vigorously. Methanol served as the blank and DPPH in methanol, without the plant extracts, served as the positive control. After 30 min incubation of samples at 25˚C in the dark, the absorption was measured at 517 nm. The optical density was recorded and % inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = 100-(A-B/A) × 100

Where A = optical density of the blank and B = optical density of the sample

3.5.2. Reducing power assay:

This assay was carried out following (Oyaizu, 1986) standard protocols. Reaction mixtures were prepared by adding 2.5ml of phosphate buffer (0.2m, PH 6.6), 2.5 ml potassium ferricyanide (1%) and varying concentrations of extracts (20-100mg/ml). After the reaction mixtures were incubated at 50˚c in water-bath for 30 minutes, allowed to cool at room temperature (28˚c) and 2.5ml of 10% TCA were added to each reaction mixture, and then centrifuged at 2000rpm for 10minutes. The supernatant (2.5ml) was separated in the test tube and added with 2.5ml of distilled water and 0.5ml Fecl3 (1.0%) and allowed to react for 10 min at room temperature and absorbance was measured at 700nm.

3.5. 3. Ferric reducing antioxidant power (FRAP) assay:

The FRAP (ferric reducing ability of plasma) assay was performed according to the previously reported method of Dehghan and Khoshkam, 2012. FRAP reagent was prepared by mixing of 2.5 ml of solutions TPTZ (10 mm, (40 mm) HCL, and FeCL3 (20 mm) in 25 mm of acetate buffer (300 mm, pH 3.6), the light blue reagent contains Fe3+-TPTZ that changes to Fe2+-TPTZ as dark blue. These changes were due to the absorbance increase as monitored at a wavelength of 593 nm for different concentrations of *Mussaenda frondosa* leaf extracts in FRAP reagent .

3.6. Spectral characterization:

Spectral analysis of plant extract would help in determining various biological activities of bioactive constituents. Spectroscopic ultraviolet-visible, Fourier transforms infrared (UV-Vis) methods and GC-MS together or separate would be useful in the identification of bioactive compounds, when compared to conventional methods.

3.6.1 UV-Visible spectral analysis:

UV –Vis spectrophotometer related to the spectroscopy of photons in the UV-Visible region or its adjacent ranges. In the present study ,to detect the UV-VIS spectrum profile of *Mussaenda frondosa* plant the extract were scanned in the wavelength ranging from 100-700nm by using UV spectrophotometer (Shimadzu, Japan) and the characteristic peaks were detected. The peaks values of the UV-VIS were recorded.

3.6.2. FT-IR spectral analysis:

FT-IR measurements were performed on spectroscope (Shimadzu, Japan) using 500-4000nm. To prepare sample for FT-IR test a small amount (about one gram) of potassium bromide KBr_3 was mixed with the sample and compressed in order to make a suitable capsule for FT-IR device.

3.7. GC-MS Analysis:

GC-MS model GC-MS-QP 2010S was used in the analysis that employs fused silica column and the components were separated using helium as a carrier gas at a constant flow of 1 ml/min. The 1µl sample extract was injected into the instrument. The initial temperature was set at 100°C, where as the injector temperature was set at 250°C and throughout the process temperature flow was set at the speed of increasing 10°C/min. The actual separation was observed at 24^{th} minute, for which final temperature was adjusted to 280°C and run for 5min (Gopalakrishnan & Vadivel, 2011).

3.8. *In silico* Molecular Docking analysis

3.8.1. Schrodinger suite

Maestro is Schrodinger's powerful, unified, multi-platform graphical user interface (GUI). It is designed to simplify modeling tasks, such as molecule building and data analysis, and also to facilitate the set up and submission of jobs to Schrodinger's computational programs. The main Maestro features include a project-based data management facility, a scripting language for automating large or repetitive tasks, a wide range of useful display options, a comprehensive molecular builder, and surfacing and entry plotting facilities.

3.8.2. LigPrep

LigPrep module was used to retrieve all molecules from GC-MS results and processed it to be docked with diseased protein. LigPrep is a robust collection of tools designed to prepare high quality, all-atom 3D structures for large numbers of drug-like molecules, starting with 2D or 3D structures in SD or Maestro format. The resulting structures can be saved in either SD or Maestro format. The simplest use of LigPrep produces a single, low-energy, 3D structure with correct chiralities for each successfully processed input structure.

3.8.3 .Evaluation of drug likeliness and toxicity prediction

The drug likeliness of the compounds was analyzed using Lipinski's rules. This rule describes molecular properties important for a drug's pharmacokinetics in the human body and provides the information regarding the utilization of the ligands as a drug (Lipinski *et al*., 1997). The rules are molecular weight < 500 daltons, number of hydrogen bond donors <5 and number of hydrogen bond acceptors < 10, calculated water partition coefficient (LogP) < 5. The ligands passing the Lipinski properties were taken for docking studies.

3.8.3.1. QikProp

Toxicity of the selected molecules was performed using QikProp module from Schrodinger. This includes property prediction and principal description of the molecule. QikProp is a quick, accurate, easy-to-use absorption, distribution, metabolism, and excretion (ADME) prediction program designed by Professor William L. Jorgensen. QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in batches. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs.

3.8.4. Molecular docking

3.8.4.1. Protein Data Bank:

The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. In the present study, the three dimensional structure of the diseased protein namely PTEN (ID: 1D5R) was selected from the protein data bank.

3.8.4.2. Ligand docking

Glide ligand docking jobs required a set of previously calculated receptor grids and one or more ligand structures. Preparation of the ligands before docking is strongly recommended. If a correct Lewis structure cannot be generated for a ligand, it is skipped by the docking job. Glide also automatically skips ligands containing unparametrized elements, such as arsenic, or atom types not supported by the OPLS force fields, such as explicit lone pair atoms. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor were represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses

3.8.4.3. Examining glide data

Glide results were examined with an emphasis on visual rather than numerical appraisal. The first set of exercises used the Project Table to display the results of the SP Glide docking job, examined individual ligand poses and their contacts with the input receptor structure.

3.9. Statistical Analysis

All the experiments were carried out in triplicate. The results of antioxidant activities were given as mean ± standard deviation (SD).

III. RESULTS

The results pertaining to the study **"Phytochemical profiling and *in vitro* free radical scavenging activities of *Mussaenda frondosa L.* leaf extract"** are presented in the following headings

4.1 .Preliminary phytochemical screening of *Mussaenda frondosa:*

Preliminary phytochemical screening of eight different chemical compounds (Flavonoids, Alkaloids, steroids, polyphenols, tannins, glycosides, saponins and carbohydrates) were tested for their presence or absence in four different extracts of *Mussaenda frondosa.* The results of phytochemical test have been summarized in Table 1.

Table 1.Qualitative Phytochemical analysis of *Mussaenda frondosa* leaf extract

Phytochemical	**Chloroform**	**Ethyl acetate**	**Hydro ethanol**	**Methanol**
Flavonoids	+ - -	+ - -	+ + +	+ + +
Alkaloids	+ + +	+ + -	+ - -	+ + -
Steroids	+ + -	+ - -	+ - -	- - -
Poly phenols	+ - -	+ + -	+ + +	+ + +
Tannins	+ - -	+ + +	+ - -	+ + -
Glycosides	+ + -	- - -	+ + +	+ + -
Saponins	+ + +	- - -	- - -	+ - -
Carbohydrates	+ + +	+ + -	+ - -	+ - -

Key: - absence, + presence, + + fairly good amount, + + + good amount.

Among the tested solvents, methanol extract showed considerably good amount of phytochemicals when compared to other solvent extracts. Hence in further, methanol extract was chosen for *in vitro* assays.

4.2. Free Radical Scavenging assays:

4.2.1 DPPH radical scavenging activity:

The antioxidant activity of extract was measured in terms of radial scavenging ability of the extract. Methanol solution of DPPH shows a strong purple color with strong absorption 517nm (Fig. 2) and when reduced showed yellow color. Free radical scavenging activity of DPPH radical was found to increase with increase in concentration, showing maximum of 1.131 at 100µg/ml (Fig. 1). Observations of DPPH reduction are shown in Table 2.

Table 2. DPPH radical scavenging activity of methanolic extract of *Mussaenda frondosa*

	Concentration (µg/ml)	**% inhibition**
1	20	1.027 ± 0.0064
2	40	1.052 ± 0.0076
3	60	1.041 ± 0.0046
4	80	1.085 ± 0.0085
5	100	1.131 ± 0.0178

