ANTIOXIDANT &ANTIMICROBIAL EFFICACY OF

Ficus religiosa L. *& Ficus benghalensis* L. PLANT

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CERTIFICATE

This is to certify that the thesis entitled "**Antioxidant and Antimicrobial efficacy of *Ficus religiosa* L. & *Ficus benghalensis* L. plant"** submitted to National Institute of Technology; Rourkela for the partial fulfilment of the Master degree in Life science is a faithful record of bonafide and original research work carried out by Rashmi Rekha Sahoo under my supervisions and guidance.

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DECLARATION

I here declare that the thesis entitled **"Antioxidant & Antimicrobial efficacy of *Ficus religiosa* and *Ficus benghalensis* plant",** submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfilment of the Master Degree in Life Science is a faithful record of bonafide and original research work carried out by me under the guidance and supervision of Dr. Bismita Nayak ,Assistant Professor ,Department of Life Science , National Institute of technology ,Rourkela. To the best of my knowledge and belief, this work has not been submitted to any other University or Institution to confer any Degree or Diploma.

Date

Miss Rashmi Rekha Sahoo
Master of Science

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ABSTRACT

Medicinal plants are used to cure variety of diseases for long years. The aim of the study was to identify such plants with antimicrobial and antioxidant efficiency for controlling some disease causing pathogens. The locally available plants like *Ficus religiosa and Ficus bengalensis* have the capability to cure diseases traditionally since long years, due to this reasons these two plants were selected for the study. Various methods are tried to standardize the extraction of antimicrobial activity. It was found that methanol extraction of two plants that is *Ficus religiosa* and *Ficus benghalensis* showed high antimicrobial activity than the aqueous extract against *Klebsilla pneumonia species* and *Bacillus subtilis species*. The plant extracts was used to study their photochemical compositions i.e. total phenols contents, flavonoids contents, terpenoids content and proteins contents. Most of the biologically active phytochemicals presents in the methanol extract. For *invitro* antioxidant activities including 1, 1 -Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and reducing power activity was performed. In DPPH activity ,when the decrease in absorbance at 517nm it increases the scavenging activity of the plants sample, then the plants sample colour is decolorized due to presence of antioxidant. For DPPH experiment ascorbic acid taken as the standard solution. The another antioxidant activity is reducing power, the result obtained that is all the sample have increased absorbance of reaction mixture and it was indicated the greater reducing power. The results obtained in this study clearly indicated that leaf and bark of *Ficus religiosa and Ficus benghalensis* have a significant role to use as a natural antioxidant. It experimentally reported by the various extract concentration from *Ficus religiosa* leaf have interesting antioxidative properties and symbolize a potential source of medicine for the treatment of inflammatory activity and wound healing properties. The results obtained that the antioxidant activity of these plant samples and could be utilized as potential source of natural antioxidant in the food or in pharmaceutical industries.

1. INTRODUCTION

The Plants have been used in conventional medicine for several thousand years awareness of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. It was reported that usually 2500 plant species and 100 species of plants serve as a regular sources of medicines. During the few centuries there has been an increase attention in the study of medicinal plants and their conventional utilization in different parts of the world. The native information through ethnobotanical studies is significant in favour of the conservation as well as consumption of biological resources. Now a day's according towards the World Health Organization (WHO), as many as 80% of the world's people depends on natural drug for their main healthcare desires. There is significant economic profit during the growth of original medicines and the use of medicinal flora for the conduct of a variety of diseases. Due to fewer communication means, deficiency, ignorance and unavailability of new health services, mainly rural people are still practice the traditional medicines for their common day's diseases. A huge awareness of how to use the plants against different disease can be normal to have accumulate in that areas where the utilization of plants is still of huge importance.

Plants are the foundation of existence on world and are central to people's livelihoods. India is rich in therapeutic plants. More than 2500 plant species which contain medicinal values. A huge number of medicinal plants are being exploited from the natural plants for the industrial production of drugs. Our body is show a huge number of foreign chemicals every day. Usually of which are man- made, our inability to properly metabolize them negatively affects for health by the generation of free radicals. Free radicals also are generated during the normal metabolism of aerobic cells. The oxygen utilization inside in cells growth leads to the generation of series of oxygen free radicals. Extremely dynamic free radicals and their uninhibited manufacture are responsible for many pathological processes such as cell tumour (prostate and colon cancers) coronary heart diseases, bleeding wounds, constipation, dysentery, boils and mumps.

Various reactive species are Reactive nitrogen species and Reactive oxygen species which are a family of antimicrobial molecules, it includes- superoxide anaions, hydrogen peroxide, and hydroxyl, nitric acid & peroxyoxide nitrite radicals, which produces enzymatic activity. Reactive nitrogen species and Reactive oxygen species act together with Reactive

oxygen species to damage the cell and play a vital role in oxidative stress related to the pathogenesis of various important diseases. These species causes the cellular damages by reacting with various bio molecules are proteins, membrane lipids, enzymes and nucleic acid. This damage is the major contributor of the production of free radicals in healthy individuals and is balanced by the antioxidative defense mechanism.

The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several diseases and means to delay aging symptoms. Due activity of antioxidant it is important to prevention of various human diseases. In nature antioxidants present in leafy vegetables and seeds, such as ascorbic acid, vitamin E & phenolic compound possess the ability to reduce the oxidative damages diseases like cancer, cardiovascular diseases, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing.

Natural products includes:(1) a whole living thing (e.g., a plant, an animal, or a microorganism) that has not been subjected to some type of production conservation (e.g., drying), (2) division of an life form (e .g plants or flowers of a plant, an cut off animal organ), (3) an extract of an living being or part of an living being, and exudates, and (4) pure compounds (e.g., alkaloids, coumarins, flavonoids, glycosides, lignins, steroids, sugars, terpenoids, etc.) isolated from plants, animals, or microorganisms. However, in most of the term natural products refers to secondary metabolites, small molecules (mol wt<2000 AMU) formed by a living being that are not severely essential for the continued existence of the creature. Concepts of secondary metabolism contain products of spread out metabolism as an effect of nutrient limitation, metabolism formed during idiophase, defense mechanism supervisor molecules, etc. Usual products can be from any global or oceanic resource: plants (e.g., paclitaxel from *Taxusbrevifolia*), animals (e.g., vitamins A and D from cod liver oil), or microorganisms (e.g., doxorubicin from *Streptomyces peucetius).* All *Ficus* species have medicinal activities so *Ficus religiosa* and *Ficus benghalensis* is one of the most medicinal plant for treatment of diseases. *Ficus religiosa* is a Bo or peepal tree locally available in India where it grows up to 5,000ft. It is said that Buddha was born under this tree. This plant having attractive heart shaped glossy leaves and it does not reproduce sexually, it must be propagated from cuttings. *Ficus benghalensis* it is commonly known as Banyan is a large and growing tree. Another plant from *Ficus* species is *F.benghalensis, it* produces propagating roots which grow downwards, known as aerial roots. Once these roots contact with the ground, they develop into woody trunks that can become identical from the main trunk.

Description of *Ficus religiosa* plant

KINGDOM	**Plantae**
DIVISION	**Magnoliophyta**
CLASS	**Magnoliopsida**
ORDER	**Rosales**
FAMILY	**Moraceae**
GENUS	***Ficus***
SPECIES	***Religiosa***
SCIENTIFIC NAME	***Ficus religiosa***
OTHER NAME	**Bo tree, Budhi tree, Scared tree, Peepal, Jari, Arani , Ashvattha**

Fig:-1 showing leaf and bark of *Ficus religiosa* plant

Table. 1:- Taxonomy of *Ficus religiosa* plant

Taxonomy of *Ficus religiosa* plant

Ficus, the genus, consists of over 800 species and is one of concerning 40 genera of the mulberry family. The species of maximum industrial significance is *Ficus carica*. (The common plant). Excellent species of Ficus are *Ficus religiosa* (the Bo tree which is the Buddha tree as he defined the "Truths"), *Ficus benghalensis* (the banyan tree) and *Ficus racemosa* (glomerata, the giant cluster tree). All the *Ficus species* have latex-like material within their vasculature; afford defence and self-healing from physical attacks. *Ficus religiosa (*Moreaceae) usually recognized as pepal tree and are spread all over India, Pakistan, China and other humid countries. The bark is reported to possess wound healing activity, anti-inflammatory activity, analgesic, anti-lipid peroxidation activity. The leaf shows the occurrence of steroids, saponins, tannins, terpenoids and phenols from the phytochemical analysis.

