INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.9.3.2019.948

A STUDY ON THE PHYTOCHEMICAL SCREENING, ANTIBACTERIAL ACTIVITY AND SILVER NANOPARTICLE SYNTHESIS FROM *FICUS RACEMOSA* L

P. Rama Bhat[1]*, Laxmi P Gadiga[1] and Vernon BSD Silva[2]

[1]Department of PG Studies and Research in Biotechnology, Alva's College, Moodbidri- 574 227, Karnataka, India.
[2]Alva's College of Medical Laboratory Technology, Moodbidri- 574 227, Karnataka, India.

ABSTRACT

In the present investigation, preliminary phytochemical screening of *Ficus racemosa* leaves has been done in methanol and aqueous extract, showed the presence of phytoconstituents namely carbohydrates, flavonoids, saponins, steroids, tannins and the absence of alkaloids and amino acids. Silver nanoparticles are synthesized using the leaf extract of *F. racemosa* by mixing dried leaves powder with aqueous solution of 1mM silver nitrate and by incubating it at 95˚C for 15 min. The colour change in the solution from colorless to dark brown indicates naoparticle synthesis which was later characterized. Maximum absorption peak was observed at 400 nm and synthesized nanoparticles are cubic shaped with a diameter varies from 2.04µm- 3.64µm. The antimicrobial activity of the aqueous, methanol and $AgNO_3$ leaf extracts were tested against 5 bacterial species viz., *Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* by agar well diffusion method with amoxicillin as a positive control. Methanol extract were found to be more active against all the organisms than the aqueous and $AgNO_3$ extract. The present study included the bio-reduction of silver ions through medicinal plants extracts and testing for their antibacterial activity. The aqueous silver ions exposed to the extract the synthesis of silver nanoparticles were confirmed by the change of color of plant extracts. These environmentally benign silver nanoparticles were further confirmed by using UV-Vis spectroscopy and FTIR analysis. The results indicated that silver nanoparticles have good antibacterial activity against different microorganisms. Nanotechnology is creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Silver ion has been known to be effective against a broad range of new microorganisms. Silver nanoparticles are well known as one of the most universal antimicrobial substances in the field of biology and medicine also reported to possess antifungal, anti-inflammatory, anti-viral, anti-angiogenesis and anti-platelet activity. Thus various formulations have varied levels of medicinal properties and can be used to cure diseases caused by different pathogens.

Keywords: Antimicrobial activity, *Ficus racemosa*, phytoconstituents and silver nanoparticles.

INTRODUCTION

In India, the *Charak Samhita* and *Sushrut Samhita* described the medicinal properties of 500 and 700 plants respectively under 37 classes or "*Ganas*" (Ahmad et al., 2001). The oldest record of medicinal use of plants is found in the *Rig Veda*, which is approximately 8000 years old. In *Atharva Veda* remarkable description of Indian medicinal plants were provided by ancient Indian scholars. *Ayurveda,* an *Upaveda,* composed around 2500 BC deals with medicine, healthcare and treatment of disease from indigenous drugs. From *Vedas* it is learnt that Indo-Aryans used the *'Soma'* (a

plant product) as a revitalizing agent, which exhibits an amazing stimulating effect (Kumar et al*.,* 2002). More than 90% of the formulations under the Indian Systems of Medicine i.e. Ayurveda, Siddha, Unani and Homoeopathy (AYUSH), predominantly contain plant based raw materials. In India Unani, Siddha and Homeopathy prescriptions constitute about 95% of traditional based medicines **(**Kirankumar et al., 2013). According to the World Health Organization's statement; the traditional healing provides the primary health care needs for a large section (80%) of the population.

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Ahmad et al*.,* 1992). However, the situation is alarming in developing as well as, AIDS and cancer patients (Banerjee et al*.*, 2014). In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune compromised antimicrobial substances from other sources including plants.

India has an ancient heritage of traditional medicine. The *Materia Medica* of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various systems including Ayurveda, Siddha, Unani and Homoeopathy. Plants are one of the most important sources of medicines. Today the large numbers of drugs in use are derived from plants. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability. *Ficus racemosa* have the various pharmacological activities like antioxidant, cardio-protective, mosquito larvicidal and gastro protective (Anitharani et al., 2011). Most of the natural products isolated from medicinal plants are the secondary metabolites, which include alkaloids, tannins, flavonoids, steroids, terpenoids, phenylpropanoids and anthraquinones, saponins etc. Some of the products have nutritive value and antifungal and antibacterial activities. Alkaloids possess antimicrobial, anticancer, antimalarial and cytotoxic properties whereas; flavonoid compounds exhibit inhibitory effect against bacteria. Flavonoids, hydroxyl groups on their β-rings are more active against microorganisms and have also been found that the more hydroxylation, the greater the antimicrobial activity. Tannin has high activities against bacterial and viral infections and also acted as strong antioxidant (Poongothai et al., 2011).