Values are given as mean ± standard deviation

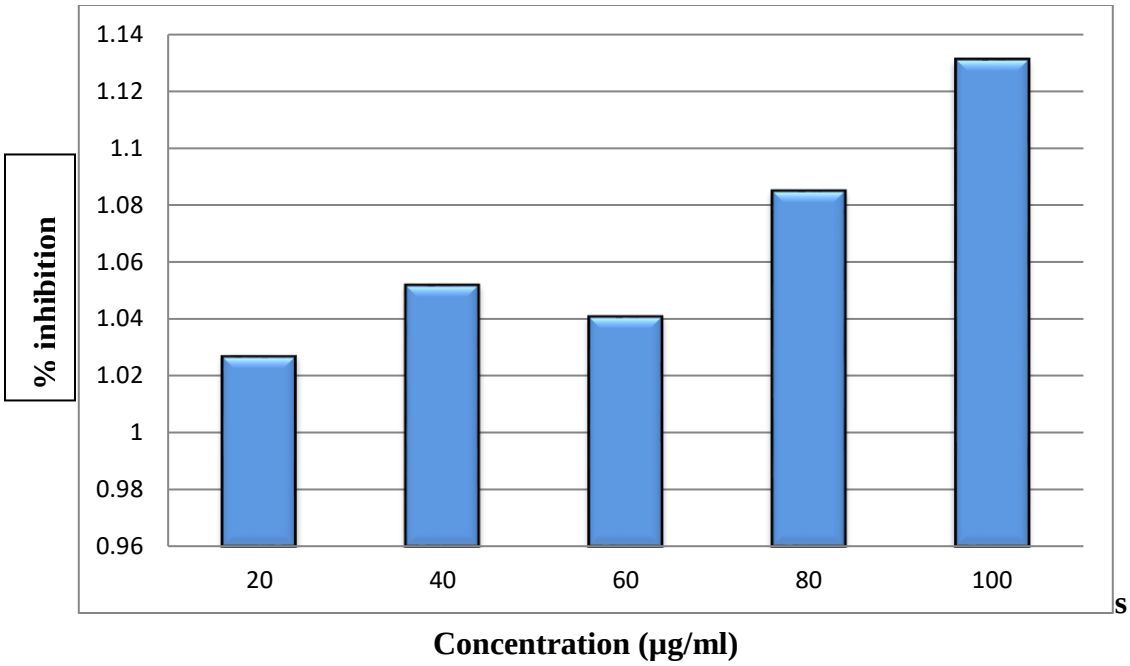

Fig 1: Radical scavenging activity of methanol extract of *Mussaenda frondosa*

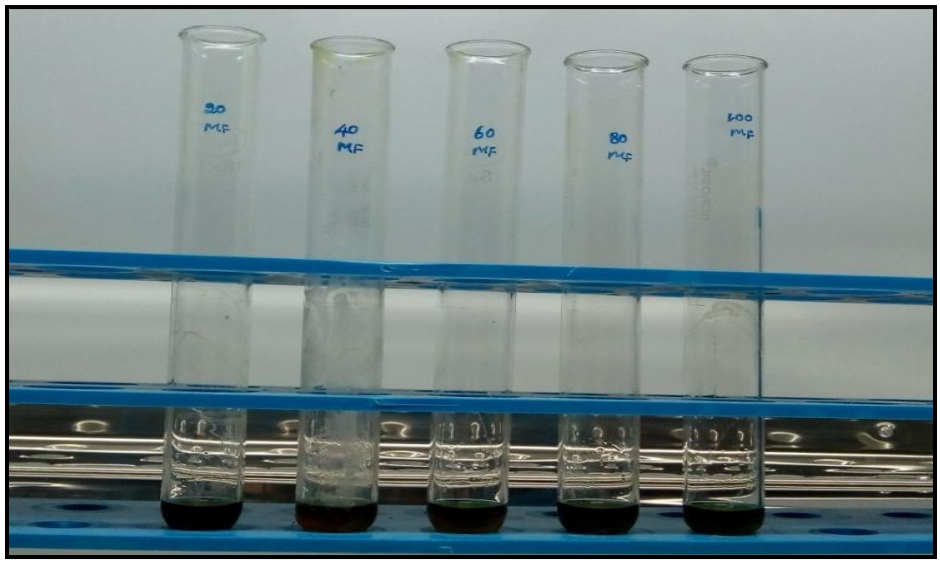

Fig 2: DPPH reduction of methanol extracts of *Mussaenda frondosa*

4.2.2 Reducing power activity of methanol extract of *Mussaenda frondosa*

The reducing power of different concentration of *Mussaenda frondosa* was found to be remarkable and the absorbance of each concentration was found to rise as the concentration gradually increases. The reducing power of the extract was found to be high at a concentration of 100µg/ml. The remaining concentrations showed activity in the following order 20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml as shown in the Table 3.

Table 3: Reducing power of methanol extract of *Mussaenda frondosa*

S. No	Concentration (µg/ml)	Absorbance (700nm)
1	20	0.544 ± 0.0393
2	40	0.728 ± 0.0273
3	60	0.830 ± 0.0334
4	80	1.133 ± 0.0087
5	100	1.255 ± 0.0167

Values are given as mean ± standard deviation

Compounds with reducing power indicate that they electron donors and can reduce the oxidized intermediates of free radical reactions, so that they can act as primary and secondary antioxidants. From the graph (Fig. 3) it is clear that as the absorbance of the extracts increased, the reducing power ability also increased suggesting the presence of electron donors in the extract which act as intermediates for radicals scavenging reaction.

Also antioxidants present in the leaf extracts of *Mussaenda frondosa* reduced Fe^{+2} ferricyanide complex to the ferrous form Fe^{+3} / ferricyanide complex to the ferrous form (Fe^{+2}) resulting in formation of Perl's Prussian blue color which was read at 700nm (Fig. 4).

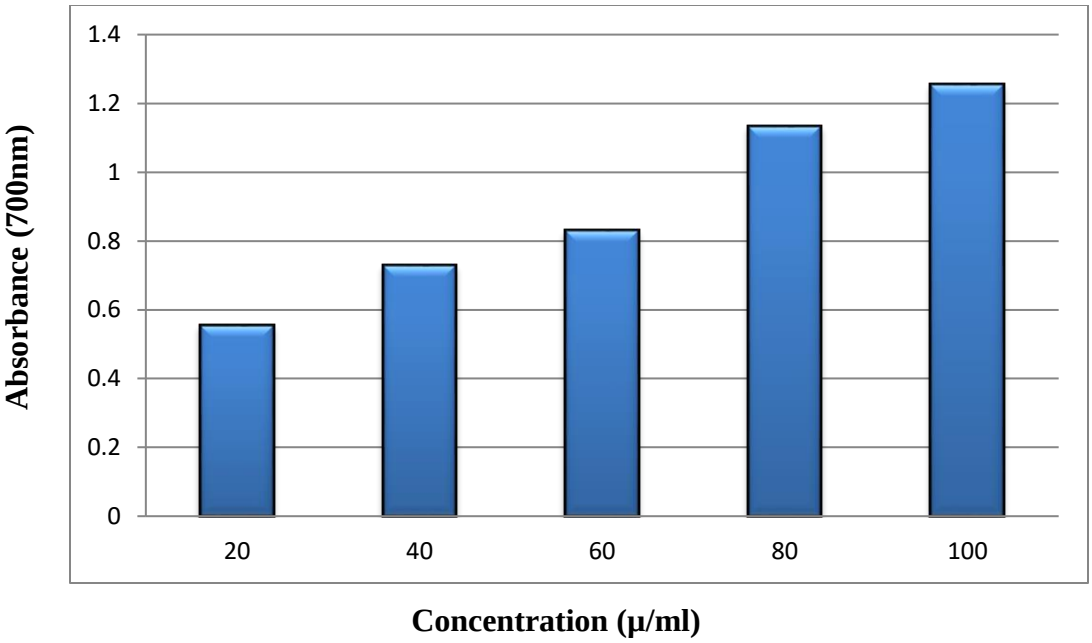

Fig 3: Reducing power assay of methanol extract of *Mussaenda frondosa*

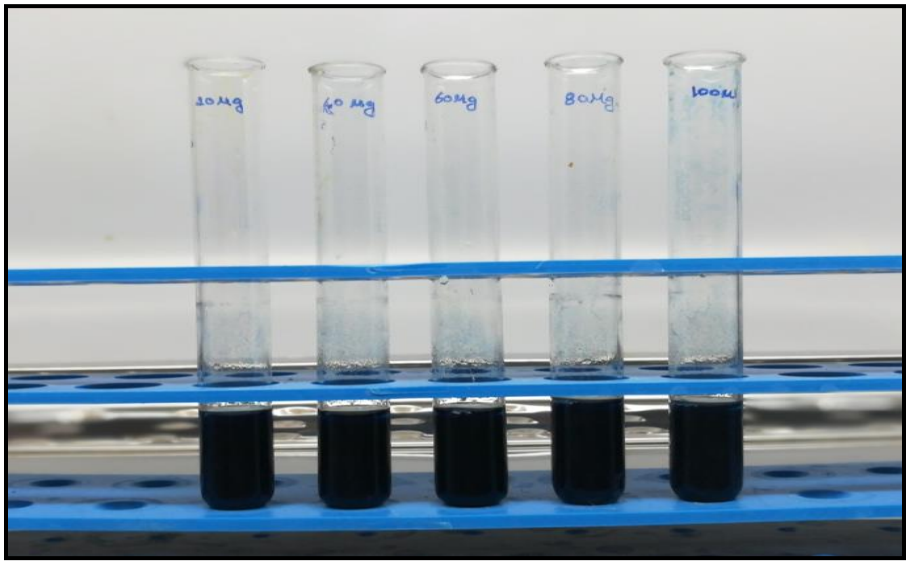

Fig 4: Reducing power ability of methanol extract of *Mussaenda frondosa*

4.2.3. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was measured by ability to reduce the ferric 2, 4, 6 tripyridyl - S - triazine complex (Fe^{3+} - $(TPTZ)_2)^{3+}$ to blue colored ferrous complex (Fe^{2+} -$(TPTZ)_2$ $)^{2+}$ in acidic medium at 593nm absorbance. In the present investigation, the methanol extract absorbed very low, and reducing ability varies at 20-100g/ml (Fig. 5). Variations in observation are recorded in Table 4.

Table 4: FRAP of methanol extract of *Mussaenda frondosa*

S. No	Concentration (µg/ml)	Absorbance (593nm)
1	20	0.709 ± 0.0714
2	40	0.795 ± 0.0176
3	60	1.240 ± 0.0114
4	80	1.159 ± 0.0170
5	100	1.393 ± 0.0150

Values are given as mean ± standard deviation

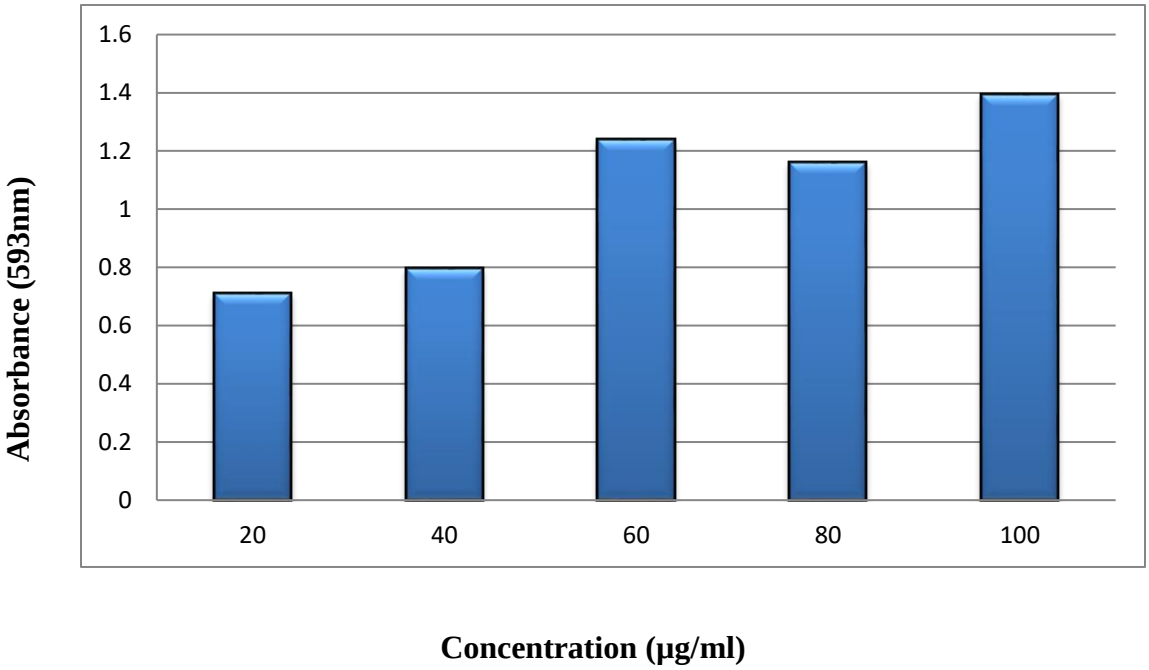

Fig 5: FRAP assay of methanol extract of *Mussaenda frondosa*

4.3. Spectral characterization

4.3.1. UV – Visible spectral analysis

The qualitative UV- VIS profile of *Mussaenda frondosa* methanolic extract was taken at 200nm to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 782,662,607,410,326 and 284 nm with the absorption 0.002, 0.021, 0.007, 0.073, 0.289 and 0.346 respectively (Fig. 6).