The leaves of *Ficus religiosa* tree are rubbery; heart shaped long tipped, extended slim petioles and purple fruits rising in pairs. The tree is regarded as a blessed tree to both Hindus as well as Buddhists. It has got mythological spiritual and medicinal significance in Indian civilization since earliest times. Leaves also contain campestrol, stigmasterol, isofucosterol, α-amyrin, lupeol, tannicacid, arginine, serine, aspartic acid, isoleucine, leucine, n-nonacosane, n-hentricontanen, hexa-cosanol and n-octacosan.

Many studies by scientist has too exposed that the methanolic leaf extract of *F.religiosa*, which include high total phenolic and exhibited high antioxidant activity.
Although its bark is widely used in conventional drug as analgesic and anti-inflammatory properties. Wound healing is a complex multi fractional process that results the contraction of wound and restoration of a functional barriers. It agreed that reactive oxygen species (ROS) are damage the wound healing effects on cells and tissues. Early steps involves an acute

inflammatory phases followed by synthesis of collagen and other extracellular macromolecules. Inflammation occurs can be due to released of histamine, kinins, serotonins and prostaglandin. Anti inflammatory agents are the agents which usually reduce the release of these inflammatory intermediates. So the *Ficus religiosa* plants use conventionally developed.

Ficus benghalensis

KINGDOM	**Plantae**
ORDER	**Rosales**
FAMILY	**Moraceae**
GENUS	***Ficus***
SPECIES	***Benghalensis***
OTHER NAME	**Bara, banyan**

Table.2:-Taxonomy of *Ficus benghalensis* plant

Fig: - 3 *Ficus benghalensis* plant

Taxonomy of *Ficus benghalensis* plant

Ficus benghalensis (Moraceae, Mulberry family) is usually identified as Banyan tree or Vata or Vada tree in Ayurveda. There are more than 800 species and 2000 varieties of *Ficus* species, Generally *Ficus benghalensis is* an excellent tree of India sends down its twigs and huge number of shoots. The bark contains glucoside, 20-tetra triaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirolalpha-D-glucose and meso-inositol. Leaves contain proteins 9.63%, fibres-26.84%, CaO-2.53%, and Phosphorus-0.4 %. The latex contains Caoytchoue (2.4%), Resin, Albumin, Cerin, Sugar and mallic acid. It is used in Ayurveda for the treatment of diarrhoea, dysentery and piles, teeth disorders, Rheumatism, skin disorders like sores, to boost resistant system, as a hypoglycaemic. The extracts of *Ficus benghalensis* were also reported to reduce insulinase action from liver and kidney. Fruit extracts exhibited anti-tumour activity in the potato disc bioassay. Two flavanoids compounds, viz. 5, 7-dimethyl ether of leucopelargonidin 3-0-alpha-L rhamnoside and 5, 3'-dimethyl ether of leucocyanidin 3-0-alpha-D galactosylcellobioside were obtained from the bark of *F. benghalensis* and were evaluated for antioxidant activity in hyperlipidemic rats. It was also established to inhibit the lipidperoxidation. Various extracts

of *Ficus benghalensis* was screened for its anti allergic and anti pressure potential in asthma by milk induced leucocytosis and milk induce deosinophilia.

Oxidation process is one of the most important routes for producing free radicals in foods, medicines and even living system. Free radical causes more than one hundred disorders in humans including diseases atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS. Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as substantial pressure, reason depletion of immune system antioxidants, alter in gene expression and induce abnormal proteins. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to non radical forms and function as usual antioxidants in human body.

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid & radicals, such as the hydroxyl radicals (OH) and superoxide anion (O_2^-), are usual substance of cellular metabolism. Rapid formation of free radicals is able to lead the oxidative damage to bio molecules and may causes many disorders for example cancer, diabetes, inflammatory diseases, asthma, cardiovascular diseases ,neurodegenerative diseases, and premature aging.

Plant-derived antioxidants, particularly, the phenolics include gained significant meaning due to their possible health benefits. Currently available artificial antioxidants like butylatedhydroxy anisole (BHA), butylatedhydroxy toluene (BHT), tertiary dutylated hydroquinone and Gallic acid esters, have been supposed to reason or punctual negative strength effects. Thus, any phytochemical analysis of a known plant reveals only extremely contracted spectrum of its constitution. Plants can be significant foundation of potentially constructive structures for the expansion of recent chemotherapeutic agents. The genus *Ficus* includes some 750 species of forested plants happening in most tropical and subtropical forests all over the world. The genus is significant for the huge difference in the habits of its species.

Ethno botany of plants studies

Ficus plants are originate throughout the world as sensible woody plants or trees. It has a huge conventional role in indigenous structure of medicine in ayurveds, siddha, unani and homeopathy. *Ficus* species, namely, *F. benghalensis* and *F. recemosa*, *F. religiosa* are significant ingredient in a lot of ayurvedic and conventional formulations. The barks, leaves fruits and latex are measured to be very effectual in different treatments, such as diabetes, skin diseases, ulcers, dysentery, diarrhoea, stomach, piles and as carminative, astringent, anti inflammatory antioxidant and anticancer agents. *Ficus benghalensis* used in Ayurveda for

conduct of diarrhoea, piles, teeth and skin disorders. The plant parts are used in diseases of blood, vaginal uterus, & leucorrhoea, burning sensation, gonorrhoea, diarrhoea, dysentery, haemorrhoids, gastrohecosis.

The bark is used in puffiness, enlargement at neck, gonorrhoea, scabies mouthwash for tooth ache, and for support gums, and steeped freshly burnt bark has been said to cure cases of fixed hitch. The latex is used in inflammations and haemorrhages. Malparmaram is a significant collection of ayurvedic formulation that constitutes the barks of, *Ficus religiosa* and *Ficus benghalensis* widely used in the action of skin diseases and also used in a variety of ailments.

2. OBJECTIVES

- The major objective of present study was to investigate the *in vivo* antioxidant potential methanolic extraction of *Ficus religiosa* and *Ficus benghalensis*, the study of their phytochemical compositions, total phenol content, & flavonoid contents. Then study of antimicrobial properties against microbial pathogens and antioxidant activities including 1, 1-Diphenyl -2-picrylhydrazyl (DPPH) radical scavenging and reducing power activity.
- The medicinal plants are used in traditional treatments to cure various diseases for thousands of years. The aim of this study was to identify such plants with antimicrobial and antioxidant efficacy for controlling some diseases through presence of scavenging activity.

3. REVIEW OF LITERATURE

Sawarkar et al., 2011 reported that numerous plants synthesize substances that are useful in the maintenance of health in humans and animals.

With a view to increasing the wide range of medicinal uses, now the present day entails new drugs with more potent and desired activity with lesser no side effects against particular disease (Roy et al., 2009).

The genus *Ficus* (Moraceae) constitutes one of the largest genera of angiosperms includes with more than 800 species and 2000 varieties of *Ficus* genus, occur in the most tropical and subtropical forests (Hamed, 2011).

(Sirisha et al., 2010) reported that All *Ficus* species possess latex-like material within their vasculatures that provide protection and self-healing from physical assaults..

Various studies indicated that *Ficus* species are widely used in the management of various types of diseases like respiratory diseases, sexual disorders, central nervous system diseases (CNS), cardiovascular disorders (CVS), gastric problems, skin infections and diabetics etc (Sirisha et al., 2010 Vinutha et al., 2007).

It was found that *Ficus religiosa* exhibit the antidiabetic activity by increasing the serum insulin level, body weight and glycogen content and also shown anti lipidperoxidative effect against streptozotocin induced diabetic rats (Pandit et al., 2010).

Kunwar and Bussmann, (2006) reported that In Nepal leaf juice with honey is used for multipurpose such as for diarrhoea, asthma, cough, earache, toothache, and migraine, in gastric problems and in haematuria .

The paste of powdered form of bark is used in cases of anal fistula and as absorbent for inflammatory swellings and also used in burns (Nadkarni, 1954, Warrier et al., 1995).

(Khan et al., 2011), (Kalyon et al., 2009), was reported that the bark of *Ficus religiosa* to possess antiulcer and wound healing activities.

(Pandit et al., 2010) reported that it is used in diabetics, diarrhoea, and leucorrhoea, anxiety, for vaginal and other urinogenital diseases and to improve the complexion.

It was reported that *Ficus religiosa* act as cardiac tonic and is useful to cure the diseases of vagina. It also cures vomiting, anorexia and edema (Singh, 2006).

(Sirisha et al., 2010) reported that, the fruit extract of *Ficus* species have anti tumour and antibacterial activity.

The hydro alcoholic extract of leaves of leaves of *Ficus religiosa* also exhibited antiulcer activity (Saha and Goswami, 2010).

The methanol extract of stem and bark of *Ficus religiosa* has inhibitory effect on carrageenan-induced inflammation in rats due to the inhibition of the enzyme cycylo oxygenase (COX) leading to inhibition of PG's synthesis. Further, various studies revealed that tannin present in the bark possess anti-inflammatory effects (Sreelekshmi et al., 2007).

