



RESEARCH ARTICLE

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *FICUS MACEMOSA* FRUITS NANOPARTICLE SYNTHESIZED EXTRACTS IT'S FROM EASTERN GHATS OF TAMIL NADU STATE IN INDIA

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ABSTRACT

We investigation report the biosynthesis of silver and gold nanoparticles using antibacterial and antifungal activity of *Ficus macemosa* fruits extract. Decidedly that *Ficus macemosa* fruits extract the antibacterial and antifungal activity of the synthesized Ag and Au nanoparticles were tested again gram-positive and gram negative determine seven human pathogenic organisms using disc diffusion method. The different concentration of synthesis of silver and gold nanoparticles *Ficus macemosa* fruits extract was taken 1mM and 2mM. The result obviously that silver and gold nanoparticles was 1mM extract highly zone of inhibition *Bacillus subtilis* 12cm against than *Staphylococcus aureus* 7cm, *Escherichia coli* 6cm, *Kilebsiella pineumoneae* 5cm, *Pseudomonas aeruginosa* 4cm. The 2mM silver and gold extract were moderate activity then *Staphylococcus aureus* 2cm, *Pseudomonas aeruginosa* 1cm, *Bacillus subtilis* 10cm, *Kilebsiella pineumoneae* 3cm and *Escherichia coli* 6cm. The antifungal activity of *Aspergillus niger*, *Aspergillus flavus* Small inhibition of synthesis silver and gold nanoparticles. The report recorded indicate that *Ficus macemosa* fruits synthesized silver and gold extract also has excellent potent in antibacterial activity and small zone of inhibition in all extract in antifungal activity.

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INTRODUCTION

India, traditional ayurvedic medicines are still used widely with gold taken as a 'rejuvenator' by millions of people each year. In China, gold was used in the treatment of ailments such as smallpox, skin ulcers and measles. In Japan, thin gold foils placed in tea, sake and food were seen as beneficial to health. Over the years, the gold nanoparticles have become more precious than pretty gold. Most important reason, for this has been their wide uses and applications in the fields of diagnostics, surgery and medicine has had greatest impact in biology & medicine. The Shevaroy's hills range located near Yercaud of Salem district in Tamil Nadu, India. The total extends of Yercaud taluk (Subdistrict) is 382.67 km. including reserve forest. The National Orchidarium and Botanical Garden is situated in Yercaud, it is maintained by Southern Circle of Botanical Survey of India. The area lies between 11° 48' N latitude and 78° 11' E latitude at an altitude ranging from 1200 -1500 m. The maximum temperature ranges between 25°C and 30°C and minimum between 13° and 16°C. The average annual rainfall is around 1750 mm. The highest peak of the hills is 1700 m. The native tribal people of Shervaroy hills are called *Malayali*. The Yercaud in fact, there

are several neighboring habitations in these hill ranges, which are prominently inhabited by the *Malayali*. They are Tamil speaking hill tribes and all are basically depend on agricultural of coffee estate, orange, pepper, pineapple, guava, custard apple, banana, pomegranate ---cultivated and forest resources for their survival. Most of the *Malayali* tribes have a great knowledge of medicinal plants that are used for first aid remedies to treat cough, cold, fever, headache, poisonous bites, cancer and some other human pathogenic organisms. The *Ficus macemosa* is belonging to the family Moraceae. The genus *Ficus* is species of *macemosa*. This is native to the Indian subcontinent. It is unusual in this plant that its figs grow on or close to the tree trunk. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. The plant grows in eastern ghats of Yercaud forests in hills. It is frequently found around the water streams and is also cultivated. Leaves are 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. It is seen dwelling in areas up to 1200 m altitude on hilltop. This requires well-drained, medium to heavy soils for its successful cultivation and comes up in all kinds of soils except in water logged and clay soil. The plant is propagated by using cuttings of stem and root suckers. Seeds can also be used for propagation.