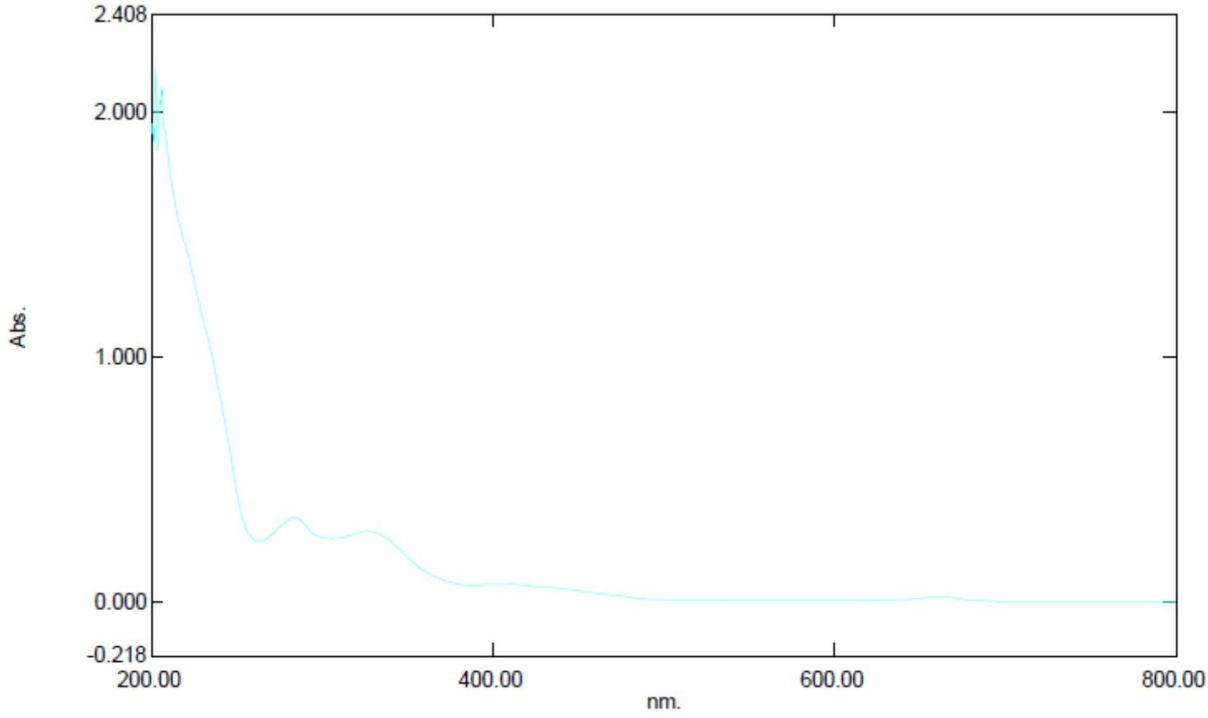

Fig. 6: UV- Visible spectrum of methanol extract of *Mussaenda frondosa*

4.3.2. FT-IR spectral analysis

Fourier transform infrared spectroscopy (FT-IR) is a high-resolution analytical technique to identify the chemical constituents/ functional groups and the structural compounds. The results of the analysis confirmed the presence of phenols, alkanes, alkynes, alkenes, aromatic compounds, ester, phosphorous compounds which shows major peaks at 3745.76, 2943.37, 2360.87, 1516.05, 1454.33, 1111.00, 1022.27 (Fig. 7).

The characteristic vibration mode of identified compounds was as follows: O-H stretching, C-H stretching, C≡C stretching, C=C stretching, C=H stretching, C-O stretching, and P-O stretching.

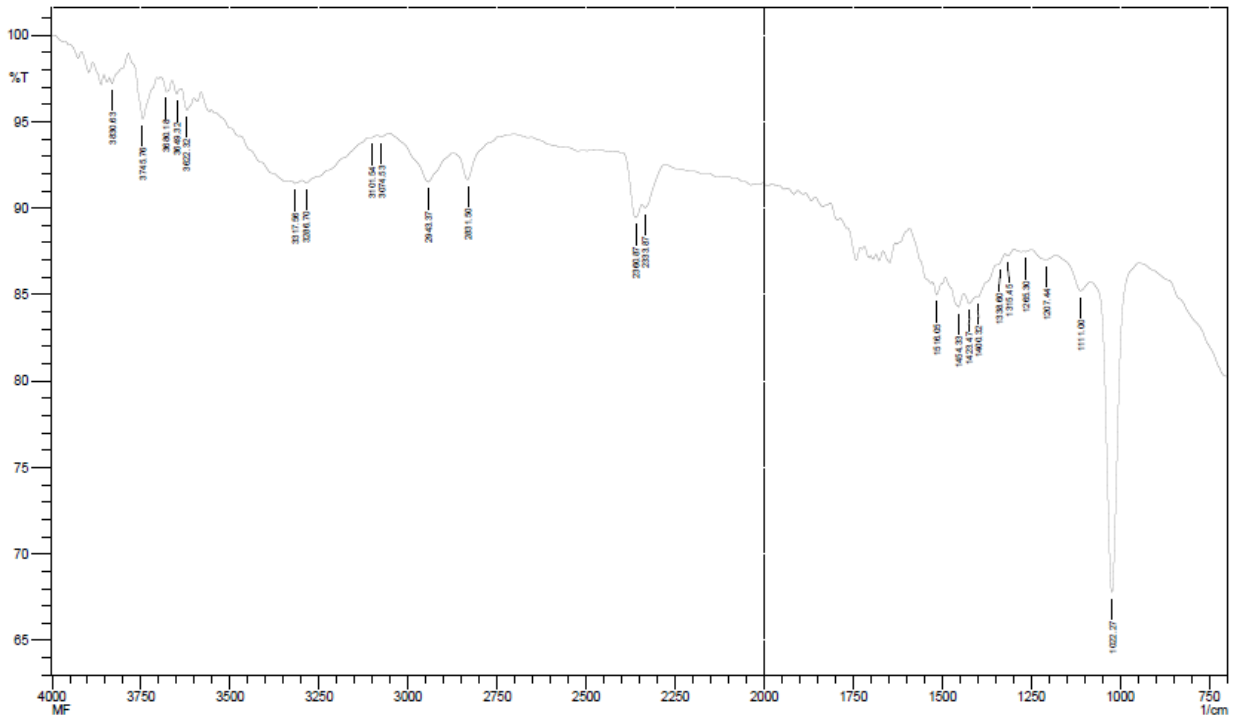

Fig 7: FT-IR spectrum of methanol extract of *Mussaenda frondosa*

4.4. GC – MS analysis of *Mussaenda frondosa*

The methanol extract of *Mussaenda frondosa* was analyzed by GC-MS to detect various compounds with help of NIST library. Totally 17 compounds were identified which have been listed in table 5 .The chromatograph showed 20 peaks with 17 compounds (Fig. 8). The major constituents identified in the extract were 1, 3, 4, 5-Tetra Hydroxy- Cyclohexane carboxylic Acid (13.17%), 3-O-Methyl-d-glucose (8.09%), n-Hexadecanoic acid (11.26%), phytol (18.92%), 7, 10, 13 - Hexadecatrienal (14.55%), 9 - Octadecenoic acid (5.66%) and many other compounds were identified as low level.

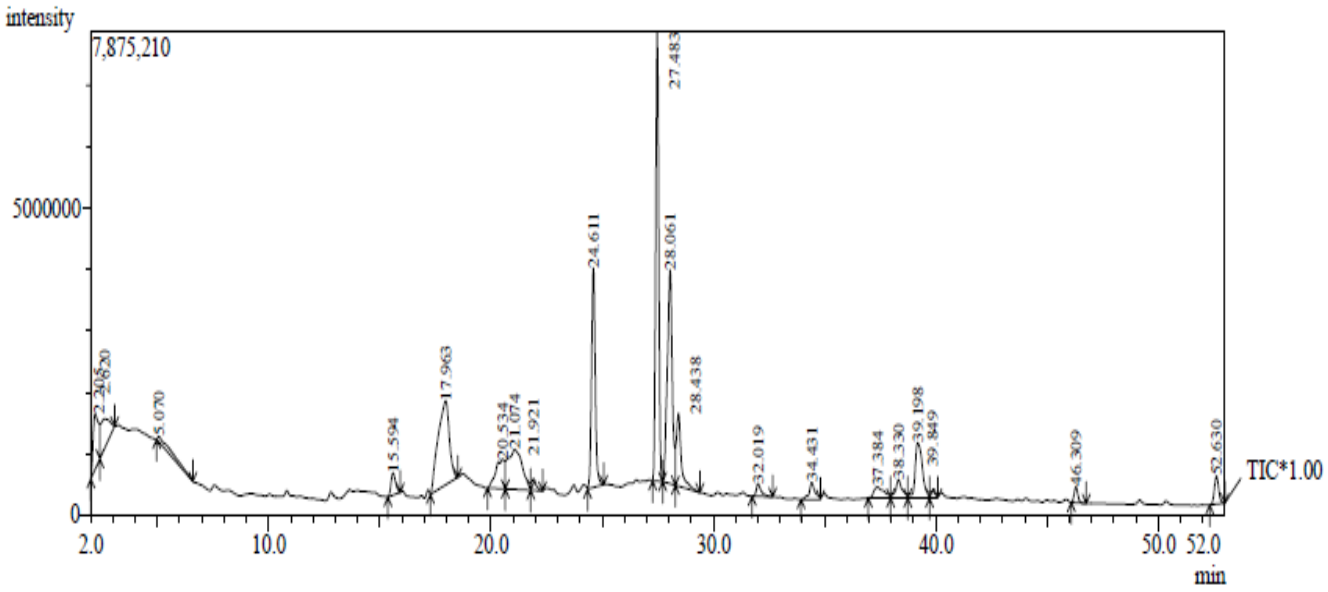

Fig 8: GC-MS chromatogram of methanol extract of *Mussaenda frondosa*

Table 5. Phytocomponents identified in the methanol extracts of *Mussaenda frondosa*