Viswanathan et al investigated the anti-inflammatory and mast cell proliferative effect of aqueous extract of bark of *Ficus religiosa (*Viswanathan et al., 1990).

It was studied that the aqueous extract of fruit of *F. religiosa* has shown potent anthelmintic activity as compared to her species of Ficus against Pheretimaposthuma (earthworms) (Sawarkar et al., 2011).

(Akhtar et al., 2000) reported that Stem and bark extract of *Ficus religiosa* was also found lethal to Ascaridiagalli (Parasitic worm belonging to phylum nematoda).

It has been found that *Ficus religiosa* is used as a national therapy against various infectious disorders. The antibacterial potential of *Ficus religiosa* was investigated by this study the chloroform extract of the leaves of *Ficus religiosa* inhibited the growth of various Salmonella species, *P. vulgaris, E. coli, B. Subtilis* and *K. Pneumonia* etc which revealed the antibacterial potential of the plant (Hemaiswarya et al., 2009).

(Uma et al., 2009) reported that study different extracts (methanol, aqueous, chloroform) of the bark of *Ficus religiosa* has inhibitory effect on the growth of three enteroxygenic *E. coli*, isolated from the diarrhoea patients.

(Pandit et al., 2010) experimented on the aqueous bark extract of *Ficus religiosa* has also shown antidiabetic activity against streptozotocin-induced diabetic rats.

(Yadav et al., 2011) has evaluated the nephro protective effect of methanolic extract of *Ficus religiosa* latex at a dose level 400 mg/kg.

(Sirisha et al., 2010) has reported that *Ficus religiosa* has also shown antioxidant activities.

The preliminary phytochemical analysis of the methanol extract of *Ficus religiosa* bark studied by Uma et al., showed the presence of flavonoids, saponins and tannins. (Uma et al 2009).

The bark of *Ficus benghalensis* exhibited significant antibacterial activity against pathogenic bacteria like *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Gayathri, 2009).

According to Preeti et al, *Ficus religiosa* aqueous extract showed the high antimicrobial activity. Higher activity was found against *Bacillus subtilis* and *Pseudomonas aeruginosa*, (multi-drug resistant) by (Preeti et al., 2010) (Iqbal et al., 2001) studied *in vitro* antihelmintic activity of *Ficus religiosa*.

It was evident by (Manimozhi et al., 2012) that aqueous and methanolic extracts of *Ficus* species contain phytochemicals of therapeutic value. Since the study was conducted in a controlled manner, the phytochemicals can be used for the formulation of compound drugs.

The Ficus plant extracts were found to inhibit the growth of Gram positive bacteria as well as the Gram negative bacteria and also the fungal species and the methanolic extract was comparably more effective to inhibit the growth of microbes than Aqueous and Chloroform extracts. (Aswar et al., 2008).

The antioxidant activity of the aqueous extraction of *F. religiosa* was investigated in streptozotocin-induced to diabetic rats. Since the oxidative stress is the major cause of diabetes. *Ficus religiosa* is a widely branched tree with leather type, heart shaped, long tipped leaves, used in the India as medicine, besides which tradition of medicines also claims to use in diarrhoea, diabetes, urinary disorder, burns, gastrohelcosis, skin diseases, tuberculosis, fever, paralysis, oxidative stress, bacterial infection.(Makhija et al.,2010).

(Vinutha et al., 2010) reported that methanolic extract of *Ficus benghalensis* is most effective than the aqueous extract in inflammatory reaction.

Indicated by (Sreelekshmi et al., 2009) that *F. religiosa* living on AH is under severe drought stress as evidence by the elevated production of H_2O_2 and the associated with stress enzymes, which detoxify the lethal effect of ROS.

(Kumar Hement et al., 2011) indicated that the extract was investigated for its antioxidant activity by DPPH radical scavenging activity, hydroxyl radicals scavenging activity, reducing power capacity, hydrogen peroxide activity, determination of total phenol content using the Folin – Ciocalteu phenolic reagent. This finding of the study explored that antioxidant potential of the plant extract by 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity and hydrogen peroxide activity was more effective in *Ficus* plants.

Plant antioxidants are composed of a broad variety of different substances like ascorbic acid and tocopherols, polyphenolic compound. They perform several important functions in plants and humans. (Kumar Hement et al., 2011).

F. religiosa contains several phyto constituents like β-sitosteryl-d-glucoside, vitamin K, n-octacosanol, kempeferol, qercetin, and myricetin. Then the plant has been studied for

their various pharmacological activities like antimicrobial, antifungal, immune modulator, antioxidant, hypoglycaemic, hypolipedemic, anthelmintics, and wound healing activities (Vinutha et al., 2010).

Ahmad et al reported that the alcoholic extract was tested, in *F.religiosa* leaf alcohol was found to be a better solvent for extraction of antimicrobial active substances compared to hexane and water. (Ahmed et al., 1998).

(Verma and Bhatia., 1986) reported that the *F. religiosa* leaf contain a higher amount of L-cystine, L-lysine, L-arginine, DL-serine aspartic acid, glycine, DL-theonine, DL-∞alanine, L-proline, tryptophan, DL-methionine, DL-valine, DL-isoleucine,and L-leucine.

A variety of proteins and carbohydrates are present in the leaves of Ficus species, makes them a good fodder (Bhadauria et al., 2002) and (Bamikole et al., 2003).

In case of *Ficus religiosa* infectious diseases, combined with therapy expands with the antimicrobial spectrum and prevents the emergence of resistance (Aiyegoro and Okoh., 2009) reported in *F. religiosa* warrant detailed investigation for its potential against cancer, cardiovascular disorders, neuro inflammatory disorders, neuropsychiatric disorders, oxidative stress related disorders and parasitic infections. The results of these studies will further expand to the existing therapeutic potential of *F.religiosa* and it provides a convincing support to its future clinical uses in modern medicine.

Nair and Chanda (2006) investigated that the antibacterial effect of the aqueous and ethanolic bark extracts of *F. religiosa* plant against microbes like *Pseudomonas aeruginosa, Pseudomonas testosterone, Proteus mirabilis, Proteus vulgaris, Enterobacter aerogenes, Escherichia coli, Citrobacterfreundii, Staphylococcus epidermidis, Baccillus cereus, Streptococcus fecalis, Streptococcus cremoris* and *Steptococcus agalactiae,* is ineffective when the extract in aqueous solution, while the ethanolic extract inhibited the growth of two tested bacterial strains. (Kamra et al., 2008) indicated that the ethanolic, methanolic and aqueous leaf extracts of *F.benghalensis* exhibited the inhibitory effect on methanogenesis caused by methanogens (methane producing microorganisms).

Sharma and Gupta (2007) investigated the *in vitro* antioxidant effect of the ethyl acetate root extracts of *F. religiosa* by using diphenylpicryl-hydrazyl (DPPH) radical scavenging activity, hydroxyl radical scavenging activity, reducing capacity and hydrogen peroxide scavenging assay.

Phytochemical research experimented on *F. religiosa* had led to the isolation of few classes of plant metabolites. However, the vast traditional use proved that pharmacological activities of *F. religiosa* are a huge scope still exists for its phytochemical study. The result of such phytochemical studies may further expand its existing therapeutic potential (Goel et al., 2010).

(Khan et al., 2011) reported that the anti-ulcer potential of the ethanol extract of stem & bark of *F. religiosa* against *in vivo* indomethacin and cold restrained stress-induced gastric ulcer. The extract (100, 200 and 400 mg/kg) is significantly ($P<0.05$) reduced the ulcer index in all assays used and increased the pH of gastric acid while at the same time reduced the volume of gastric juice. In conclusion, this study provide preliminary data on the antiulcer potential of *F. religiosa* stem, bark it support the uses of the plant for the treatment of gastric ulcer. (Khan et al., 2011) suggested that the bark of *F. religiosa* possesses significant anti-ulcer activity in animal models. It has a gastric anti secretory and acid neutralize the effect that are comparable to reference drug ranitidine. The anti-ulcer activity is probably due to the presence of bioactive compounds like flavonoids, saponins and tannins.

Review of various extraction process and phytochemicals of plants

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plants (and animals) tissues using selective solvents through the standard procedures. The products are obtained froms plants are relatively complex mixtures of metabolites, in liquid or semisolid state or (after removing the solvent) in powdery form and are proposed for oral or external use. These include the classes of preparation known as decotions, infusions, fluid extracts, semisolid extracts or powdery extracts. Such types of preparations are popularly called galenicals, named after Galen, the Greek physician. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During the extraction process, solvents diffused into the solid plant material and solubilise compounds with similar polarity.