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value (Kumar et al., 2012). The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains. Presently 80% of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases (Hemraj et al., 2012).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments. Extracts of plants were used for the treatment of various diseases and this forms the basis for all Indian systems of Medicine. However, this area is not much developed when compared to modern system of medicine, mainly because of the lack of scientific documentation in this field (Hemraj et al*.,* 2012, Prasoon and Chittaranjan, 2012).

In the past few years, there has been an increasing interest in silver nanoparticles on account of the antimicrobial properties that they display (Dinesh et al*.,* 2012). They are even being projected as future generation antimicrobial agents. Nanoparticles (NPs) of noble metals like gold, silver and platinum are well recognized to have significant applications in electronics, magnetic, optoelectronics and information storage. One such important member of the noble metal NPs are silver NPs (Ag NPs). They are also broadly applied in shampoos, soaps, detergents, cosmetics, toothpastes and medical and pharmaceutical products and are hence directly encountered by human systems (Deb, 2014).

Silver nanoparticles are of interest because of the unique properties (*e.g.,* size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles (Banerjee et al*.,*

2014). Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry). Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Nanoparticles and nanostructure are become developing in human medical application, including imaging or the delivery of therapeutic drugs to cell, tissues and organs. Many drug loaded nanoparticles are interacts with organ and tissues and are taken up by cells. Several studies have shown that the tissue, cell and even cell organelle distribution (Kumar, 2012; Prasoon and Chittaranjan, 2012) of drugs may be controlled and improved by their entrapment in colloidal nanomaterials, like micellar structure, such as nanocontainer.

A variety of techniques including physical and chemical methods have been developed to synthesize silver nanoparticles. Therefore, there is a growing need to develop environmentally friendly nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocols. Group of researchers developed silver nanoparticles being extensively synthesized using various plants. Different types of plants are being currently investigated for their role in the synthesis of nanoparticles. Nanoparticles of silver, nickel, cobalt, zinc and copper have also been synthesized inside the live plants of *Brassica juncea* (Indian mustard), *Medicago sativa* (Alfa alfa) and *Helianthus annuus* (Sunflower). Certain plants are known to accumulate higher concentrations of metals compared to others and such plants are termed as hyper-accumulators. Of the plants investigated, *Brassica juncea* had better metal accumulating ability and later assimilating it as nanoparticles. Recently much more work has been done with regard to plant assisted reduction of metal nanoparticles and the respective role of phytochemicals. The main phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids in the light of IR spectroscopic studies. The size of the nanoparticles synthesized studies were using xerophytes, mesophytes and hydrophytes were in the range of 2- 5nm (Reddy et al., 2013).

Silver nanoparticles can be produced either intra- or extra-cellularly by using living organisms. Over the past decade, a variety of microorganisms such as bacteria, fungi and yeast have been used to synthesize silver nanoparticles. Green synthesis of silver nanoparticles and their antimicrobial activities have been done using plants. Most of the reported biological synthesis methods using plants took more than 1 hr for the formation of colloidal silver (Mittal, 2012).

Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly as well as intracellarly. Silver nanoparticles shows very strong bactericidal activity against Gram positive as well as Gram negative bacteria including multi-resistant strains, and also it was found to be in few studies (Senapathi, 2015).

Ficus racemosa L. (*F. glomerata* Roxb.) belonging to the family Moraceae. The genus *Ficus* includes 750 species (Tanvir et al., 2010), widely distributed in different habitats. *F. racemosa* is popularly known as the Cluster Fig Tree (English), Goolar (Gular), Fig (Hindi), Udumbar (Sanskrit), Atti (Kannada) and Audumbar (Marathi). Medium to moderate sized deciduous tree with reddish grey or grayish green bark, soft surface, uneven and often cracked and taste mucilaginous without any characteristic odour. Unlike the banyan, it has no aerial roots, roots are long, brownish in colour. The leaves (Plate 1A) are simple, dark green, 7.5-10 cm long, glabrous; in large clusters from old nodes of main trunk, ovate, ovate-lanceolate or elliptic, sub acute, entire and petiolate and are shed by December and replenished by January and April, when the tree becomes bare for a short period. The fruits (Plate 1B) receptacles are 2-5 cm in diameter, in large clusters, arising from main trunk or large branches. The fruit of *F. racemosa* is 2-5 cm long, circular and grows directly on the trunk. The seeds are tiny, innumerable- and grain-like. The plant is native to Australia, Malaysia, South-East Asia and the Indian subcontinent.

F. racemosa is a popular medicinal plant in India, which has long been used in Ayurveda, the ancient system of Indian medicine, for various diseases/disorders including diabetes, liver disorders, diarrhea, inflammatory conditions, hemorrhoids, respiratory, and urinary diseases. *Ficus racemosa* is pharmacologically studied for various activities including antidiabetic, antipyretic, anti-inflammatory, hepatoprotective, and antimicrobial activities (Padmaa, 2009; Joseph et al., 2010; Anitharani et al., 2011; Ganatra et al., 2012; Rishikesh et al., 2012; Mishra et al., 2013; Rageeb et al., 2014).