S. No	Name of the Compounds	Formula	MW	Area %	RT(min)
1	3- Butyn-1-ol	C_4H_6O	70.091	4.14	2.205
2	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.126	1.39	5.070
3	Cyclopentan-1-al,4-isopropylidene-2-methyl	$C_{10}H_{16}O$	152.233	1.40	15.594
4	1,3,4,5-TETRA HYDROXY-CYCLOHEXANECARBOXYLIC ACID	$C_7H_{12}O_6$	192.167	13.17	17.963
5	Methoprene	$C_{19}H_{34}O_3$	310.478	3.70	20.534
6	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.183	8.09	21.074
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	11.26	24.611
8	Phytol	$C_{20}H_{40}O$	296.539	18.92	27.483
9	7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234.383	14.55	28.061
10	Octadecanoic acid	$C_{18}H_{36}O_2$	284.484	4.89	28.438
11	9-OCTADECENOIC ACID	$C_{18}H_{34}O_2$	282.468	0.85	32.019
12	Methyl(Z)-5,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	318.501	1.55	34.431
13	3-Heptanol, 2,4-dimethyl-	$C_9H_{20}O$	144.258	1.70	37.384
14	9-OCTADECENOIC ACID	$C_{18}H_{34}O_2$	282.468	5.66	38. 198
15	17-Pentatriacontene	$C_{35}H_{70}$	490.945	0.51	39.849
16	Vitamin E	$C_{29}H_{50}O_2$	430.717	0.81	46.309
17	STIGMAST-5-EN-3-OL, (3.BETA.,24S)	$C_{29}H_{50}O$	414.718	1.66	52.630

4.5. *In silico* molecular docking Analysis

4.5.1. Selection of Ligands according to ADMET analysis

A total of 17 compounds from the GC-MS spectrum, 12 molecules were selected having CAS number. Then, ADMET properties of the 12 molecules were assessed by using of Qik-Prop tool from Schrödinger suite (Table 6). Out of 12 molecules, only 7 molecules were found to satisfy drug-like properties based on Lipinski's rule of five.

Table 6: Bio-ligands selected for molecular docking

SI. No.	Bio-Ligands
1	1,3,4,5-TETRA HYDROXY-CYCLOHEXANECARBOXYLIC ACID- 1064
2	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-119838
3	3-O-Methyl-d-glucose-8973
4	3-Heptanol, 2,4-dimethyl-40545
5	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-131632909
6	Methyl (Z)-5,11,14,17-eicosatetraenoate-5367364
7	n-Hexadecanoic acid-985
8	Vitamin E-14985
9	3- Butyn-1-ol-13566
10	Methoprene-5366546
11	Phytol-5280435
12	7,10,13-Hexadecatrienal-556280

These selected compounds were further evaluated for their drug-like behavior through analysis of bioactivity scores (G - Protein Coupled Receptor ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitors) are depicted in Table 7.

Table 7: Bioactivity score of selected bio-lignds

Bio-ligands	GPCR Ligands	Ion channel modulator	Kinase Inhibitor	Nuclear receptor ligands	Protease inhibitor	Enzyme inhibitor	Blood brain barrier
1,3,4,5-Tetra HydroxyCyclohexane carboxylic acid	-0.24	0.10	-0.77	0.16	-0.26	0.60	-
3-O-Methyl-d-glucose	-0.63	-0.15	-0.85	-0.66	-0.35	0.20	No
n-Hexadecanoic acid	0.02	0.06	- 0.33	0.08	- 0.04	0.18	No
Phytol	0.11	0.16	-0.32	0.35	0.00	0.31	Yes
7,10,13-Hexadecatrienal	0.17	0.46	-0.34	0.05	0.07	0.45	-
Octadecanoic acid	0.11	0.05	-0.20	0.17	0.06	0.20	No
9-Octadecenoic acid	0.17	0.07	-0.22	0.23	0.07	0.27	No

4.5.2. Toxicity risk assessment

4.5.2.1. Principal descriptors

Twelve principal descriptors were calculated for these molecules. Principal descriptors included in the study are Stars, Molecular weight (MW), Volume, Role of five, HBA (Hydrogen Bond Acceptor), HBD (Hydrogen Bond Donor), MW (Molecular Weight), PSA (Polar surface area), SASA (Solvent accessible surface area), FOSA (Hydrophobic component of SASA), FISA (Hydrophilic component of SASA), PISA (Pi component of SASA) and WPSA (Weakly polar component of SASA) (Table 8).

Table 8: Compliance of Principal descriptors

Parameters with Range in 95% drugs	Lig 1	Lig 2	Lig 3	Lig 4	Lig 5	Lig 6
Stars(0 – 5)	1	2	1	5	6	1
MW(130 - 725)	192.168	144.127	194.184	144.256	414.713	318.498
Volume (500 - 2000)	587.311	493.82	645.003	662.898	1424.786	1292.822
Donor HB (0 - 6)	5	2	4	1	1	0
Acceptor HB (0 - 10)	7.85	5.2	10.5	1.7	1.7	2
SASA(300 - 1000)	359.03	321.521	388.445	405.538	719.632	710.964
FOSA(0 - 750)	112.352	165.952	175.98	375.101	642.464	567.169
FISA (7 - 330)	246.678	142.94	212.465	30.438	46.986	86.143
PISA (0 - 450)	0	12.628	0	0	30.182	57.652
WPSA (0 - 175)	0	0	0	0	0	0
PSA (7-200)	131.332	80.347	123.847	18.88	21.641	40.149
Rule of five	0	0	0	0	1	1

Ligand 1:1, 3, 4, 5-Tetra Hydroxy-Cyclohexanecarboxylic Acid; Ligand 2: 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl; Ligand 3: 3-O-Methyl-d-glucose; Ligand 4: 3-Heptanol, 2, 4-dimethyl; Ligand 5: Stigmast-5-En-3-Ol, (3. Beta, 24s); Ligand 6: Methyl (Z)-5, 11, 14, 17-eicosatetraenoate

Table 8: Compliance of Principal descriptors (continue)

Parameters with Range in 95% drugs	Lig 7	Lig 8	Lig 9	Lig 10	Lig 11	Lig 12
Stars (0 – 5)	3	6	9	1	2	1
MW(130 - 725)	256.428	430.713	70.091	310.476	296.535	234.381
Volume (500 - 2000)	1100.464	1604.144	362.22	1195.137	1243.181	1056.378
Donor HB (0 - 6)	1	1	1.5	0	1	0
Acceptor HB (0 - 10)	2	1.5	1.7	2.75	1.7	2
SASA(300 - 1000)	648.29	828.793	264.547	645.646	693.519	635.218
FOSA(0 - 750)	552.383	767.004	146.964	554.109	645.526	516.484
FISA (7 - 330)	95.907	35.723	56.562	66.661	41.394	72.042
PISA (0 - 450)	0	26.066	61.022	24.876	6.599	46.691
WPSA (0 - 175)	0	0	0	0	0	0
PSA (7-200)	48.807	23.449	23.095	43.724	19.759	35.703
Rule of five	1	1	0	1	1	0

Ligand 7: n-Hexadecanoic acid; Ligand 8: Vitamin E; Ligand 9: 3- Butyn-1-ol; Ligand 10: Methoprene; Ligand 11: Phytol; Ligand 12: 7, 10, 13-Hexadecatrienal

4.5.2.2. Property Predictors

Also eleven property predictors were calculated for these molecules. The major properties analyzed in the study are Stars, CNS activity, Metabolism, blood brain barrier, HERG, Caco-2 transporter, MDCK cell permeability, binding value to human serum albumin, Skin permeability and solubility (Table 9).

Table 9: Compliance of Property predictors

Parameters	Lig 1	Lig 2	Lig 3	Lig 4	Lig 5	Lig 6
CNS activity (-2.0 / 2.0)	-2	-1	-2	1	0	-2
Metabolism (1 / 8)	4	3	5	1	3	7
QPlogBBB (-3.0 / 1.0)	-1.581	-0.636	-1.679	0.015	-0.291	-1.162
QPlogHERG (Below -5)	-0.62	-2.567	-2.674	-3.043	-4.088	-4.926
QPPCaco (< 25 poor > 500 great)	11.488	436.915	95.74	5096.367	3550.839	1510.117
QPPMDCK (< 25poor > 500great)	5.037	202.144	39.177	2876.173	1946.224	772.395
QPlogKhsa (-1.5 / 1.5)	-0.953	-0.802	-1.137	-0.128	1.92	1.242
Human oral absorption (1 – Low, 2 - Medium3 - High)	2	2	2	3	1	1
% Human oral absorption (>80% high < 25% poor)	38.787	70.835	51.382	100	100	100
QPlog S (-6.5 / 0.5)	-0.695	-0.792	-0.156	-1.431	-7.033	-4.909
QPlog Kp (-8.0 / -1.0)	-5.586	-3.917	-4.475	-1.6	-1.607	-1.56

Ligand 1:1, 3, 4, 5-Tetra Hydroxy-Cyclohexanecarboxylic Acid; Ligand 2: 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl; Ligand 3: 3-O-Methyl-d-glucose; Ligand 4: 3-Heptanol, 2, 4-dimethyl; Ligand 5: Stigmast-5-En-3-Ol, (3. Beta, 24s); Ligand 6: Methyl (Z)-5, 11, 14, 17-eicosatetraenoate

Table 9: Compliance of Property predictors (continue)