The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract obtained after standization, may be used as medicinal agents in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsule. These

products have a complex mixture of many medicinal plant metabolites. The general techniques of medicinal plant extraction includes maceration, infusion, percolation, digestion, deoctition, hot extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave assist extraction , ultrasound extraction (sonication),super critical fluid extraction, and phytonic extraction with the hydro fluorocarbon solvents. For aromatic plants, hydro distillation techniques (water distillation, steam distillation), hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction) can be employed. Some of the new extraction methods for aromatic plants are head space trapping, solid phase micro extraction, protoplast extraction, micro distillation, thermo micro distillation and molecular distillation.

The basic parameters enhancing the quality of an extracts are:-

1. Plant part used as starting material

2. Solvent used for extraction

3. Extraction procedure

Phytochemicals of plants extracts depends on:

1. The nature of the plant material

2. Its origin

3. Degree of processing

4. Moisture content

5. Particle size

The variation of different extraction methodology that will affect quantity and secondary metabolite composition of an extract depends upon:

1. Type of extraction

2. Time of extraction

3. Temperature

4. Nature of solvent

5. Solvent concentration

6. Polarity

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the procedure of extraction. Property of a good solvent in plant extractions includes low toxicity, evaporation of low heat, and promotion of rapid physiologic absorption of the extract, preservative action, and inability to turn the extract to complex or dissociates form. The factors are affect the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extract, diversity of inhibitory compounds extracted, in case of subsequent handling of the extracts, toxicity of the solvent in the bioassay process. The choice of solvent is influenced by which is intended with the extract. The solvent should be non toxic and should not interfere with the bioassay. The choice will also depend upon the targeted compounds to be extracted.

Phytochemicals	Activity	Mechanism of action
Quinones	Antimicrobial	Inactivates enzymes
Flavonoids	Antimicrobial Anti diarrhoeal	Complex with cell wall, binds to adhesions Inhibit the release of autocoids and prostaglandins, normalization of the dearranged water transport across the mucosal cells, Inhibits GI of acetylcholine.
Polyphenols and Tannins	Antimicrobial Anti diarrhoeal Antheimintic	Binds to adhesions, enzyme inhibition ,substrate deprivation, complex with cell wall, membrane disruption , metal ion complexion Metals intestinal mucosa more resistant & reduces secretion, suppression of heat labile enterotoxin-induced diarrhoea, astringen action. Increases supply of digestive protein of animals by forming protein complexes in rumens, interferes with energy generation by uncoupling oxidative phosphorylation, causing a decrease in GI metabolism.
Phytochemicals	Activity	Mechanism of action
Caumarin	Antiviral	Interaction with eukaryotes DNA
Terpenoids and essential oil	Antimicrobial Anti diarrhoeal	Membrane disruption Inhibits release of autocoids and prostaglandins
Alkaloids	Anthelmintic	Paralysis
Lectins and polypeptides	Antiviral	Blocks the viral infections
Glycosides	Anti diarrhoeal	Inhibits release of autocoids and prostaglandins
Saponins	Anti diarrhoeal Anticancer Anthelmintic	Increase histamine release Possesses membrane permealizing properties Leads to vacuolization
Steroids	Anti diarrhoeal	Enhance intestinal absorption of NA & water

Table. 3:-Mode of action of Phytochemicals activity of plants

Plant tissue homogenization in solvent has been widely used by researchers. Dried or wet, fresh plant parts are grinded in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract is filtered. The filtrate then may be dried under reduced pressure and re dissolved in the solvent to determine the concentration. Some researchers however centrifuged the filtrate for clarification of the extract.

Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped, in terms of phenomenology, by the soap-like foaming they produce when shaken in aqueous solutions, and, in terms of structure, by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. (Hostettmann, K. A., and Marston., 1995).

They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack. These compounds can also be regarded as preformed, since the plant enzymes that activate them are already present in healthy plant tissues (Osbourn, 1996). The natural role of saponins in plants is thought to be protection against attack by pathogens and pets (Price et al. 1987; Morrissey and Osbourn., 1999). These molecules also have considerable commercial value and are processed as drugs and medicines, foaming agents, sweeteners, taste modifiers and cosmetics (Mukharji et al., 1998).

Saponins are glycosylated compounds that are widely distributed in the plant kingdom and can be divided into three major groups; a triterpenoid, a steroid, or a steroidal glycoalkoloid. Terpenoids and saponins are found primarily in dicotyledonous plants but also in some monocots, whereas steroid saponins occur mainly in monocots, such as the Lilliaceae, Droscoraceae and Agavaceae and in certain dicots, such as foxglove (Hostettmann and Marston, 1995). Oats (*Avena* species) are unusual because they contain both triterpenoid and steroid saponins (Price et al 1987). Steroidal glycol alkaloids are found primarily in members of the family Solanaceae, which includes potato and tomato. The saponins produced by oats and tomato have been studied in detail in relation to their potential role in the defense of plants against phytopathogenic fungi (Osbourn, 1996).

Various studies have shown the effect of saponins on the immune system. Saponins induce a strong adjuvant effect to T-dependent as well as T-independent antigens & it also

induces strong cytotoxic $CD8^+$ lymphocyte responses and potentiate the response to mucosal antigens (Kensil C.R., 1996) Saponin based adjuvants have the ability to modulate the cell mediated immune system as well as to enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity (Oda K., et al 2000)

However, saponins are surface active agents and cause haemolysis of red blood cells in vitro, although haemolysis does not appear to be correlated with adjuvant activity (Kensil C.R., 1996). Saponins have been widely used as adjuvants for many years and have been included in several veterinary vaccines. The adjuvant action of saponins was not so pronounced in some of the non-mammalian species tested (Cossarini-Dunier M 1985), (Grayson T.H., et al 1987) Saponin not only has stimulatory effects on the components of specific immunity, but also presents some non-specific immune reactions such as inflammation(de Oliveira C.A.C., et al 2001) and monocyte proliferation (DelmasF.,et al 2001).The mechanisms of immune-stimulating action of saponins have not been clearly understood, Saponins reportedly induce production of cytokines such as interleukins and interferon that might mediate their immune stimulant effects, (Kensil C.R.,1996), saponins have been shown to intercalate into cell membranes, through interaction with cholesterol, forming 'holes' or pores. It is currently unknown if the adjuvant effect of saponins is related to pore formation, which may allow antigens to gain access to the endogenous pathway of antigens presentation, promoting cytotoxic T-lymphocyte (CTL) response (Sjölander A.et al 2001). It was believed that the adjuvant activity of saponins could be related to branched sugar chains or aldehyde groups or to an acyl residue bearing the aglycone (Kensil C.R., 1996). Latter, soyasaponins and lablabosides were found to show strong adjuvant activity despite lacking acyl residues and possessing only un-branched sugar chains Oda et al. concluded that not only the functional groups themselves, but the overall conformation of such functional groups, affected adjuvant activity of saponins.

Tannins are naturally occurring plant polyphenols. Their main characteristic is that they bind and precipitate proteins. They can have a large influence on the nutritive value of many foods eaten by humans and feedstuff eaten by animals. Tannins are common in fruits (grapes, persimmon, blueberry, etc.), in tea, in chocolate, in legume forages (trefoil, etc.), in legume trees in grasses (sorghum, corn, etc).

Tannins contribute too many aspects of our daily lives. They are responsible for the astringent taste we experience when we partake of wine or unripe fruits, and for the enchanting colours seen in flowers and in autumn leaves.

The word tannin is very old and reflects a traditional technology. "Tanning" (waterproofing and preserving) was the word used to describe the process of transforming animal hides into leather by using plant extracts from different plant parts of different plant species. Tannins are one of the many types of secondary compounds found in plants

Characteristics of tannins: Oligomeric compounds with multiple structure units with free phenolic groups, molecular weight ranging from 500 to >20,000, soluble in water, with exception of some high molecular weight structures, ability to bind proteins and form insoluble or soluble tannin-protein complexes.

Tannins are usually subdivided into two groups:

Hydrolyzable tannins (HT)

Proanthocyanidins (PA) (often called Condensed Tannins)

Plant parts containing tannins include bark, wood, fruit, fruit pods, leaves, roots, and plant galls. Examples of plant species used to obtain tannins for tanning purposes are wattle (Acacia sp.), oak (*Quercus species*), eucalyptus (*Eucalyptus spices*), birch (*Betula species*), willow (*Salix caprea*), pine (*Pinus species*), quebracho (*Scinopsis balansae*). Tannins are phenolic compounds that precipitate proteins. They are composed of a very diverse group of oligomers and polymers. There is some confusion about the terminology used to identify or classify a substance as tannin, In fact, not only tannins bind and precipitate proteins (other phenolics such as pyrogallol and resorcinol also have this property), not all polyphenols precipitate proteins or complex with polysaccharides.