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The flowers are pollinated by very small wasps. It has evergreen leaves, if it is close to a water source. Otherwise it sheds its leaves in January. Thin sticks can be joined by inserting them in gular figs to make interesting shapes. *F. macemosa* can grow over 30 feet tall and 20 to 30 feet wide. The tree is medium to moderate sized deciduous. The rich green foliage provides a good shade. The leaves are dark green, 7-9 cm long, glabrous; receptacles small subglobose or piriform, in large clusters from old nodes of main trunk. The fruits Gular fig or Cluster fig, which is considered sacred, has reddish pink coloured exudates. The fruits receptacles are 5-8 cm in diameter, pyriform, in large clusters, arising from main trunk or large branches. The fruits resemble the figs and are green when raw, turning orange, reddish pink or dark crimson on ripening. Bark is white grey or grayish green, soft surface, uneven and often cracked, 0.5-1.5 cm thick, on rubbing white papery flakes come out from the outer surface, inner surface light brown, fracture fibrous, taste mucilaginous without any characteristic odour. The roots of *F. macemosa* are long, brownish in colour. It's having characteristic odour and slightly bitter in taste. Roots are irregular in shape.

Ficus macemosa L traditionally acclaimed to be very effective in the management of liver diseases. Different parts of tree have been found to possess medicinal properties. Used in the treatment of liver disorders, wounds, diarrhoea, skin diseases haemorrhages and rheumatism. It has been investigated that *familyis* used for anti-inflammatory, anthelmintic, antimicrobial, anti-diarrhoeal, antioxidant and in inflammatory bowel diseases. Ayurveda medicine the roots are useful in treating hydrophobia, and the bark for gynaecological disorders, whereas the fruits are astringent to the bowels, styptic, and useful in the treatment of many diseases including urinary discharges, leprosy and intestinal worms. According to the leaves are astringent to the bowels and useful in treating bronchitis, whereas the fruits are useful in the treatment of a dry cough, loss of voice, diseases of the kidney and spleen, _____ the bark is useful for treating asthma and piles; the latex is applied externally on chronic infected wounds to relieve the pain and assist the healing; and the tender leaf buds are applied to the skin, in the form of a paste, to improve the complexion. In the Indian subcontinent, the figs are traditionally used by the children as playthings. They can be used to join thin sticks together to make interesting shapes. The tree can often be seen standing alone in agricultural areas, being the only species that the loggers do not cut: the wood is not considered useful either for construction or for firewood. The tree is said to be an important food source for many bird and mammal species and serves as a food plant for the caterpillars of the Two-brand Crow butterfly.

MATERIALS AND METHODS

Plant material

The plant *Ficus macemosa*. Linne (Tamil vernacular name – anai, atti, malaiyin munivan, and utumparam) belongs to the family moraceae was at Yearcuad hills of Salem District, Tamil Nadu, Eastern Ghats of India. Herbarium specimens were prepared and taxonomic identification of the plant was confirmed at the Rapinat Herbarium and Centre for Molecular Systematic, Tiruchirappalli, Tamil nadu, India. With the voucher number: PA 001. A voucher specimen of Plant was deposited to that the Rabbinate Herbarium for future reference.

Scientific classification

Kingdom: Plantae
 Division: Magnoliophyta
 Class: Magnoliopsida
 Order: Rosales
 Family: Moraceae
 Genus: *Ficus*
 Species: *F. macemosa* Linn



Fig.1. The habitats of *Ficus macemosa* Linn closely image of plants

Bio-synthesized Silver and Gold Nanoparticles

Chemical

Silver nitrate (AgNO_3), Chloroauric acid (HAuCl_4) was purchased from Sigma-Aldrich Chemical Ltd. (St Louis, MO, USA) and another chemicals AR grade Hi Media, Mumbai, India.

Preparation of plant extracts

The fruits of *Ficus macemosa*. Linne fruits were washed thoroughly thrice with distilled water and were shade dried for 10 days. The fine powder was obtained from the dried plant materials by using kitchen blender. The plant powder was sterilized at 121°C for 15 minutes. 50 g of powder was taken and mixed with 200 mL of Milli Q water and kept in boiling

water bath at 60 °C for 10 minutes. The extracts were filtered with whatman filter paper No. 1. The filtered extract residue was removed by then centrifuged at 500g for 10 min. The supernatant was collected and used for further studies. Double-distilled de-ionized water was used in all experiments.



Fig.3. The image of *Ficus macemosa* fruits in closely

Antimicrobial Property in Bio-synthesized Silver and Gold Nanoparticles

Collection of micro organisms

The microbial strains employed in the biological assays were bacteria strains: *Salmonella typhi* (MTCC 734), *Pseudomonas aeruginosa* (MTCC 2474), *Escherichia coli* (MTCC 119). Fungal Strains: *Aspergillus niger*, *Aspergillus flavus* obtained from Microbial type culture collection (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh, India.