Parameters	Lig 7	Lig 8	Lig 9	Lig 10	Lig 11	Lig 12
CNS activity (-2.0 /2.0)	-2	0	0	-1	-1	-1
Metabolism (1 / 8)	1	5	2	3	3	5
QPlog BBB (-3.0 / 1.0)	-1.292	-0.592	-0.054	-0.724	-0.705	-0.87
QPlog HERG (Below -5)	-3.039	-4.899	-2.973	-4.182	-4.699	-5.158
QPPCaco (< 25 poor > 500 great)	309.04	4540.881	2880.885	2310.771	4011.993	2054.576
QPPMDCK (< 25 poor > 500 great)	176.83	2538.871	1552.541	1223.287	2220.808	1077.372
QP logKhsa (-1.5 / 1.5)	0.471	2.215	-0.848	0.885	1.099	0.683
Human oral absorption (1–Low, 2 –Medium, 3- high)	3	1	3	3	1	3
% Human oral absorption (> 80% high < 25% poor)	88.939	100	92.875	100	100	100
QPlog S (-6.5 / 0.5)	-3.576	-7.808	-0.193	-4.391	-4.516	-3.123
QPlog Kp (-8.0 / -1.0)	-1.943	-0.838	-2.059	-1.604	-0.915	-1.627

Ligand 7: n-Hexadecanoic acid; Ligand 8: Vitamin E; Ligand 9: 3- Butyn-1-ol; Ligand 10: Methoprene; Ligand 11: Phytol; Ligand 12: 7, 10, 13-Hexadecatrienal

4.5.3. Molecular Docking

The diseased protein, Phosphatase and tensin homolog (PTEN- 1D5R) was selected from protein data bank. Protein was interacted with screened ligands from *Mussaenda frondosa*. The entire docked complex was visualized using XP visualizer. The hydrogen bonding interactions between the ligands and the proteins were also visualized (Fig. 9).

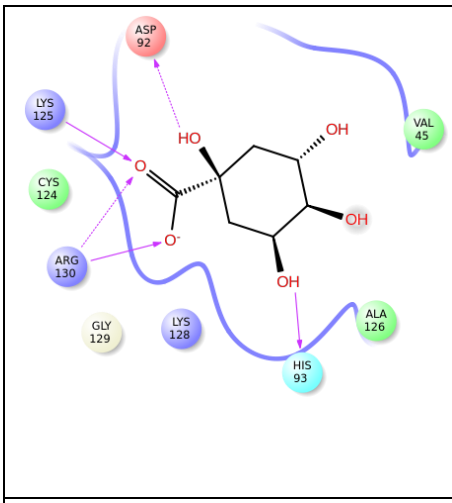	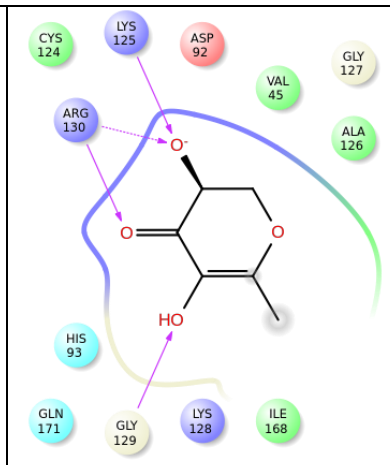	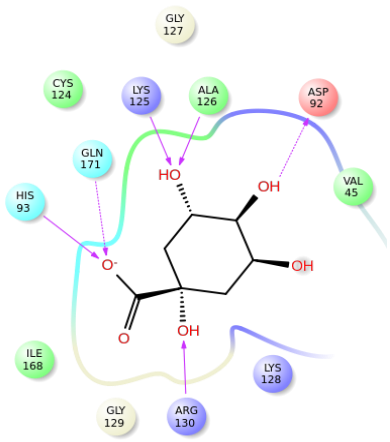
1, 3, 4, 5 - Tetra Hydroxy Cyclo hexane carboxylic acid	4H – pyran – 4 - one, 2, 3 – dihydro - 3, 5 – di hydroxy – 6 – methyl	1, 3, 4, 5 - Tetra Hydroxy Cyclo hexane carboxylic acid (multiple interaction)

Fig.9: Interaction profile of bio-ligands

The results of the docking studies showed that the ligands 1, 3, 4, 5-Tetra Hydroxy-Cyclohexane carboxylic Acid and 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl exhibited gliding interaction with the Glide score of -7.67 and -7.42. The ligand 3-O-Methyl-d-glucose also showed glide score of - 4.56 but less compared to others. Remaining ligands showed glide score in the range of -2.75 to 1.29. Among these 7, 10, 13-Hexadecatrienal was the least effective compound in the extract. The interaction profile of the ligands with the protein is depicted in Table 10.

Table 10: Glide Score of bio-ligands

S. No	Compounds	Glide score
1	1,3,4,5-Tetra Hydroxy-Cyclohexanecarboxylic Acid	-7.67
2	4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	-7.42
3	3-O-Methyl-d-glucose-8973	-4.56
4	3-Heptanol, 2,4-dimethyl-40545	-2.91
5	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-131632909	-2.75
6	Methyl (Z)-5,11,14,17-eicosatetraenoate-5367364	-2.24
7	n-Hexadecanoic acid-985	-2.19
8	Vitamin E-14985	-2.10
9	3- Butyn-1-ol-13566	-1.76
10	Methoprene-5366546	-1.56
11	Phytol-5280435	-1.03
12	7,10,13-Hexadecatrienal-556280	1.29

V. DISCUSSION

Medicinal plants and their extracts play a vital role within the medicine system to preserve our health. India being a medico diverse country in which the traditional systems of Ayurveda, Homeopathy and Unani recognize the importance for medicinal plant extract in various origins. Researchers have great interest in products which are derived from plants because of their versatile applications. Various phytochemicals can be obtained from plants which are very beneficial for mankind and medicinal plants have become the richest biological resource of such chemicals, used in manufacturing of traditional drugs as well as in modern nutraceuticals, food supplements, medicines, folk medicines, as raw material and pharmaceutical intermediates for synthetic drugs (Tumwine, 2011).

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression and neurodegenerative diseases (Young and Woodside, 2001). Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994).

A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is phytocomponents in the plant extracts which may elicit anti oxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties.

The preliminary phytochemical screening and qualitative analysis of *Mussaenda frondosa* extracts showed that the leaves were rich in alkaloids, flavanoids, polyphenols and glycosides in all the extracts especially in methanol and hydro ethanol. Saponins and sterols were found to be negligible in all the tested extracts. Presence of tannins was prominent in the ethyl acetate fraction of the leaf extracts. Carbohydrates are present in a good amount in non polar solvent like chloroform and its intensity was found to be less as the polarity increased.

It should be noted that flavanoids, alkaloids and polyphenols are of greater importance in the oxidative stress problems and much interest in pharmacy due to their role in alleviating the ROS attack by scavenging the free radicals. In a recent study by Mahesh and Niharika (2017) the preliminary phytochemical investigation showed the prominent presence of flavanoids and phenolic compounds, glycosides, carbohydrates in *Mussaenda frondosa* leaf extracts and

concluded that ethyl acetate and petroleum ether extracts of Mussaenda frondosa are effective against inflammatory diseases.

Flavonoids are polyphenolic compounds and consist of flavones, flavonols, flavanols, flavanone and flavanonols. These compounds represent the majority of plant secondary metabolites and have shown to possess remarkable health promontory effects. Flavonoids on the other hand are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity (Salah *et al.,* 1995: Del-Rio *et al.,* 1997: Okwu, 2004). They exhibit marked physiological activity when administered to animals. Tonthubthimthong *et al.* (2001) reported that the beneficial medicinal effects of plant materials typically result from the combination of their secondary products.

Free radical induced per-oxidation has gained much importance because of their involvement in several pathological conditions. This study provides the evidence that free radical scavenging potential possessed by methanolic extract of *Mussaenda frondosa in vitro* in different models and may be due to the presence of flavanoids and other major phytochemicals such as polyphenols and glycosides reported. The remarkable results are in agreement with normalizing affect of *Mussaenda frondosa* in rats against a variety of stressors, thereby indicating its adaptogenic potential (Koul and Chaudhary, 2011).

Similar observations were reported by Siju *et al*. (2010) in whole plant extracts of *M. frondosa*. The study provides the *in vitro* antioxidant effects of the ethyl alcohol and aqueous extracts of *Mussaenda frondosa* thereby preventing cancer and other dreadful diseases. Maiti *et al.* (2013) described the antioxidant activity of *Mussaenda* Roxburghii Hook. F.using DPPH assay. The *n*-butanol and chloroform fractions showed significant antioxidant activity against DPPH and superoxide radicals. Both the fractions also exhibited moderate inhibition of α-glucosidase activity.

The spectral properties of plant leaves have been obtained for ultraviolet, visible, and infrared frequencies. The spectral reflectance, transmittance, and absorbance for *Mussaenda frondosa* is unique depend on its components. The mechanism by which radiant energy interacts with a leaf is discussed, and examples are given below. The evolution of the spectral properties of plant leaves during the early growing season is given as well as the colorimetric behavior during the autumn. Plants depend upon radiant energy for the energy necessary to carry on photosynthesis and other physiological processes.

All green plant has been called the converter of solar energy. In the presence of sunlight it synthesizes complex organic compounds such as sugars, fats, proteins, etc., from simple inorganic compounds such as water, carbon dioxide, minerals, salts, etc. The interaction of plants with radiant energy is of interest to the botanist, forester, geographer, biophysicist, biochemist, ecologist, hydrologist and agronomist.

Previous reports of spectral characterization revealed the presence of a number of bioactive components from *Mussaenda frondosa*. Gopalakrishnan and Vadivel (2011) reported the spectral studies using GC-MS and twenty chemical constituents have been identified. The major chemical constituents are (-)-Quinic acid, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Naphthalene, decahydro- 2-methoxy and 1, 2, 3-Benzenetriol. Other reported phytochemicals were Ursanes and Oleananes. These are derivatives of urs-12-en-28-oic acid as well as their glucosides and are identified according to their carbon skeletons (Kim *et al*., 1999).

Triterpenoids form the largest class of compounds isolated from *Mussaenda.* These are identified by spectral studies. These occur in *Mussaenda* either in the Free State or as saponins. The saponins of *Mussaenda* are generally called mussaendosides. Mussaendosides yield the respective aglycones on hydrolysis. Triterpenoids isolated from *Mussaenda* belong to ursanes, oleananes and cycloartane groups (Dev and Nagasampagi, 1989).