One of the most satisfactory definitions of tannins was given by Horvath (1981):

"Any phenolic compound of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups (i.e. carboxyl) to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied"

Currently there is an increasing interest in tannins as bioactive component of foods as well as biological antioxidants. Tannins are a unique group of waters soluble phenolic metabolites of relatively high molecular weight and having the ability to complex strongly with carbohydrates and proteins. In the past, tannins have been viewed as one of the anti nutrients of plant origin because of their ability to precipitate proteins, inhibit the digestive

enzymes and decrease the absorption of vitamins and minerals (Khattab et al., 2010). However, recently several health benefits have been attributed to intake of tannins and some epidemiological correlations with the decreased incidence of chronic diseases have been established (Serrano and others 2009). Numerous studies have demonstrated potentially significant biological effects of tannins such as antioxidant or radical scavenging activity as well as inhibition of lipid peroxidation and lipoxygenases *in vitro,* antimicrobial and antiviral (Dolara and others 2005; De Bruyne and others 1999), antimutagenic (Dolara et al., 2005); Carlsen et al.,), and antidiabetic properties. The antioxidant activity of tannins results from their free radical- and reactive oxygen species-scavenging properties, as well as the chelation of transition metal ions that initialize the oxidation process (Serrano et al., 2009). Antioxidants have also been reported to provide synergistic benefits for the treatment of diabetes because of their insulin enhancing potential (Madhujithand et al., 2004).

Terpenes are the most numerous and structurally diverse plant natural products. For this reason, a system of nomenclature has been established. The nomenclature of terpene compounds is ostensibly complex, yet can be quickly elucidated upon closer examination. The isoprene unit, which can build upon it in various ways, is a five-carbon molecule. The single isoprene unit, therefore, represents the most basic class of terpenes, the hemiterpenes. An isoprene unit bonded with a second isoprene is the defining characteristic of terpene, which is also a monoterpene (C10). While sesquiterpenes contain three isoprene units (C15), diterpenes (C20) and triterpenes (C30) contain two and three terpeneunits, respectively. Tetraterpenes consist of four terpeneunits and polyterpenes are those terpenes containing more than four terpene units (i.e., more than eight isoprene units).

Many of the terpenoids are commercially interesting because of their use as flavour sand fragrances in foods and cosmetics (e.g. menthol, nootkatone and sclareol) or because they are important for the quality of agricultural products, such as the flavour of fruits and the fragrance of flowers (e.g. linalool) (Aharoni, A. et al., 2004). In addition, terpenoids can have medicinal properties such as anti-carcinogenic (e.g. Taxol and perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity. The terpenoid have also been shown to be of ecological significance (Rodriguez-Concepcion, M. (2004). Compounds such as the bitter tri terpenoid cucurbitacins and the pungent di terpenoidpolygodial have been shown to be involved in insect resistance (Balkema et al., 2003). Other terpenoid compounds are involved in interactions between plants, between

plants and microorganisms, and between plants and arthropod herbivores [e.g. (E, E)-a-farnesene, which is induced in cucumber by spider mite feeding] (Arimura, G. et al., 2000).

The commercial and ecological importance of terpenoids makes their metabolic engineering an attractive subject for investigation (Galili, G. et al., 2002). On the one hand, engineering could lead to the improvement of many input and output traits in crops. These include disease and pest resistance, weed control (e.g. by producing allelopathic compounds), improved fragrance of ornamentals and pollination of seed crops (both by altering floral scent), enhanced aroma of fruits and vegetables, and the production of pharmaceuticals in plants. On the other hand, transgenic plants with modified terpenoid production could make an important contribution to fundamental studies of the biosynthesis and regulation of these compounds and their importance in ecological relationships.

Phenolic compounds exist in most plant tissues as secondary metabolites, i.e. they are not essential for growth, development or reproduction but may play roles as antioxidants and in interactions between the plant and its biological environment. Phenolics are also important components of the human diet due to their potential antioxidant activity (Martin et al., 2010), their capacity to diminish oxidative stress induced tissue damage resulted from chronic diseases (Bravo, L., 1998), and their potentially important properties such as anticancer activities (Harris, C.S., 2004).The structure of phenolics consists of an aromatic ring carrying one (phenol) or more hydroxyl (polyphenol) moieties. Several classes can be distinguished according to the number of phenol rings and to the structural elements that join these rings (Stalikas C.D., 2007). Two main groups of polyphenols, termed flavonoids and non-flavonoid polyphenols have been adopted in the literature (Rosa L.A. et al., 2010). The flavonoid group, including flavanoles, flavones, dihydro flavonols, flavonols, flavan-3-ols, isoflavones, anthocyanidins, proanthocyanidins and chalcones, comprises those compounds with a C6-C3-C6 structure.

Flavonoids, a group of naturally occurring benzo-g-pyrone derivatives, have been shown to possess several biological properties (including hepatoprotective, anti-thrambotic, anti inflammatory, and antiviral activities), many of which may be related, partially at least, to their antioxidant and free-radical-scavenging ability. (Robak, et al., 1988) The antiradical property of flavonoids is directed mostly toward HO; and 02 - as well as peroxyl and alkoxyl radicals.(Husain et al.,1987). Furthermore, as these compounds present a strong affinity for iron ions (which are known to catalyze many processes leading to the appearance of free

radicals), their anti peroxidative activity could also be ascribed to a concomitant capability of chelating iron.(Afanas'av, I.B.et al., 1989) (Morel, I.et al., 1993).

Cardiac glycosides are complex triterpene molecules, created by plants and amphibians that exert intense biological effects in humans and many other organisms. While extremely toxic, these molecules often have therapeutic use when dosed appropriately in minute quantities. The structure of a cardiac glycoside begins with a 30-carbon triterpene, which is then modified with an unsaturated lactones ring at carbon 17. This large molecule is referred to as an aglycone or genin, and to it are attached sugar molecules. The glycone portion exerts much of the physiological activity and the sugars contribute to water solubility and absorption when ingested. These compounds should not be confused with alkaloids since they do not contain nitrogen. In humans, small amounts of cardiac glycosides slow down and strengthen the beat of the heart. They do this by blocking the sodium-potassium pumps of heart cells which leads to a delay in the electrical signal between the atrium and ventricles. Excess intracellular sodium that builds up due to the blockage is then exchanged for extracellular calcium by another system, which induces stronger heart muscle contractions. These compounds also increase the sensitivity of the ventricular pacemaker (sinus node) in the heart to the neurotransmitter acetylcholine, and have an effect on the central vital nuclei in the medulla (a centre of autonomic activity). In larger amounts, cardiac glycosides may be extremely toxic, rapidly inducing drowsiness, colour vision disturbances, slow and irregular heart rate, followed by death. Plants and animals create cardiac glycosides as a strategy for protection against being eaten by other creatures. Some animals, like monarch butterflies (Danausplexippus), have evolved immunity to the toxins. They consume plants like milkweed (Asclepiassyriaca) that are high in cardiac glycosides and store the toxins in their tissues. This makes them poisonous to other predators. Cardiac glycosides are also created in very small amounts in mammalian systems and may have a regulatory role.

4. Materials and methods

Extraction

The plant materials were collected from the locality of Rourkela. The leaves and barks were initially separated from the main plants body and rinsed with distilled water, and dried under shade paper towel in laboratory and then homogenized into fine particles and stored in air tight bottles and were used for all the extraction process.

4.1 Preparation of extracts

- **Extraction of aqueous component**

Cold aqueous extraction

10g of each flower and leaves air dried powder was weighed and soaked separately in 50ml cold water in a conical flask stopper with rubber cork and left uninterrupted for 24 hrs and then filtered off using sterile filter paper (What Man No: 1) into a sterile conical flak and subjected to water bath evaporation, where the solvent was evaporated at its boiling temperature 100°C. The extract was got with the help of muslin cloth and was subjected to centrifugation at 5000Xg for 5 rpm and the supernatant was obtained and stored at 4°C for further use (Farombi et al., 2003).

- **Solvent extraction**

4.2 Methanol extract

10g of each leaf and flower air dried powder was weight and was placed in 100ml of organic solvent (methanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 hrs after 24 hrs it was filtered with the help of muslin cloth and centrifuged at 5000Xg for 15 rpm. The supernatant was collected and the solvent was evaporated to make the final volume of one-fourth of the original volume, giving a concentration of 40 µg/0.1ml stored at 40°C in air tight bottles for further studies (Ikram et.al., 1984).

A. Phytochemical screening of plants

Phytochemical analysis of plants was carried out for all the extracts as per the standard methods.