The objectives of the present study was to analyse phytochemical constituents in aqueous and methanol extract of the leaves of *Ficus racemosa,* their antibacterial activity against selected bacterial strains, synthesis of silver nanoparticles and their characterization.

(A)

(B)

Plate 1: Leaves (A) and Clusters of fruits on main trunk (B) of *Ficus racemosa*

MATERIALS AND METHODS

Collection of plant sample

Ficus racemosa leaves were collected from Shobhavana campus at Mijar, Moodbidri. The leaves were cleaned by washing several times with de-ionized water and allowed it to shade dry for a week. The leaf material was kept in hot air oven at 60°C for 24-48 hours until it was dried completely. Then it was powered using mixer to obtain fine powder and it is then sieved using 150 µ size sieves.

Test organisms

Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa were used as bacterial strains for the study collected from Dept of Microbiology, Alva's College of Medical Laboratory Technology and microscopic examination was done for the confirmation, and were maintained in slants.

Preparation of aqueous extract

Twenty five grams of plant sample was taken in a conical flask with 150 ml of distilled water and this was mixed and covered with aluminum foil. Then it was kept in water bath at a temperature of 60°C for 6-7 hours, the mixture was filtered using Whatman No. 1 filter paper and filtrate was put in a weighed china dish. This was kept in water bath open, till all the filtrate evaporated leaving behind a film of plant residue. The obtained residue was dissolved in 10 ml of distilled water to obtain aqueous extract of plant sample. The extract was stored at 4°C for further use.

Preparation of methanol extract

Twenty five grams coarse powder of the leaf was extracted by soxhlet process using 150ml methanol. This was followed by distillation and the extract obtained was stored at 4°C until further use.

Preparation of silver nitrate solution

1mM aqueous extract of silver nitrate was prepared by using 300ml distilled water.

Synthesis of silver nanoparticles

For biosynthesis of nanoparticles, 100 ml of 1mM $AgNO_3$ was taken in a conical flask and 3g of leaves powder was added, kept aside for 30 minutes and centrifuged at 3000rpm for 15 minutes. The supernatant were collected and heated at 95°C. A change in the colour of the solution was observed after heating the mixture within 15 min. The extracts were stored at 4°C for further use.

Phytochemical screening of the plant extract

Phytochemical tests for flavonoids, tannins, amino acids, alkaloids, saponins, carbohydrates and steroids were carried out

on the aqueous and methanol extracts with respective solvents using standard protocols (Poongothai et al., 2011; Tiwari et al., 2011).

Characterization of Silver nanoparticles

UV-VIS spectra analysis: The reduction of pure Ag^+ ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample with distilled water. UV-VIS spectral analysis was done by using UV-VIS spectrophotometer.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy was used to recognize the functional groups bound to the silver surface and involved in the formation of silver nanoparticles. The lyophilized powder sample was used and examined by Infrared (IR) spectrum at the spectral range of 1000-4000cm^{-1}

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using SEM machine. Thin films of the sample was prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 minutes.

Antibacterial screening of leaf extracts

Preparation of inoculums

A loop full of culture was inoculated into nutrient broth and incubated at 37°C for 24 hours to obtain a bacterial culture.

Antibacterial activity test by Agar well diffusion method

Petri dishes were plated with nutrient agar media and allowed to solidify for 30mins. The test organisms were then spread on the surface of the media using sterile swab stick. Cork borer (7mm) was used to bore wells in media. The aqueous extract of different concentrations viz., 50µl, 100µl and 150µl were dispensed into the wells using a micropipette. A negative control of water and a positive control of amoxicillin were kept and the extract was allowed to diffuse for one hour at room temperature. Then the plates were incubated at 37°C for 24hours. Zones of inhibition were measured. Similarly antibacterial activities of methanol and silver nanoparticle extracts were studied.

RESULTS

Phytochemical screening

The plant extract showed positive result for carbohydrates, tannins, flavonoids and saponins whereas negative result for amino acids and alkaloids (**Table 1: Phytochemical analysis for methanolic and aqueous leaf extracts of *F racemosa***).