Molecular docking is a method used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Recently docking ligands to receptors utilizing rational drug design is on the increase owing to few problems in the conventional methods of drug designing (Kitchen *et al*., 2004). Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

In the present investigation, the results demonstrated that the investigated compounds were biologically active and produced the physiological actions by interacting with target protein, PTEN. The PTEN protein is a lipid phosphatase with putative tumor suppressing abilities. Inactivating mutations or deletions of the PTEN gene are increasingly being reported in breast cancer and have been related to features of poor prognosis and resistance to chemotherapy. Frequent genetic inactivation of PTEN occurs in glioblastoma, endometrial

cancer and prostate cancer; and reduced expression is found in many other tumor types such as lung and breast cancer (Chen *et al*., 2005).

The detailed intermolecular interactions between the ligand and the target protein revealed that out of the 12 complexes docked with Schrodinger Glide module, 3 complexes such as 1, 3, 4, 5-Tetra Hydroxy - Cyclohexane carboxylic Acid, 4H-pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl and 3-O-Methyl-d-glucose showed highest glide score of -7.67, -7.42 and -4.56 correspondingly. Based on this observation, it can conclude that 1, 3, 4, 5-Tetra Hydroxy - Cyclohexane carboxylic Acid, 4H-pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl and 3-O-Methyl-d-glucose can be considered as good inhibitors for phosphatase and tensin homolog.

Various literature reports also validate our findings. Khan *et al*. (2015) investigated the chemotherapeutic potential of 27 dietary phytochemicals against breast cancer proteins such as NF-kB, EGF, Bcl-2, HER-2, Cyclin D1, 5-LOX, COX-2 and VEGF. The docking results revealed that quercetin exhibited better binding interaction to NF-kB than its known inhibitors. Similarly the bioactive compounds from *Alpinia purpurata* may act as novel inhibitors for CXCR4, a receptor for chemokine (Anusooriya *et al*., 2015). Suganya *et al.* (2014) reported that docking results revealed the binding interactions between the Human Estrogen Receptor protein and the 19 natural flavanoid compounds.

VI. SUMMARY AND CONCLUSION

Phytochemicals are non-nutritive plant chemicals that contain certain protective, disease preventing compounds. More than 900 different phytochemicals have been identified as components of food, and many more phytochemicals continue to be discovered today. Researchers have long known that there are phytochemicals present for protection in plants, but it has only been recently that they are being recommended for protection against human diseases.

It is of great concern about flavanoids, alkaloids and polyphenols in the oxidative stress problems especially cancer and much interest in pharmacy due to their role in alleviating the ROS attack by scavenging the free radicals. In the present investigation, preliminary phytochemical investigation showed the prominent presence of above mentioned phytochemicals in a good amount in polar solvents. The advantage of using phytochemical compounds for various treatments (including cancer) is their relatively non-toxic nature and availability in an ingestive form. Many of the phytochemicals present in human diet have been identified as potential chemo preventive agents too.

Free radical induced per-oxidation has gained much importance because of their involvement in several pathological conditions. This study provides the evidence that free radical scavenging potential possessed by leaf extract of *Mussaenda frondosa in vitro* in different models and may be due to the presence of flavanoids and other major phytochemicals (polyphenols, glycosides) reported.

Spectral characterization of the plant extracts serves as a quantitative chemical analysis of the secondary metabolites. In the present investigation, UV-Visible spectrum indicates the possible presence of flavanoids and polyphenols from the characteristic absorption spectra. FT-IR spectrum confirmed the functional groups (flavanoids and phenolics) according to the characteristic vibration profile of each molecule from the sample. And GC-MS spectrum profile provides a plethora of bioactive compounds which may be act as bio-ligands for further investigation.

Docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking analysis. In the present investigation, selected ligands from the leaf extract were found to be effectively inhibiting the target protein,

Phosphatase and tensin homolog and thus throw light on the possible role of *Mussaenda frondosa* biocandidates in future therapies.

REFERENCES

Abdel-Aziz, M.S., Shaheen, M. S., Nekeety, E. I., Abdel-Wahhab, M. A. 2014. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. *Journal of Saudi Chemical Society*, 18: 356–363.

Abootalebian, M., Keramat, J., Kadivar, M., Ahmad, F., Abdinian, M. 2016. Comparison of total phenolic and antioxidant activity of different *Mentha spi*cata and *M. longifolia* accessions. *Annals of Agricultural Science*, 61:175–179.

Abruzzo, J. 2005. The natural history of medicinal plnts. Eukaryon. 1, 10.

Adebiyi, O. E., Olayemi, F. O., Ning-Hua, T., Guang-Zhi, Z. 2017. *In vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. *Beni-Suef University Journal of Basic and Applied Sciences*, 6:10–14.

Almeida, M.M.B., de Sousa, P. H. M., Arriaga, A. M. C., do Prado, G. M., de Carvalho, D. M., Maia, G. A., de Lemos, T. L. G. 2011. Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International*, 44: 2155–2159.

Al-Owaisi, M., Al-Hadiwi, N., Khan, S. A. 2014. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. *Asian Pac J Trop Biomed.*, 4(12): 964-970.

Andreetta, C., Minisi, A.M., Misicoria, M and Puglisi, F. 2010. First-line Chemotherapy with or Without Biologic Agents for Metastatic Breast cancer. *Critical Reviews in Oncology/Hematology*, 76(2): 99-111.

Anjum, F., Razvi, N., Masood, M. A and Sajid, M. 2017.Life style characteristics: Risk factor for Brast Cancer. *European Journal of Pharamaceutical and Medical Research.* 4(4): 629-641.

Anusooriya, P., Arulraj, C., Chella Perumal, P., Sowmya, S., Vidya, B., Pratibha, P. and Gopalakrishnan, V. K. 2015. Screening of novel CXC chemokine receptor 4 inhibitors from ethyl acetate extract of *Alpinia purpurata* using GC-MS analysis and its molecular docking studies. *International Journal of Pharmacognosy and Phytochemical Research*, 7(3): 480-488.

Apata, L.1979. *Practice of Herbalism in Nigeria*. University of life Press, Nigeria.

Arab, F., Alemzadeh, I., Maghsoudi, V. 2011. Determination of antioxidant component and activity of rice bran Extract. *Scientia Iranica,* 18 (6): 1402–1406.

Aslam, M., Naveed, S., Ahmed, A., Abbas, Z., Gull, I. and Athar, M. 2014. Side Effects of Chemotherapy in Cancer Patients and Evaluation of Patients Opinion about Starvation Based Differential Chemotherapy. *Journal of Cancer Therapy*. 5:817-822.

Auddy, B., Ferreira, M., Blasina, F., Lafon, L., Arredondo, F., Dajas, F. 2003. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *J. Ethno-pharmacol*., 84(2-3): 131–138.

Baba, S. A., Malik, S. A. 2015. Determination of total phenolic and flavonoid content, anti-microbialand antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*. 9: 449–454.

Bargah, R. H. 2015. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma Gaertn. Journal of Pharmacognosy and Phytochemistry*, 4(1): 07-09.

Basu, B.D., Kirtikar, K.1998. Indian Medicinl plants. Vol. II, M/S Bishen Singh, Mahendra Palsingh., New Delhi.

Boonsong, S., Klaypradit. W., Wilaipun, P. 2016. Antioxidant activities of extracts from five edible mushrooms using different extractants. *Agriculture and Natural Resources,* 50: 89-97

Bouhlali, E. T., Bammou, M., Sellam, K., Benlyas, M., Alem, C., Filali-Zegzouti,Y. 2016. Evaluation of antioxidant, anti-hemolytic and antibacterial potential of six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. *Journal of King Saud University – Science*, 28: 136–142.

Boutennoun, H., Boussouf, L., Rawashdeh, A., Al-Qaoud, H., Abdelhafez, S., Kebieche, M., Madani, K. 2017. *In vitro* cytotoxic and antioxidant activities of phenolic components of Algerian *Achillea odorata* leaves. *Arabian Journal of Chemistry*, 10: 403 - 409

Caia, Y., Luob, Q., Sunc,M., Corkea, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences.* 74:2157–2184.

Calvo, E., Baselga, J. 2006. Ethnic differences in response to epidermal growth factor receptor tyrosine kinase inhibitors. *J Clin Oncol.*, 24 (14): 2158-213.

Chaouche, T. M., Haddouchi, F., Ksouri, R., Atik-Bekkara, F. 2014. Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from South Algeria. *Journal of the Chinese Medical Association*.77: 302-307.

Chen, Z., Trotman, L.C., Shaffer, D., Lin, H.K., Dotan, Z.A. and Niki, M. 2005. Crucial role of p53-dependent cellular senescence in suppression of PTEN deficient tumorigenesis. *Nature*, 436 (7051): 725-730

Chigayo, K., Mojapelo, P. E. L., Mnyakeni-Moleele, S., Misihairabgwi, J. M. 2016. Phytochemical and antioxidant properties of different solvent extract of *Kirkia wilmsii* Tubers. *Asian Pac J Trop Biomed.*, 6(12): 1037–1043.

Christopher, A., Lipinski F. L. B. W., Dominy, P. J. Feeney. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 23 (1–3): 3-25.

Cragg, G.M and Newman, D. J. 2005. Plants as a source of Anti-cancer agents. *J Ethno pharmacol*, 100:72-79.

Dai, X., Li, T., Bai, Z., Yang, Y., Liu, X., Zhan, J., Shi, B. 2015. Breast cancer intrinsic subtype classification, clinical use and future trends, 5(10): 2929- 2943.

Deepti, K., Umadevi, P., Vijayalakshmi, G., Vinod polarao, B. 2012. Anti-microbial Activity and Phytochemical Analysis of *Morinda tinctoria Roxb*. leaf Extracts. *Asian Pacific Journal of Tropical Biomedicine*, S1440-S1442.

Dehghan, G., Khoshkam, Z. 2012. Tin (II) – quercetin complex: Synthesis, spectral characterisation and antioxidant activity. *Food Chem*., 131: 422-426.

Del-Rio, A., Obdululio, B.G., Casfillo, J., Martin, T.G. 1997. Properties of Citrus flavonoids. *Journal Agric. Food. Chem*., 4505 - 4515.