1.**Detection of alkaloids**: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The yellow coloured precipitate was indicated that presence of alkaloids.

1. **Detection of carbohydrates**: Extracts were dissolved individually in 5 ml distilled water and filtered it. The filtrates sample was used to test for the presence of carbohydrates.

Fehling's Test: Filtrates samples were hydrolysed with the dilute Hcl, and neutralized with alkali then heated with Fehling's A and B solutions. The red coloured precipitate indicated that presence of reducing sugars.

Detection of glycosides: Sample extracts were hydrolysed with the dilute Hcl, and then subjected to test for glycosides.

4. Detection of steroids and terpenoids

In 1 ml of methanol plant extract 1ml of chloroform was added and 2-3 ml of acetic anhydride was mixed then 1-2 drops of concentrated H_2SO_4 was added. Then dark green colouration of the solution indicated that the presence of steroids and pink or red colouration of the solution indicated that presence of terpenoid.

5. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes only. Then formation of 1 cm layer of foam indicated that presence of saponins.

Foam Test: 0.5g of plant extracts was shaken with 2 ml of water. The foam was produced persists for 10 minutes it was indicated that the presence of saponins.

6. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The bluish black colour was indicated that presence of phenols.

7. Detection of proteins and amino acids

Xanthoproteic Test: The extracts were treated with few drops of concentrated nitric acid. Then the formation of yellow colour indicated that presence of proteins.

8. Detection of flavonoids

In Methanol extract 10% NaOH was added and dilute Hcl was added to that solution. The change of colour from yellow to colourless provides the positive result.

B. Anti Microbial Test

The microbial strains are standard which were obtained from IGH, Rourkela .The bacterial strains studied are *Bacillus subtilis, Klebsiella pneumonia, pseudomonas vulgaries, E.coli.* and *Proteus.*

Culture preparations for Antimicrobial Assay

The cultures were grown on Trptic soya broth 37 °C for 24 hours in the test tube in an incubator. The turbidity was adjusted at 0.5 Mac Far land standard (108 CFU/ml).

Agar Well Diffusion Method:

Muller Hinton agar plates were prepared and wells of 6mm were cut and swabbed with different cultures and the cut wells were then filled with 50µl. (Artizzu et al., 1995).

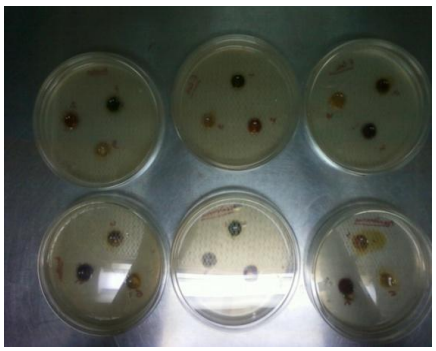

Fig:-4 showing agar well diffusion

C. Assay of free radical scavenging activity

By DPPH method:-

The antioxidant activities were determined using DPPH, (Sigma-Aldrich, Germany; M.W.394.32M) as a free radical. Then 1µg/ml solution of plant extract in methanol was prepared & 6×10^{-5} mol/L DPPH was prepared in methanol. 0.1 ml of plant sample extracts was added to 3.9 ml of DPPH solution. Then the decrease in absorbance at 517nm was recorded at 1 min interval up to 15 minute or until the reaction is reached a level. Firstly, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control. Ascorbic acid (Merck; M.W.176.13) was used as a standard. The experiment was carried out in triplate. Then the free radical scavenging activity was calculated by the following formula:

Percentage (%) DPPH radical scavenging activity = [(Absorbance of control -Absorbance of test Sample) / (Absorbance of control) × 100

Fig:-5 (a) Showing decolourization of plant sample by DPPH

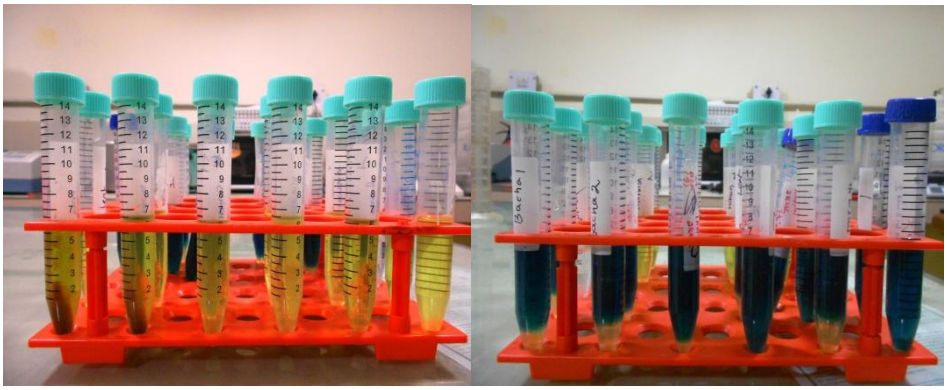

Fig:-6 Reducing power activity

Assay of reducing power

The reductive capability of the extract was quantified by the method of (Oyaizu., 1986).1 ml of extract (100, 200 and 300 µg/ml) mixed in distilled water then mix 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K_3 Fe (CN) $_6$]. Similar concentrations of standard routine were used as standard. The mixture was incubated at $50^{\circ C}$ for 20 minute. Then, the reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid. The upper layer of solution (2.5 ml) was mixed with the distilled water (2.5 ml) and 0.5 ml of 0.1% $FeCl_3$ was added. Blank reagent is prepared as above without adding any extract. Then the absorbance was measured at 700 nm in a spectrophotometer against a blank sample. Result was found that increased absorbance of the reaction mixture indicated greater reducing power.

5. RESULTS

Table .4:- 5.1 Results of phytochemical analysis:-

Sl.No	Phytochemicals	*Ficus religiosa(leaf)*	*Ficus religiosa(bark)*	*Ficus benghalensis(leaf)*	*Ficus benghalensis(bark)*
1	Tannins	+ve	+ve	+ve	+ve
2	Saponin	+ve	+ve	+ve	+ve
3	Flavonoids	+ve	+ve	+ve	+ve
4	Cardiac glycosides	-ve	-ve	-ve	-ve
5	Steroids	+ve	+ve	-ve	-ve
6	Terpenoids	+ve	+ve	+ve	+ve
7	Carbohydrates	-ve	-ve	-ve	-ve
8	Phenols	+ve	-ve	+ve	-ve
9	Proteins	+ve	-ve	+ve	-ve
10	Alkaloids	-ve	-ve	-ve	-ve

Fig.7 (a) saponin

fig: 7(b) tannins

fig: 7(c) Terpinoid

fig:7(d) protein

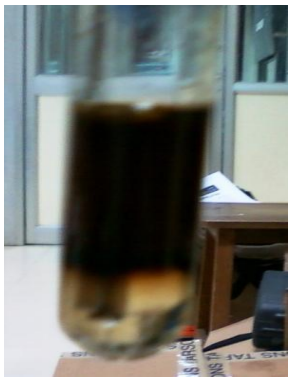

Fig: 7(e) Cardiac glycosides

fig:7(f) carbohydrate

fig:7(g) Alkaloid

fig:7(h) flavonoid

Fig7 (i) phenols

fig: 7(j) steroids

Fig:-7 (a-i) Showing change of colour in phytochemicals analysis

5.2 Results of antimicrobial activity

Methanol extracts Table no:-5(a)

Plant	*Escherichia coli*	*Pseudomonas vulgaries*	*Klebsiella pneumonia*	*Bacillus subtilis*	*Proteus*
Ficus religiosa(leaf)	+ve	-ve	-ve	-ve	-ve
Ficus religiosa(bark)	-ve	-ve	+ve	+ve	-ve
Ficus benghalensis(leaf)	+ve	-ve	+ve	-ve	-ve
Ficus benghalensis(bark)	-ve	-ve	-ve	-ve	-ve

5.3 Aqueous extract Table:-5(b)

Plant	***Escherichia coli***	***Pseudomonas*** *vulgaries*	***Klebsiella*** *pneumonia*	***Bacillus*** *pneumonia*	***Proteus***
Ficus religiosa(leaf)	**+ve**	**-ve**	**-ve**	**-ve**	**-ve**
Ficus religiosa(bark)	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**
Ficus benghalensis(leaf)	**+ve**	**-ve**	**+ve**	**-ve**	**-ve**
Ficus benghalensis(bark)	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**

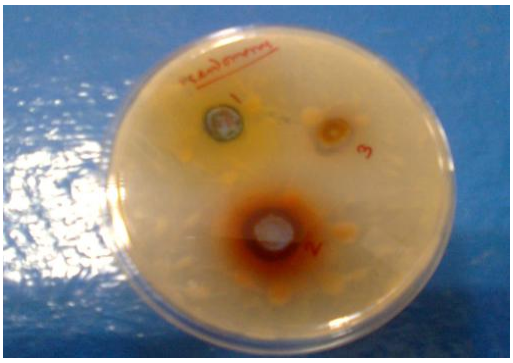

Fig:-8(a) Microbial activity of *F.religiosa* leaf & *F. benghalensis* leaf on *Klebsilla pneumonia*