Determination of antibacterial activity by agar well – diffusion assay

For the biosynthesis silver nanoparticles, 1.5 ml of plant extracts is mixed with 30 ml of AgNO_3 solution (1 mM) (2mM) and incubated at 28 °C for 24 hours. Whereas same procedure for gold extract prepared. Small aliquot of solution is used for the procedure is followed Agar well disc diffusion method was performed as follows: Muller –Hinton Agar (MHA) plates were swabbed (sterile cotton swabs) with 8 – 12 hours old broth cultures of the respective bacteria. A sterile

cork borer was used to place four wells, each measuring 8 mm diameter, in each of the plates. About each of 50 mg/ml of different concentrations of the solvent extracts were added into the wells using sterilized dropping micropipettes and allowed for diffusion at room temperature for 2 hours. The plates were incubated at 37 °C for 24 hours. The solvent without extracts served as negative control. Standard antibiotics of DMSO 25 mg/m, ceftriaxone 10mg disc and were used as positive controls. After 24 hours of incubation, diameter of the inhibition zone was recorded in mm. The experiment was repeated thrice and the average values were calculated for antibacterial activity.

Determination of antifungal activity by agar well diffusion method

Antifungal activity for the biosynthesis silver nanoparticles, 1.5 ml of plant extracts is mixed with 30 ml of AgNO_3 solution (1 mM) (2mM) and incubated at 28 °C for 24 hours. Whereas same procedure for gold extract prepared. Small aliquot of solution is used for the procedure is followed Agar well disc diffusion method was performed as follows: Sabouraud dextrose agar (SDA). The Sabouraud Dextrose Agar plates were swabbed (Sterile cotton swabs) with 8 hours old broth culture of the respective fungi. A sterile cork borer was used to place four wells, each measuring 8 mm in diameter, in each of the plates about 1mM, 2mM each of 50 mg/ml of the concentrations of the solvent without extracts served as negative control. Standard antibiotics of DMSO 50 mg/ml were used as positive controls and ceftriaxone 10mg disc. The added into the wells using sterilized dropping micropipettes and allowed for diffusion at room temperature for 2 hours. The plates were incubated at 28 °C for 18 – 24 hours the zone of inhibition was observed respectively. The formed around each disc were measured in mm and recorded.

RESULTS

Green Biosynthesis of Silver and Gold Nanoparticle Using *Ficus macemossa* Fruits Extract

Antibacterial activity of biosynthesized silver nanoparticle in *Ficus macemossa* fruits extracts different concentration of 1mM and 2mM. The *Bacillus subtilis* is highest zone of inhibition 12mm than that against *Staphylococcus aureus* 7mm, *Pseudomonas aeruginosa* 4mm, *Kilebsiella pineumoneae* 5mm and *Escherichia coli* 6mm in fruits silver extract. The small zone of inhibition is moderate activity of *Staphylococcus aureus* and *Escherichia coli*. The low zone of inhibition *Pseudomonas aeruginosa* and *P. aeruginosa* in the samples. Whereas antibacterial activity of biosynthesized gold nanoparticle in *Ficus macemossa* fruits extracts different concentration of 1mM and 2mM. The *Bacillus subtilis* is highest zone of inhibition 10mm against other human pathogenic organisms. The moderate zone of inhibition *Escherichia coli* 6mm. the low zone of inhibition *Staphylococcus aureus* 2mm, *Pseudomonas aeruginosa* 1mm and *Kilebsiella pineumoneae* 3mm in the gold synthesis plant extract. Whereas antifungal activity of synthesis silver fruits extract were carried out the two fungal human pathogens. The *Aspergillus niger* and *Aspergillus flavus* 2mM silver synthesized plant extract is the maximum activity of sample. But 1mM silver synthesized plant extract no activity in other samples.