Dev, S., Nagasampagi, B. A. 1989. CRC Handbook of Terpenoids, 1, 43.

Dilip, C., Ameena, K., Saraswathi R., Krishnan, P.N., Simi, S.P and Sanker, C. 2010. Evaluation of a new tablet excipient from the leaves of *Mussaenda frondosa*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 1(3): 401 - 411.

Dinda, B., Debnath, S., Majumder, S. 2005. Chemical constitutes of *Mussaenda incana*. *Ind J Chem*., 44B (11): 2362-2365.

Do, D. Q., Angkawijaya, A.E., Tran-Nguyen, P. L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., Ju, Y. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and anti-oxidant activity of *Limnophila aromatica*. *Journal of food and drug analysis*, 22: 296-302.

Edeoga, H.O., Okwu, D. E and Mbaebie, B.O. 2005. Phytochemical constituents of some Nigerian medicinal Plants. *African Journal of Biotechnology*, 4 (7): 685-688.

Foo, S. C., Yusoff, F., Ismail, M., Basri, M., Khong, N. M. H., Chan, K. W., Yau, S. K. 2015. Efficient solvent extraction of antioxidant - rich extract from a tropical diatom, *Chaetoceros calcitrans (*Paulsen) Takano. *Asian Pac J Trop Biomed.*, 5(10): 834–840.

Gamulin, M., Garaj-Vrhovac, V., Kopjar, N., Rami, S., Viculin, T., Jureti, A., Grgi, M. 2010. DNA and cytogenetic damage in white blood cells of postmenopausal breast cancer patients treated with radiotherapy. *J Environ Sci Health A Tox Hazard Subst Environ Eng.*, 45(3): 292-304.

Gopalakrishnan, G and Vadivel, E. 2011. GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* Linn. *International Journal of Pharma and Bio Sciences*, 2, 1.

Gopalakrishnan, G and Vadivel. E. 2011. DNA Cleavage studies of the ethanolic extracts of the bark of *bauhinia tomentosa L.* and the whole plant *of Mussaenda frondosa L. International Journal of Pharmacy,* 2 (9):153-154.

Guarneri, V and Conte, P. E. 2004. The Curability of Breast cancer and the treatment of advanced disease. *European journal of nuclear medicine and molecular imaging*, 31(1): 149-161.

Guggilla, S and Ramesh, A. 2017. Anti sezure activityof Mussaenda frondosa extracts cobalt-induced epileptogenic cortex in rats. *World Journal of Pharmacy and Pharmaceutical Sciences.* 6(7): 844-849.

Halliwell, B. 1994. Free radicals, antioxidants, and human disease. *Lancet,* 344: 721-724.

Harbome. J.B. 1988. Phytochemical Methods, Chapman and Hall. New York.

Hassan, B. A. 2012. Medicinal plants (Importance and uses). *Pharmaceut Anal Acta* 3,139. Doi: 10.4172/2153-2435.1000e139.

Hidayat, M. A., Fitri, A., Kuswandin, B. 2017. Scanometry as microplate reader for high throughput method based on DPPH dry reagent for antioxidant assay. *Acta Pharmaceutica Sinica B,* 7(3): 395–400.

Hunt, D. 2000. Ecological ethano botany: stumbling toward new practices and paradigms. *Model Assisted Statistics and Applications,* 16:1-13.

Iriti, M and Faoro, F. 2009. Chemical diversity and defense metabolism: how plants cope with pathogens and ozone pollution, a review. *International journal of Molecular Science.* 10: 3371-3399.

Jackson, S.P., Bartek, J. 2009. The DNA- damage response in human biology and disease, 461:1071-1078.

Jacob, R. A. 1995. The Integrated Antioxidant System. *Nutr Res*., 15(5): 755-766.

Jayasinghe, U.L.B., Jayasooriya, C.P., Bandara, B.M.R., Ekanayake, S.P., Merlini, Assante L. G. 2002. Anti-microbial activity of some Sri Lankan *Rubiaceae* and *Meliaceae*. *U.L.B. Fitoterapia,* 73: 424 - 427

Jayathilake, C., Rizliya, V and Liyanage, R. 2016. Antioxidant and free radical scavenging capacity of extensively used medicinal plants in Sri Lanka. *Procedia Food Science,* 6: 123 – 126

Joshy, C., Thahimon, P. A., Kumar, A. R., Carla, B., Sunil, C. 2016. Hepatoprotective, anti-inflammatory and antioxidant activities of *Flacourtia montana* J. Grah leaf extract in male wistar rats. *Bulletin of Faculty of Pharmacy, Cairo University*, 54: 209–217.

Kanjilal, U. N., Kanjilal, P.C, De, R.N. and Das, A. 1938. *Flora of Assam: Caprifoliaceae to Plantaginaceae.* Omsons publication, Govt, of Assam, Shillong. Pp: 11-95.

Karur, C and Kapoor, H. C. 2001. Antioxidants in fruits and vegetables: The millennium's health. *Int. J. of Food Sci. And Tech*., 36: 703-725.

Kaur, H., Datt, M., Ekka, M.K., Mittal, M., Singh, A. K., Kumaran, S. 2011. Cys-Gly specific dipeptidase Dug1p from *S. cerevisiae* binds promiscuously to di, tri, and tetra-peptides: Peptide-protein interaction, homology modeling, and activity studies reveal a latent promiscuity in substrate recognition. *Biochimie* 93(2):175-86.

Kesari, A and Maiti, B. C. 2015. Anthelmintic activity of the leaves of *Murraya koenigii linn* and *Mussaenda frondosa* linn. *World Journal of Pharmaceutical Research*, 4(5): 502-509.

Khan, M.K.A., Siddiqui, M.H., Akhtar, S., Ahmad, K., Hassan Baig, M. and Osama, K. 2015. Screening of plant - derived natural compounds as potent chemotherapeutic agents against breast cancer: An *in silico* approach. *J. Chem. Pharm. Res*., 7(1): 519-526.

Kim, N. C., Desjardins, A. E., Wu, C. D., Kinghorn, A. D. 1999. Activity of triterpenoid Glycosides from the Root Bark of *Mussaenda macrophylla* against Two Oral Pathogens. *J. Nat. Prod*., 62: 1379.

Kirtikar, K.R. & Basu, B.D. 1976. Indian Medicinal Plants, Vol. I-IV.Periodical Expert Book Agency, New Delhi.

Koul, S. and Chaudhary, A. 2011. Radical scavenging and antistress activity(s) of *Mussaenda frondosa* roots (r). *Pharmacologyonline*, 1: 1091-1097.

Koul, S., Chaudhary, A. 2011. Radical Scavenging and Anti stress activity(s) of *Mussaneda Frondosa* roots (Rubiaceae). *Pharmacology Online*, 1: 1091-1097.

Krishnaiah, D., Bono, A., Sarbatly, R., Anisuzzaman, S. M. 2015. Antioxidant activity and total phenolic content of an isolated *Morinda citrifolia* L. methanolic extract from Poly-ethersulphone (PES) membrane separator. *Journal of King Saud University – Engineering Sciences*. 27: 63–67

Kumar, S.R., Venkateshwar, C, Samuel, G., Rao, S.G. 2013. Phytochemical Screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. *International Journal of Engineering Science Invention*, 2(8): 2319-6726.

Kusumaa, I. W., Murdiyantoa, A.E.T., Syafrizalb, K.Y. 2014. Anti-microbial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. *Food Science and Human Wellness*, 3:191–196.

Lakshmi priya, K and Velanganni, A.J. 2015. Therapeutic potential of doxorubican loaded poly (D, L-Lacticco-Glycolic acid) against p-Dimethylaminoazobenzene induced liver cancer. Int *J pharma Bio Sci.* 6(4): 511 – 521

Loewus, F.A. & Ryan, C.A. (1965 - 1981). Recent Advances in Phytochemistry, Vol. I-XV, Plenum Press. London.

Madhu, M., Sailaja, V., Satyadev, S.S., Satyanarayana, M.V. 2016. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. *Journal of Pharmacognosy and Phytochemistry*, 5(2): 25-29.

Mahesh, G and Niharika, M.G. 2017. Phytochemical Evaluation, Anti inflamatory Activity, and Determination of Bioactive Components from Leaves of *Mussaenda frondosa*. *Indo Am. J. P. Sci*, 4(09): 3333-3341.

Mahesh, G., Niharika, M. G. 2017. Phytochemical evalution, anti-inflamatory activity, and determination of bioactive components from leaves of *Mussaenda frondosa. Indo American Journal of Pharmaceuticalsciences,* 4(09): 3333-3341.

Maiti, D., Bhattacharjee, B., Singha, A. K., Ghosh, R., Chandra De, U. 2013. Antimicrobial, Antioxidant and Anti A-Glucosidase Activities of the Leaf Extract from *Mussaenda Roxburghii* Hook. F. (Rubiaceae). *World Journal of Pharmacy and Pharmaceutical Sciences*. 2 (5): 3216-3228.

Majeed, M., Hussain, A.I., Chatha, S.A.S., Khosa, M.K.K., Kamal, G.M., Kamal, M. A., Zhang, X., Liu, X. 2016. Optimization protocol for the extraction of antioxidant components from *Origanum vulgare* leaves using response surface methodology. *Saudi Journal of Biological Sciences*, 23: 389–396.

Majumder, S., Nishteshwar, K. 2013. Pharmacognostical and phytochemical investigation of *Ipomoea seplaria* Koenig Ex. Roxb. leaf. *International Journal of Pharmacy*, 4(1): 212-217.

Malara, J., Chairman, K., Singh, A. R. J., Vanmathi, J. S., Balasubramanian, A., Vasanthi, K. 2014. Anti-oxidative activity of different parts of the plant *Lepidium sativum* Linn. *Biotechnology Reports*, 3: 95–98.

Manasa, D. J., Chandrashekar, K.R., Bhagya, N. 2017. Rapid *in vitro* Callogenesis And Phytochemical screening Of Leaf, Stem And Leaf Callus of *Mussaenda Frondosa* Linn.: A Medicinal Plant. *Asian J Pharm Clin Res.,* 10(6): 81-86.

Martinez, M.J.A., Lazaro, R.M., Del Olmo, L.M.B., Benito, P. B. 2008. Anti-infectious activity in the anthemideae tribe. In: Atta-ur- (Ed.), Studies in Natural Products Chemistry.