Antibacterial activity of leaf extract

The bacterial cultures showed varied levels of sensitivity towards different concentrations of aqueous extracts as given in table 2. *Staphylococcus aureus* showed high sensitivity for the aqueous extract of the plant. The *Salmonella typhi* was showed less sensitive. *Stapylococcus aureus* was high sensitive for the methanol extract of the plant and *Bacillus subtilis* showed less sensitivity. The antibacterial activity for different concentrations for the extract of silver nanoparticles of *F. racemosa* was showed in table 2. *Pseudomonas aeruginosa* showed higher sensitivity, whereas *Bacillus subtilis* showed less. **(Table 2: Antibacterial activities of the aqueous, methanolic extracts and silver naoparticles from leaves of *F. racemosa)***

Synthesis of silver nanoparticles

Formation of silver nanoparticles by the reduction of silver ions during the exposure to *Ficus racemosa* leaf extract was easily identified from the colour change of the reaction mixture from colorless to dark brown (Plate 2) observed in 15 min of incubation which indicated the formation of silver nanoparticles.

Plate 2: Synthesis of silver nanoparticles from *F. racemosa* leaves confirmed by change in color

Characterization of silver nanoparticles

Ultraviolet- Visible (UV- VIS) Spectra analysis

UV-VIS spectroscopy is used to analyze the formation and stability of metal nanoparticles in the solution. Silver nanoparticles are known to exhibit a UV- Visible absorption maximum in the range of 400-750nm. In this study the formation of silver nanoparticles was initially confirmed by using UV- Visible spectroscopy due to Surface Plasmon Resonance phenomenon. Absorption peak (Fig. 1) was observed at 400nm which is a characteristic of silver nanoparticles.

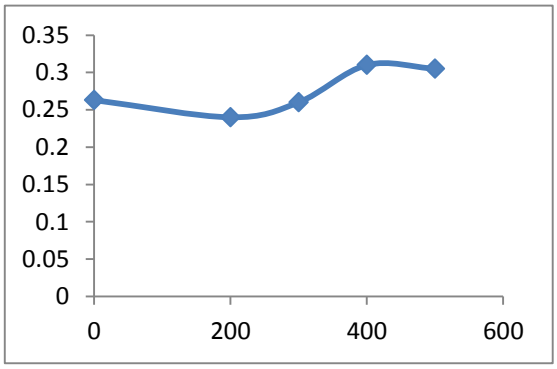

Fig. 1: UV-Vis absorption spectrum of silver nanoparticles from *F. racemosa* leaf extracts

Scanning Electron Microscopic (SEM) study

The SEM image has been employed to characterize the size, shape and morphology of synthesized silver nanoparticles. From the SEM image (Fig. 2) of synthesized silver nanoparticles, it is evident that the morphology of the synthesized silver nanoparticles are cubic shape with the diameter range 2.04µm-3.64µm.

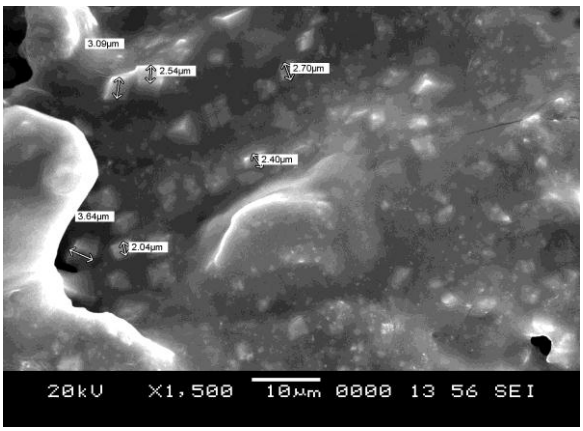

Fig. 2: SEM Image of silver nanoparticles from *F. racemosa* leaf extracts

Fourier Transform Infrared Spectroscopy (FTIR)

The lyophilized nanoparticle samples were analyzed in FTIR to identify the possible biomolecules responsible for the reduction of the silver ions by cell filtrate. The representative spectra of nanoparticles obtained manifests absorption peaks (Fig. 3) using the spectral range between 1000-4000cm^{-1}.The absorption peaks were observed at 3275.13cm^{-1}, 3062.95cm^{-1}, 1604.77cm^{-1}, 1438.90cm^{-1}, 1062.78 cm^{-1} ,821.68 cm^{-1} and 777.31 cm^{-1} can be assigned as absorption bands of –OH group of alcohol, C-H aromatic stretch of groups, C=C aromatic stretch of group, C=H alkyl Halide stretch of group, C-O alcohol stretch of group, C-H bending of alkene group, and C-Cl halide stretch of alkyl functional groups. The FTIR analysis supported the reducing property of silver nanoparticles by *Ficus racemosa* leaves extract which in turn imparted the high stability of the synthesized silver nanoparticles.