Fig.3. Bio synthesis of silver nanoparticle in *Ficus macemosa* Linn fruits extract



Fig.4. Bio synthesis of gold nanoparticle in *Ficus macemosa* Linn fruits extract

Table 3. Antibacterial activity of silver synthesized nanoparticle in *Ficus macemosa* Linn fruits extract

S.No.	Microorganism	1mM	2mM	Control	Disc
1	<i>Staphylococcus aureus</i>	7cm	2cm	Nil	Nil
2	<i>Pseudomonas aeruginosa</i>	4mm	1mm	Nil	11mm
3	<i>Bacillus subtilis</i>	12mm	10mm	Nil	1mm
4	<i>Kilebsiella pineumoneae</i>	5mm	3mm	Nil	7mm
5	<i>Escherichia coli</i>	6mm	6mm	Nil	Nil

Table 4. Antifungal activity of silver synthesized nanoparticle in *Ficcus recemosa* Linn fruits extract

S.No.	Microorgism	1mM	2mM	Control	Disc
1	<i>Aspergillus niger</i>	1mm	3mm	Nil	Nil
2	<i>Aspergillus flavus</i>	1mm	4mm	Nil	Nil

Table 5. Antibacterial activity of gold synthesized nanoparticle in *Ficus macemosa* Linn fruits extract

S.No.	Microorganisms	1mM	2mM	Control	Disc
1	<i>Staphylococcus aureus</i>	7mm	2mm	Nil	Nil
2	<i>Pseudomonas aeruginosa</i>	4mm	2mm	Nil	11mm
3	<i>Bacillus subtilis</i>	12mm	9mm	Nil	1mm
4	<i>Kilebsiella pineumoneae</i>	5mm	3mm	Nil	6mm
5	<i>Escherichia coli</i>	6mm	5mm	Nil	Nil

Table 6. Antifungal activity of gold synthesized nanoparticle in *Ficcus recemosa* Linn fruits extract

S.No.	Microorgism	1mM	2mM	Control	Disc
1	<i>Aspergillus niger</i>	1mm	1mm	Nil	Nil
2	<i>Aspergillus flavus</i>	Nil	Nil	Nil	Nil