Medini, F., Fellah, H., Ksouri, R., Abdelly, C. 2014. Total phenolic, flavonoid and tannin contents and antioxidant andantimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum. Journal of Taibah University for Science*, 8: 216–224.

Mensor, L. L., Menezes, F. S., Leitao, G. G., Reis, A. S., dos Santos, T. C., Coube, C. S., Leitao. 2001. Screening of Brazilian extracts for antioxidant activity by the use of DPPH free radical method, *Phytother. Res*., 15(2): 127-130.

Miller. L. P. 1973. Phytochernisrr. Vol 1-Ill. Van Nostrand Reinhold. NewYork.

Murugan, R., Parimelazhagan, T. 2014. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn. – An *in vitro* approach. *Journal of King Saud University – Science*, 26: 267–275.

Nifio, J., Diana, M., Narvaez, O. M. and Yaned, M. C. 2006. Anti-bacterial, Anti-fungal and Cytotoxic Activities of Eight Asteraceae and two Rubiaceae plants from Colombian Biodiversity. Brazilian Journal of Microbiology. doi.org / 10.1590 / S1517 - 83822006000400030.

Okwu, D. E. 2004. Phytochemicals and vitamin content of indigenous spices of Southeastern Nigeria. *J. Sustain. Agric. Environ.,* 6(l): 30-37.

Oyaizu, M. 1986. Studies on products of browning reactions: antioxi- dative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*. 44, 307–315.

Padma, V.V. 2015. An overview of targeted cancer therapy, Biomedicine (Taipei), 5(4): 19.

Patil, S. A., Joshi, V. G., Sutar, P. S and Joshi, N.H. 2010. Screening of alcoholic extract of *Mussaenda frondosa* leaf for wound healing and antibacterial activities in albino rats. *Pharmologyonline*, 2: 761-773.

Poojary, M.M., Kanivebagilu, A. Vishnumurthy, A.V. 2015. Extraction, characterization and biological studies of Phytochemical from *Mammea suriga*. *Journal of Pharmaceutical Analysis*, 5(3): 182–189.

Pourmorad, F., Josseinimehr, S. J., Shahabimajd, N. 2006. Antioxidant activity, phenols, flavonoid contents of seleted Iranian medicinal plants. *S. Afr. J. Biotechnol*., 5:1142-1145.

Prabhavathi R. M., Prasad, M. P and Jayaramu, M. 2016. Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis. Advances in Applied Science Research,* 7(4):11-17.

Qadir, U., Paul, V.I., Ganesh, P. 2015. Preliminary Phytochemical screening and *in vitro* antibacterial activity of *Anamirta cocculus* (Linn.) seeds. *Journal of King Saud University – Science.* 27: 97–104

Quriroga, E. N., Sampietro, A. R., Vattuone, M. A. 2010. Screening of antifungal activities of selected medicinal plants. *Journal of Ethno Pharmacology*, 74: 89-96.

Ram, J., Moteriya, P., Chanda, S. 2015. Phytochemical screening and reported biological activities of some medicinal plants of Gujarat region. *Journal of Pharmacognosy and Phytochemistry*, 4(2): 192-198.

Ranarivelo, Y., Skaltsounis, A.L., Andriantsiferana, M and Tillequin, F.1990 .Glycosides from *Mussaenda arcuata* Lam. Ex Poiret leaves. *Ann Pharm Fr*., 48(5): 273-277.

Reinhold, L. & Harbome. J. B. 1977. Progress in Phytochemistry, Pergarnon Press, Oxford.

Robbrecht, E. 2009. Rubiaceae Research at the National Botanic Garden of Belgium. *National Botanic Garden of Belgium website*. National Botanic Garden of Belgium.

Saha, S., Verma, R. J. 2016. Antioxidant activity of polyphenolic extracts of *Terminalia chebula* Retzius fruits. *Journal of Taibah University for Science*, 10: 805–812.

Salah, N., Miller, N. J., Pagange, G., Trijburg, B. G.P., Rice, E., Evans, C. 1995. Polyphenols, flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidant. *Arch. Biochem. Bioph*, 2: 339- 346.

Sambrekar, S. N. and Patil S. A. 2012. Protective effect of *Mussaenda frondosa* Linn extracts on isoniazid induced hepatotoxicity In Wistar Rats.*World journal of Pharmacy and Pharmaceutical sciences*, 3(2): 1885-1898.

Sambrekar, S. N., Patil P. A and Patil S. A. 2012. Hepato protective activity of *Mussaenda frondosa* Linn extract in ethanol treated rats. *International Journal of Drug Research and Technology*, 2(6): 446-453.

Samuelsson, G.1999. Drugs of Natural origin: a text book of pharmacognosy. 4th ed., Stockholm, Swedish Pharmaceutical Press.

Santhi, K. and Sengottuvel, R. 2016. Qualitative and Quantitative Phytochemical analysis of *Moringa concanensis* Nimmo. *Int. J. Curr. Microbiol. App. Sci.,* 5(1): 633-640.

Satyavati, G.V., Gupta, A.K., and Tandon, N. Eds. 1987. Medicinal Plants of India Volume 2. Indian Council of Medical Research. New Delhi.

Senguttuvan, J., Paulsamy, S., Karthika. K. 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for *in vitro* antioxidant activities. *Asian Pac J Trop Biomed.*, 4(1): S359-S367.

Sharma, S. K., Singh, A.P. 2012. *In Vitro* Antioxidant and Free Radical Scavenging Activity of *Nardostachys jatamansi* DC. *J Acupunct Meridian Stud*., 5(3):112-118.

Sheikh, N., Kumar, Y., Misra, K. A., Pfoze, L. 2013. Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India. *Journal of Medicinal Plants Studies*, 1(6): 62-69.

Shylaja, G., Shoba, S., Mythili, S., and Sathiavelu, A. 2015. The genus *Mussaenda*: A *phytopharmacological* review. Journal of Chemical and Pharmaceutical Research, 7(7): 1037-1042.

Siju, E.N., Rajalakshmi, G.R., Kavitha, V.P and Joseph, A. 2010. *In-vitro* Antioxidant activity of *Mussaenda frondosa*. *International Journal of Pharm Tech Research*, 2(2): 1236-1240.

Silva, J. K., Cazarin, C. B. B., Colomeu, T. C., Batista, A. G., Meletti, L.M. M., Paschoal, J.A.R., Júnior, S. B., Furlan, M. F., Reyes, F.G.R., Augusto, F., Júnior, M.R., Zollner, R. L. 2013. Antioxidant activity of aqueous extract of passion fruit (*Passiflora edulis*) leaves: *In vitro* and *in vivo* study. *Food Research International*, 53: 882–890.

Smith, G. L., Shih, Y.C., Giordano, S. H., Smith, B. D., Buchholz, T. A. 2010. A method to predict breast cancer stage using Medicare claims. *Epidemiol Perspect Innov.,* 7, 1, doi: 10.1186/1742-5573-7-1.

Somasundaram, G., Inbaraj S. D., Madhusudhanan N., Konda, V. G. A., Madhavi E. 2012. Antioxidant and free radical scavenging activity of *Nymphaea stellata*: *in vitro* study. *Journal of Pharmacy Research*, 5(3): 1350-1352.

Sreelakshmi, R., Mohammed, S. P., Jyoti Harindran, Sriganesan, P. 2015. Evaluation of Diuretic Activity of *Mussaenda frondosa*. *Asian J Pharm Clin Res.,* 8 (2): 117-118.

Stankovi, N., Mihajilov-Krstevb, T., Zlatkovi, B., Stankov-Jovanovi, V., Miti, V., Jovi, J., Comi, L., Koci, B., Bernsteine, N. 2016. Antibacterial and Antioxidant Activity of Traditional Medicinal Plants from the Balkan Peninsula. *NJAS - Wageningen Journal of Life Sciences*. 78: 21–28.

Suganya, J., Radha, M., Naorem, D.L. and Nishandhini. M. 2014. *In silico* docking studies of selected flavonoids - natural healing agents against breast cancer. *Asian Pac. J. Cancer Prev*., 15 (19): 8155-8159.

Tonthubthimthong, P., Chuaprasert, S., Douglas, P., Luewisuttichat, W. 2001. Supercritical CO_2 extraction of Nimbin from neem seeds - an experimental study. *Journal of Food Engineering*. 47: 289-293.

Tumwine, W. 2011. Implementation of the framework convention on tobacco control in Africa: Current Status of Legislation. *Int. J. Environ. Res. and Public Health*, 8: 4312-4331.

UNESCO. 1996. *Culture and Health*, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996, Paris, France, 129.

Upadhya, V., Pai, S. R., Hegde, H.V. 2015. Effect of method and time of extraction on total phenolic content in comparison with antioxidant activities in different parts of *Achyranthes aspera*. *Journal of King Saud University – Science*, 27: 204–208

Vaidyaratnam, P. S, Warier, P. K. 1993. Indian Medicinal Plants a Compendium of 500 Species. Madras: Orient Longman; P. 86-89.

Verpoorte, R. 1998. Chemo diversity and the biological role of secondary metabolites, some thoughts for selecting plant material for drug development. Proc. Phytochem Soc. Europe, Kluwe publishers. 43: 11-24.

Wesley, J. J., Jeyaananathi, J., Ansar, A. M., Ravikumar, K. 2009. Hypolipidemic effect of methanolic extract of *Mussaenda frondosa* Linn. Leaves inhigh fat diet fed rats. *J Pharm Res.*, 2 (4): 579-81.

WHO. Medicinal plants in the south Pacific. Ed. (Michael Doyle). WHO regional publications. Western packs serious, 1998: Pp-125.

Wong-paz, J. E., Rodrfguez-Herrera, R., Inungaray, M. L. C, Lopez, L. I., Nevarez Moorillon,G.V., Aguilar, C.N. 2015. Total phenolic content, *in vitro* antioxidant activity and chemical composition of plant extracts from semiarid Mexican region. *Asian Pacific journal of Tropical Medicine*, 104-111.

Yadav, R. N. S and Agarwala, M. 2011. Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3(12): 10-14.

Young, I. S., Woodside. J. V. 2001. Anti-oxidants in health and disease. *J. Clin. Pathol*., 54: 176-186.

Yusuf, A. Z., Zakir, A., Shemau, Z., Abdullahi, M and Halima, S. A. 2013. Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* linn. *Journal of pharmacognosy and phytotherpy*, 6(2):10-16.