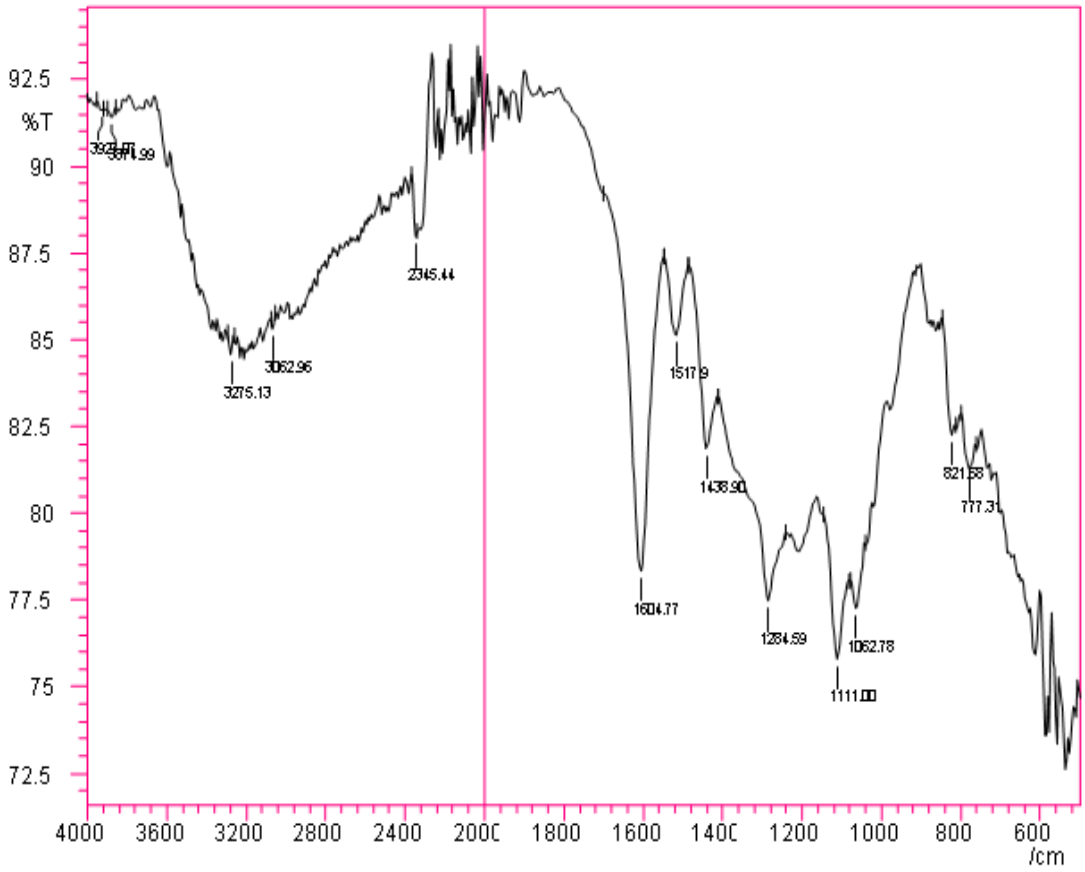

Fig. 3: Fourier Transform Infrared Spectroscopy (FTIR) image of SNP from *F. racemosa* leaf extracts

Table 1: Phytochemical analysis for methanolic and aqueous leaf extracts of *F racemosa*

Phytoconstituents	Inference	
	Aqueous extract	Methanol extract
Carbohydrates	+	+
Tannins	+	+
Alkaloids	-	-
Steroids	+	-
Flavonoids	+	+
Saponins	+	+
Amino acid	-	-

+ Present, - Absent

Table 2: Antibacterial activities of the aqueous, methanolic extracts and silver naoparticles from leaves of *F. racemosa*

Test organisms	Zone of inhibition (mm) for different concentrations								
	Aqueous extract			Methanol extract			Silver nanoparticle		
	50 µl	100 µl	150 µl	50 µl	100 µl	150 µl	50 µl	100 µl	150 µl
Bacillus subtilis	3.0	5.0	6.0	0.0	1.0	2.0	0.0	2.0	3.0
Klebsiella pneumoniae	2.0	2.0	2.0	2.0	3.0.	3.0	3.0	3.0	6.0
Pseudomonas aeruginosa	3.0	4.0	5.0	4.0	4.0	5.0	4.0	4.0	6.0
Salmonella typhi	0.0	2.0	3.0	3.0	4.0	6.0	2.0	2.0	3.0
Staphylococcus aureus	3.0	4.0	6.0	4.0	4.0	7.0	4.0	4.0	5.0

DISCUSSION

In the present investigation, preliminary phytochemical screening of methanol and aqueous leaf extract of *Ficus racemosa* showed the presence of carbohydrates, flavonoids, saponins, steroids, tannins and the absence of alkaloids and amino acids. In one of the earlier studies by Sunil et al. (2012) reported the presence of phenols, flavonoids, quinones, saponins, cardinolites, steroids, tanins and terpenoids in various extracts of *F. racemosa*. Similarly, sugar, protein, alkaloids, flavonoids, sterols and glycoside components were observed in bark of plants *F. racemosa* with solvent such as benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether (Poongothai et al., 2011). Raju and Sivaraj (2012) showed the presence of alkaloids, phenol, sugar, terpenoids, glycosides, flavonoids and tannins in the bark, stem, leaf and fruit extracts of *F. religiosa*.