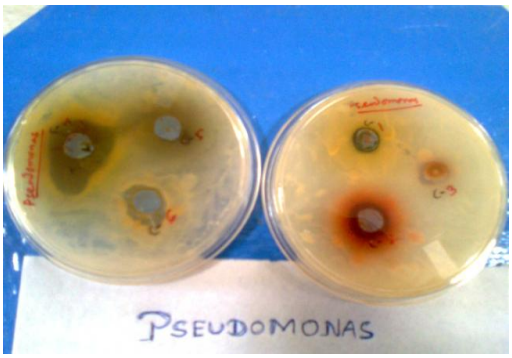

Fig: 8(a) Microbial activity of *F.religiosa* leaf & *F. benghalensis* leaf on *Pseudomonas vulgaries*

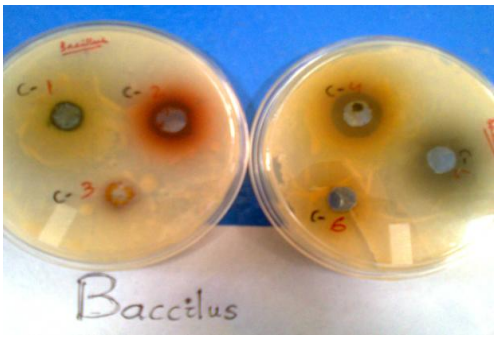

Fig:-8(b) Microbial activity of *F.religiosa* leaf & *F. benghalensis* leaf on *Baccillus subtilis*

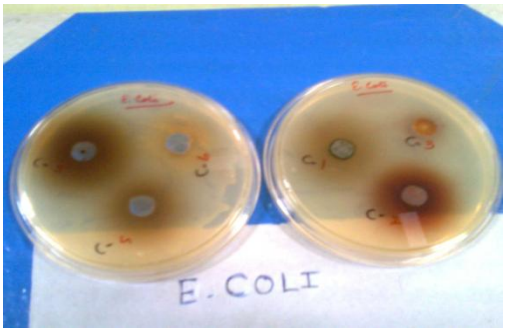

Fig:-8(c) Microbial activity of *F.religiosa* leaf & *F. benghalensis* leaf on E.coli

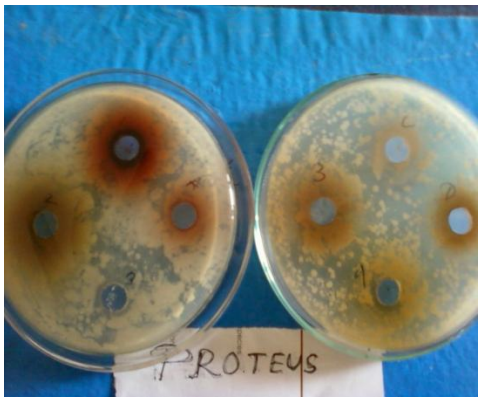

fig:-8(c) Microbial activity of *F.religiosa* leaf & *F. benghalensis* leaf on Proteus

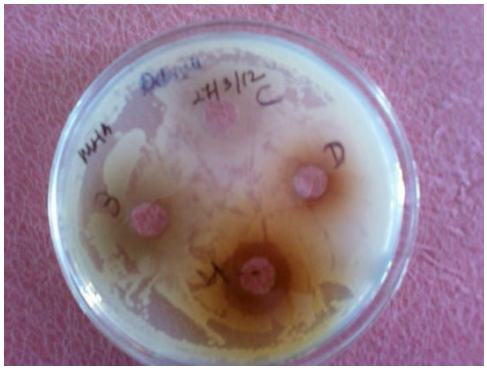

Fig:-8(d) Microbial activity of *F.religiosa* leaf & *F. benghalensis* bark on *Klebsilla pneumonia*

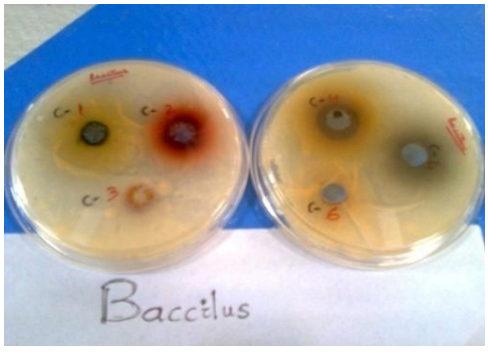

Fig:-8 (e) Microbial activity of *F.religiosa* bark & *F. benghalensis* bark on *Baccillus subtilis*

Results of reducing power activity

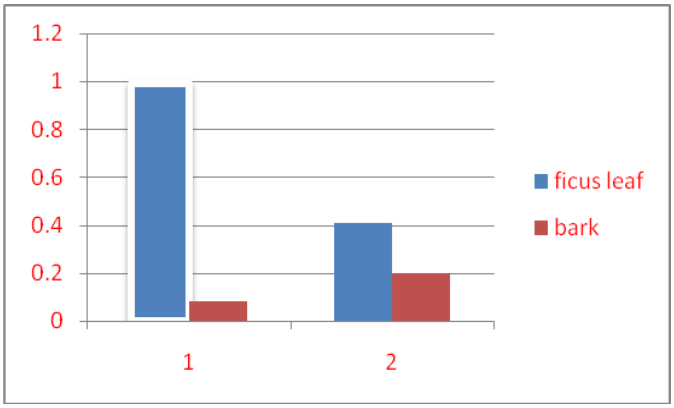

Fig: 9 graph of reducing power of *Ficus religiosa* leaf & bark

Result of DPPH ASSAY

	OD			
µg/ml	*Ficus religiosa* leaf (scavenging activity %)	*Ficus religiosa* bark (scavenging activity %)	*Ficus benghalensis* leaf (scavenging activity %)	*Ficus benghalensis* bark (scavenging activity %)
0	0	0	0	0
1	0.98	0.99	0.97	0.94
10	0.89	0.87	0.89	0.85
20	0.80	0.80	0.80	0.78
30	0.67	0.69	0.64	0.64
40	0.65	0.62	0.68	0.64
50	0.59	0.57	0.59	0.59
60	0.53	0.56	0.55	0.53
70	0.49	0.48	0.49	0.45
80	0.42	0.42	0.39	0.42
90	0.37	0.38	0.29	0.37
100	0.30	0.29	0.19	0.28
200	0.19	0.18	0.19	0.18
300	0.16	0.17	0.14	0.18
400	0.14	0.13	0.12	0.14
500	0.11	0.14	0.09	0.14
600	0.10	0.096	0.11	0.11

Table:-6 Showing OD of 4 plant samples

Table 7-(a)-(d) showing different concentration of scavenging activity% of different samples

Table:- 7(a)

µg/ml	Ascorbic acid (scavenging activity %)	Ficus leaf (%)
0	0	0
1	34.26	34.26
10	40.63	40.63
20	45.99	45.99
30	55.26	55.26
40	56.23	56.29
50	60.60	60.59
60	63.88	63.88
70	67.57	67.57
80	71.37	71.37
90	75.29	74.29
100	80.25	80.25
200	87.64	87.64
300	88.99	88.99
400	90.27	90.27
500	92	92
600	93.5	93.5

Table:-7(b)

µg/ml	Ascorbic acid (scavenging activity %)	Ficus bark %
0	0	0
1	34.26	33.44
10	40.63	41.99
20	45.99	45.49
30	55.26	53.76
40	56.29	57.79
50	60.59	62.09
60	63.88	62.32
70	67.57	68.07
80	71.37	71.35
90	75.29	74.82
100	80.25	80.78
200	87.67	88.14
300	88.99	88.99
400	90.27	90.77
500	92	90.5
600	93.5	93.6

Table: - 7(c)

µg/ml	Ascorbic acid (scavenging activity %)	*Ficus benghalensis* leaf %
0	0	0
1	34.26	34.76
10	40.63	40.13
20	45.98	45.98
30	55.26	56.77
40	56.30	54.80
50	60.59	60.60
60	63.88	62.38
70	67.57	67.57
80	71.37	71.37
90	75.29	74.29
100	80.25	80.25
200	87.64	87.64
300	88.99	89.99
400	90.27	91.77
500	92	94.5
600	93.5	92.5

Table: 7(d)