The phytochemical analysis of aqueous, acetonic, and methanolic extract was easily performed for qualitative analysis of flavonoid, phenols, saponins, cardiac glycosides, terpenoids, quinones, carbohydrates, and alkaloids. A widespread variety of vigorous phytochemicals such as flavonoids, alkaloids, and saponins have been isolated and identified in different plants of *Ficus* family. As the results obtained, it can be concluded that aqueous extract of three plants shows positive results in case of saponins and phenols, it gave a negative result in case of amino acid, as it is absent in aqueous extract of *Ficus benjamina*, *F. infectoria* and *F. krishnae*. In case of methanolic extract, all three gives a negative test for flavonoids and saponins. For carbohydrates, phenols, quinones, and cardiac glycosides *F. benjamina* and *Ficus krishnae* have a negative result while positive result is shown by *F. infectoria*. (Jassal and Sharma, 2019).

In the present study, the leaf extract with silver nitrate mixture changed its color to dark brown after 15 minutes, indicated reduction of silver ions and lead to the formation of silver nanoparticles which is due to the excitation of Surface Plasmon vibrations. There are few reports on plant extracts of leaves, bark or the seeds with silver nitrate solution, in which the color changes from pale yellow/yellow to brown or dark brown (Ankanna et al., 2010; Prathibha et al., 2015; Bhat et al., 2016). Formations of silver nanoparticles by reduction of silver nitrate during exposure to *Ficus racemosa* can be easily monitored from the change in colour of the reaction mixture. Silver nanoparticles bear characteristic dark brown colour after 15 min due to the excitation of surface plasmon vibrations.

The synthesized nanoparticles were detected by UV-VIS Spectroscopy at various nanometers and the particle has increasingly sharp absorbance peak maximum at 400nm. Some of the earlier studies reported that absorption peak of silver nanoparticles showed at 412nm (Maribel *et al*. 2009), 450nm (Geetha et al., 2012), 358 nm (Lakshmi et al., 2014) and 358nm and 450 nm (Pratibha et al., 2015). Priya et al. (2016) observed a UV-visible peak of 444nm for the silver nanoparticles synthesized from *F. racemosa* bark extracts, while Rahman and Prasanna (2018) showed the peak at 424 nm in silver nanoparticles synthesized from *Ficus religiosa* plant leaf extract.

Hemanth et al. (2010) and Christensen et al. (2011) reported the nanoparticle of 52-104nm and 10-25nm size diameter. Some of the previous studies by Lakshmi et al. (2014) and Prathibha et al. (2015) reported the particle size were in the range of 90-120nm and 80-120nm. In present study the cubic shaped nanoparticle was obtained ranging 2.04µm-3.64µm diameter. Rahman and Prasanna (2018) observed the morphology of the synthesized silver nanoparticles as spherical in *F. religiosa* leaf extract.

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. The biomolecules which are involved in the reduction of silver ions identified by absorption peaks in the spectral range of 1000-4000 cm^{-1} and the absorption bands of –OH group of alcohol, C-H aromatic stretch of groups, C=C aromatic stretch of group, C=H alkyl Halide stretch of group, C-O alcohol stretch of group, C-H bending of alkene group, and C-Cl halide stretch of alkyl functional groups. Lakshmi et al., (2014) obtained the spectra of nanoparticles manifests absorption peaks spectral range between 1000-4000cm^{-1}. In one of the earlier studies in *Ficus religiosa* leaf extract the FTIR confirmed the presence of alcohol, alkene, alkyne and alkyl Halide functional groups in the sample (Rahman and Prasanna (2018).

Hossain et al. (2014) studied the antimicrobial activity of methanolic extracts of *Ficus racemosa* fruits *in vitro* using disc diffusion method against human pathogenic bacteria fungi. The displayed a potential antibacterial activity against all the tested four Gram negative and Gram positive bacteria: *Staphylococcus aureus, Bacillus subtilis, Vibrio cholera, Bacillus cereus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Klebsiella* species and *Proteus* species as well four fungi: *Alternaria* spp., *Colletotrichum* spp., *Curvularia* spp. and

Fusarium spp. The highest zone of inhibition was found in the concentration of 200 µg/disc for *Staphylococcus aureus* (18mm) and in the concentration of 150 µg/disc for *Fusarium* spp. (12mm). The consequences of this investigation suggest that the extracts of *Ficus racemosa* can be used to discover antibacterial agent for developing new pharmaceuticals to control studied human pathogenic bacteria responsible for severe illness. In the present study, Antibacterial activity tested against *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Staphylococcus aureus, Bacillus substilis, Salmonella typhi.* The highest zone of inhibition was found in the concentration of 150 µg/ml disc for *Staphylococcus aureus* (7mm) in methanol extract and concentration of 150 µg/ml disc for *Pseudomonas aeruginosa* (6mm). Similarly, on the other hand, using the disc diffusion method the antimicrobial activity of the pathogens - *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Aeromonas hydrophila, Staphylococcus aureus*, against aqueous extract of the bark, leaves, stem and fruits of *Ficus religiosa* by Raju and Sivaraj (2012) showed that the aqueous extract of the plant have antimicrobial activity against *Streptococcus pyogenes, Aspergillus niger* and *Candida albicans* at various concentrations. The highest zone of inhibition (10 mm -15 mm in diameter) was observed in 100 mg/ml concentration in all tested microbes.