µg/ml	Ascorbic acid (scavenging activity %)	F. benghalensis bark%
0	0	0
1	34.26	36.31
10	40.63	42.13
20	45.98	47.48
30	55.26	56.76
40	56.30	56.30
50	60.59	60.59
60	63.88	63.88
70	67.57	69.07
80	71.37	71.37
90	75.29	75.29
100	80.25	81.78
200	87.64	88.14
300	88.99	88.49
400	90.27	90.27
500	92	90.5
600	93.5	92

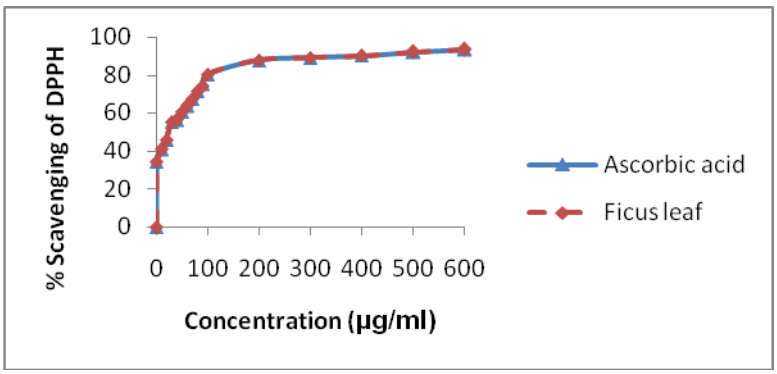

Fig: 10(a) showing concentration against scavenging activity in DPPH in *Ficus religiosa* leaf

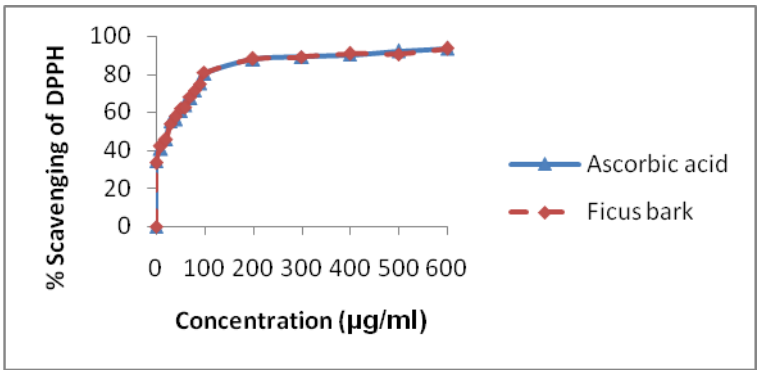

Fig10 (b) showing concentration against scavenging activity in DPPH *Ficus religiosa* bark

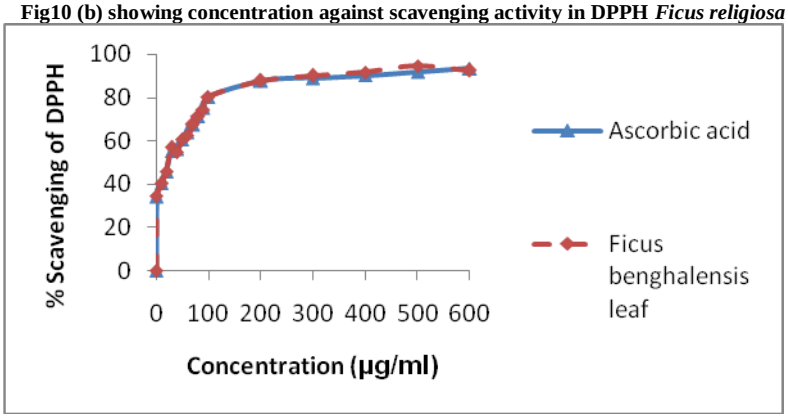

Fig: 10(c) showing concentration against scavenging activity in DPPH *Ficus benghalensis* leaf

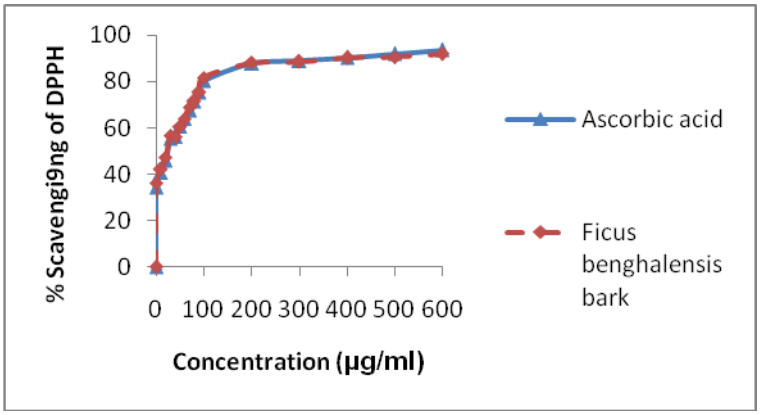

Fig: 10(d) showing concentration against scavenging activity in DPPH *Ficus benghalensis* bark

Discussion

Phytochemicals screening of aqueous and methanolic extract of *Ficus benghalensis* leaf and bark showed the presence of tannins, saponins, flavonoids & terpenoids (Table:-4). In the *F.religiosa* leaf & bark showed tannins, saponins, flavonoid, terpenoids & steroids are present. It is reported that Phytochemicals screening of aqueous and methanolic extract of *Ficus benghalensis* leaf and bark, contains tannins, saponins & flavonoid. It was reported that tannins, saponins, flavonoid, terpenoid are present in *F. religiosa* leaf and *F.benghlensis* leaf (Manimozhi et al., 2012).

DPPH is a radical that has been used widely to evaluate the antioxidant activity of various natural products. In this study, DPPH scavenging activity has been found in *Ficus religiosa* and *Ficus benghalensis* plant extract due to decolourization of purple colour to yellow. In phytochemical analysis the result obtained that phenols, proteins, saponins, tannins and flavonoids are present, and they have been shown to have multiple biological functions, including antioxidant activity. In DPPH the absorbance is decreases due to presence of antioxidant activity. Due to decrease of absorbance the purple colour was turns to yellow. It was reported by (yadav et al., 2011) that DPPH absorbance is reduced by antioxidant compound or free radicals spices to become stable diagnostic molecules resulting colour change from purple to yellow that can indicates that hydrogen denoting ability of extract sample of *Ficus religiosa* and *Ficus benghalensis*. There is a significant increase in absorbance of the reaction mixture indicates the reducing power. In this experiment Ficus *religiosa* leaf has a more reducing power than the bark as shown in graph. (Fig:-9).

Antimicrobial activity of leaf of *F.religiosa* & *F.benghalensis* showed activity against *E.coli, Klebsilla pneumonia, Baccillus subtilis* in methanolic extracts. (Table:-5, a). Aqueous extract of *Ficus religiosa* showed against *E.coli & Klebsilla pneumonia*. The bark of *F.benghalensis* has no activity against any microbes. Leaf of *Ficus* plant acted against only *E.coli & Klebsilla pneumonia* (table:-5b). It was experimentally proved that Methanol extraction of plants showed high antimicrobial activity than the aqueous extract. It was reported that methanol extract of *Ficus religiosa* & *Ficus benghalensis* leaf found to be more active against all the toxin produce *Baccillus subtilis*. (Uma, et al., 2009).The results obtained clearly indicates that leaf and bark of *Ficus religiosa* and *Ficus benghalensis* have a significant potential to use as a natural antioxidant agent. The overall results of this study indicates that the various extract concentration from *Ficus religiosa* leaf have interesting antioxidative properties and these plant samples could be utilize as potential source of natural antioxidant in the food or in pharmaceuticals industry.

Conclusion

This project comprised of plant description phytochemical constitution, antibacterial activity and antioxidant activity of leaf and bark of *Ficus religiosa* and *Ficus benghalensis* (moraceae). These plants have a great medicinal value as it has been reported to have versatile phytochemical constituents including flavonoid, phenols, saponins, tannins and saponins etc. Antioxidant activity of *Ficus religiosa* and *Ficus benghalensis* extracts has been found by means of free radical scavenging assays, reducing power assay. The plants contain high phenol & flavonoid which indicates that the sample has antioxidant effects. The antimicrobial activity of the extracts was evaluated based on the inhibition zone using the well diffusion assay. Among the extracts the methanolic fraction had a better antibacterial activity against microorganism like *Baccilus & Klebsilla*. The aqueous and methanolic extracts of Ficus species contain phytochemical therapeutic value. The result of present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations. Phytochemicals can be used for the formulation of compound drugs. The findings of this study support the view that *Ficus religiosa* and *Ficus benghalensis* are promising sources of potential antioxidant and may be efficient as preventive agents in diseases like fever, cough, wound healing property, anti-inflammatory activity, cardiovascular diseases, neuro-degerative diseases and cancer.

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