Extracts of *Ficus benjamina*, *Ficus infectoria*, and *Ficus krishnae* were screened for their antibacterial activity against bacterial strains of *C. perfringens* and *S. aureus*. Of three plant species tested, *Ficus benjamina* showed a maximum zone of inhibition against *C. perfringens* (20 mm), *Ficus infectoria* showed a maximum zone of inhibition against *S. aureus* (30.5 mm), and *Ficus krishnae* showed a maximum zone of inhibition against *C. perfringens* (28 mm) (Jassal and Sharma, 2019). The aqueous extract of *F. religiosa* has shown high antimicrobial activity against *S. aureus* and *B. subtilis* (Shamila et al., 2012). For different extracts of *Ficus*, diethyl ether extract was used and exhibited better inhibitory effect against *K. pneumoniae* (20 mm), *E. coli* (12 mm), and *P. aeruginosa* (12 mm) and minimum activity was shown against *S. aureus* (10 mm) (Tijjani et al., 2012). The presence of various phytochemicals such as saponins and alkaloids was reported in the leaf extracts of *Ficus* species (Fouche et al., 2008).

Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effects of fruits and vegetables have been attributed by phytochemicals, which are the non-nutrient plant compounds. Phytochemicals are naturally occurring; biologically active, non-nutritive chemical compounds found in plants and act as a natural defense system against various pests. Various phytochemicals have been known to possess medicinal properties and hence widely used in Indian systems of traditional medicine. Different parts of plant possess varied levels of phytoconstituents which can be explored and tested for various medicinal uses as per the knowledge of local medical practitioners or traditional practitioners. Based on the available information the crude extracts can be tested against human pathogens and initial effect on crude extracts will deal about its further teat and usage.

REFERENCES

1. Ahmad I and Arina Z. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol. 2001;7(4):113–123.
2. Anitarani, Shiksharthi and Mittal S. Ficus racemosa: Phytochemistry, Traditional Uses and Pharmacological Properties: A Review. Int J Recent Adv Pharma Res. 2011;4(2):6-15.
3. Anakanna S, Prasada T and Savitramma N. Production of Biological Silver Nanoparticles using Boswellia ovalifoliolata Stem Bark. Digest J Nanomat Biostruct. 2012; 5(2):369-372.
4. Banerjee P, Satapathy M, Mukhopahayay A and Das P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants; synthesis, characterization, antimicrobial property and toxicity analysis. Springer Open J Biores Bioproc. 2014;1(3):189-193.
5. Bhat PR, Savitri VH, Laxmi PG and Jenitta EP. A study on the phytochemical analysis, silver nanoparticle synthesis and antibacterial activity from seed extract of Areca catechu L. Int J Biochem Res Rev. 2016;9(1):1-9.
6. Christensen L, Vivekanandhan S, Misra M and Mohanty A. Biosynthesis of silver nanoparticles using Murraya koenigii (curry leaf), An investigation on the effect of broth concentration in reduction mechanism and particle

size. Adv Mat Lett. 2011;2(6):429-434.
7. Dinesh S, Karthikeyan S and Arumugam P. Biosynthesis of silver nanoparticles from Glycyrrhiza glabra root extract. Arch Appl Sci Res. 2012; 4(1):178-187.
8. Ganatra S, Durge S and Patil S. Phytochemicals Investigation and TLC Analysis of Ficus racemosa Leaves. J Chemical Pharma Res. 2012;4(5): 2380-2384.
9. Geeta N, Harini K, Jerlin J and Selva K. Biofabrication of Silver Nanoparticles using Leaf Extract of Chromolaena odorata (L.) King and Robinson. International Conference on Nuclear Energy. Environ Biol Sci. 2012;3(2):56-58.
10. Hemath N, Gaurav K, Karthik L and Rao B. Extracellular biosynthesis of silver nanoparticles using the filamentous fungus Penicillium sp. Arch Appl Sci Res. 2010;2(6):161-167.
11. Hemraj V and Anil J. Antimicrobial Activities of Medicinal Plants. Int J Res Pharma Biomed Sci. 2012;3(1):2229-2237.
12. Hossain M, Sayeed M, Nasir M (2014). In vitro antimicrobial activity of methanolic extract of Ficus racemosa L. fruits. J. Sci. Innov. Res. 3(4): 446-449.
13. Joseph B, Justin R. Phytopharmacological properties of Ficus racemosa - an overview. Int J Pharma Sci Rev and Res. 2010;3(2):134-138.
14. Kirankumar S, Umesh M and Ramesh I. phytochemical screening and antimicrobial activities of Ficus glomerata fruit extracts. Int J Pharmacy Pharma Sci. 2013;5(4): 372-375.
15. Kumar A. Rapid and Green synthesis of silver nanoparticles using the leaf extract of Parthenium husterophorus: A novel biological approach. Int Res J Pharmacy 2012;3(2):169-173.
16. Kumar DP, Subash Chandra and Santosh Kumar. Anti-inflammatory activity of methanolic extract of bark of Ficus racemosa and root of Cissampelos pareira. Int J Res Pharmacy Chem. 2012;2(4):2231-2781.
17. Krishnamurti and Upendra Kumar. Antimicrobial activity of Ficus benghalensis and F. racemosa roots L. Am J Microbiol. 2011;2(1):21-24.
18. Lakshmi N, Jenitta EP, Rama Bhat P and Jayadev K. Studies on phytochemical, antibacterial properties and synthesis of silver nanoparticles from the floral extract of Mesua ferrea L. Int J Green Herbal Chem. 2014; 3(4):1730-1739.
19. Maribel G, Dille J and Godet S. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. Int J Chemical Biol Engg. 2009;2:104-110.
20. Mishra R and Kumar A. Phytochemical and chromatographic studies in the latex of Ficus racemosa L. Asian J Plant Sci Res. 2013; 3(4):150-154.
21. Mittal S. Biosynthesis of silver nanoparticles (AgNPs) using waste fruits peel as an antimicrobial drug agent. Ph.D. Thesis. Department of Botany, Dayalbagh Educational Institute (Deemed University) Dayalbagh, Agra-282 110. 2012.
22. Padmaa M. Ficus racemosa L. - An Overview. Natural Product Radiance 2009;8(1):84-90.
23. Poongothai, A, Sreena K and Sreejith M. Preliminary phytochemical screening of Ficus racemosa bark. Int J Pharma Biosci. 2011;2(2):97-103.
24. Prathibha S, Jenitta EP, Rama Bhat P, Jayadev K and Shrinidhi Shetty. Green synthesis of silver nanoparticles from fruit extracts of Terminalia chebula Retz. and their antibacterial activity. Int J Res Biosci. 2015;4(2):29-35.
25. Prasoon P and Chittaranjan B. Green synthesis of gold nanoparticles and silver nanoparticles from leaves and bark of Ficus carica for nanotechnological applications. Int J Sci Res Publ. 2012;2(5):2250-3153.
26. Priya FJ, Vimala JR, Sathyabama R and Lavanya M. Green synthesis of silver nanoparticles using aqueous extract of Ficus racemosa bark and its antimicrobial activity. World J Pharmacy Pharma Sci. 2016;5(5): 753-765.
27. Rageeb M, Usmana M and Patil R. Medicinal uses of Ficus racemosa. Int J Pharma Arch. 2013;2(3):33-42.
28. Rahman A and Prasanna A. Characterization of silver nanoparticles biosynthesized using Ficus religiosa plant leaf extract. Int Res J Engg Technol. 2018;5(12): 1449-1452.

29. Raju R and Sivaraj R. Screeening for phytochemicals and antimicrobial activity of aqueous extract of Ficus religiosa L. Int J Pharmacy Pharma Sci. 2012;4:207-209·
30. Reddy C, Ramamurthy, Shrilakshmi and Rao S. Photosynthesis of Ecofriendly Silver nanoparticles and biological application. Afr J Biotechnol. 2014;14(3):223-247.
31. Rishikesh K, Sunil A, Nirmal and Sagar K. Potential of Ficus racemosa Bark: An immunomodulatory agent. Indian J Basic Appl Medical Res. 2012;1(2):120-127.
32. Shamila IM, Jeeva S, Sheela DJ, Brindha JR and Lekshmi NC. Antimicrobial spectrum and phytochemical study of Ficus tsiela L. (Moraceae). Drug Inven Today. 2012;4:337-339.
33. Senapati S. Biosynthesis and immobilization of nanoparticles and their applications. University of Pune. India. 2005;3(5):143-154.
34. Sunil H, Shweta P and Patil S. Preliminary phytochemicals investigation and TLC analysis of Ficus racemosa leaves. J Chemical Pharma Res. 2012;4(5):2380-2384
35. Tanvir S, Ruksana R, Kiran B, Pimprikar R and Sufiyan A. Antibacterial activity of Ficus racemosa Linn. leaves on Actinomyces viscosus. J Pharma Sci Res. 2010;2(1):41-44.
36. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H. Phytochemical screening and Extraction: A Review. Int Pharma Sciencia. 2011;1(1):98-105.