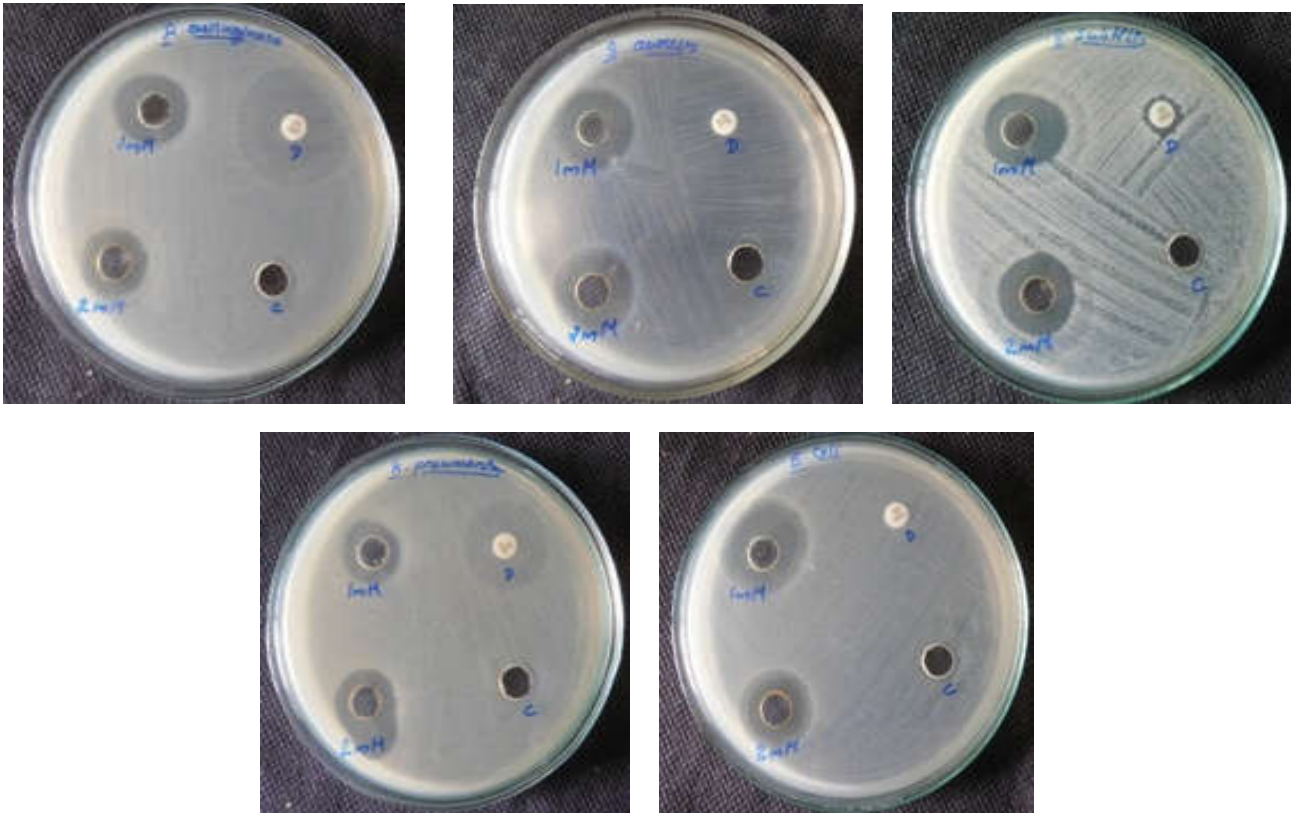


Plate 2 Antibacterial activity of silver synthesized nanoparticle in *Ficus macemosa* Linn fruits extract



Plate. 3 Antifungal activity of silver synthesized nanoparticle in *Ficus macemosa* Linn fruits extract

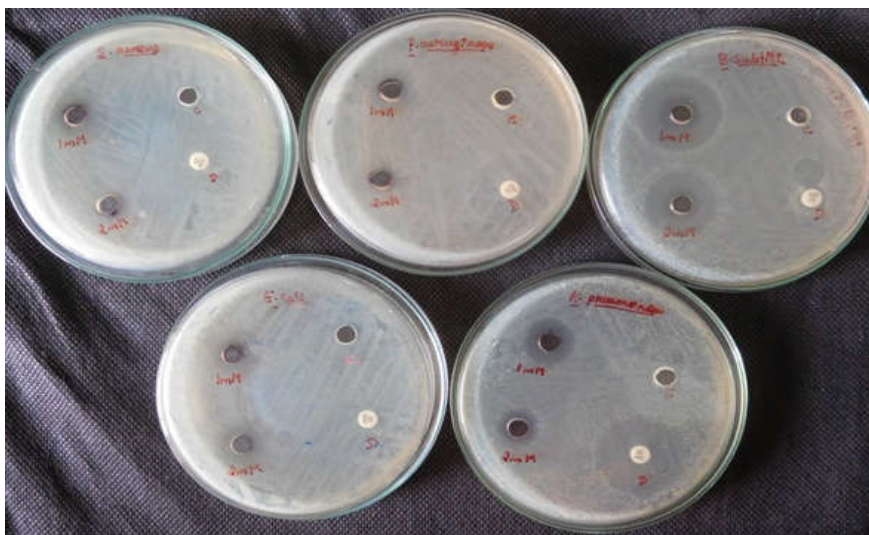


Plate 4 Antibacterial activity of gold synthesized nanoparticle in *Ficus macemosa* . Linn fruits extract



Fig.7. Antifungal activity of gold synthesized nanoparticle in *Ficus macemosa*. Linn fruits extract

Whereas gold synthesized plant fruits extract *Aspergillus niger* 1mM and 2mM low zone of inhibition another human organisms of *Aspergillus flavus* 1mM and 2mM extract is no activity in samples. Brightly, whereas antibacterial activity of biosynthesized gold nanoparticle in *Ficus macemosa* fruits extract different concentration of 1mM and 2mM.

DISCUSSION

Ficus racemosa linn Moraceae all parts of this plant leaves, fruits, bark, latex and sap of the root are medicinally important in the traditional system of medicine in India. The leaves powder and mixed with honey are given in bilious infections. The bark is antiseptic, antipyretic and vermifugal and the decoction of bark are used in the treatment of various skin disease, ulcers and diabetes. It is also used as a poultice in inflammatory swellings of piles, and is regarded to be effective in treatment of piles, dysentery, asthma, gonorrhoea, gleet, leucorrhoea and urinary diseases (Murti *et al.*, 2010). Suggested that the *Ficus religiosa* (*F. religiosa*) tree has a major role in an indigenous structure of medicine like Ayurveda, Siddha, Unani and Homeopathy. The different parts of the tree are commonly used to treat various human diseases such as diabetes, atherosclerosis, Alzheimer's, gastritis, cancer and AIDS (Chandrasekar *et al.*, 2010). Earlier report analysis the antimicrobial activity of the various solvent extracts (Petroleum ether, chloroform, ethanol and aqueous) are screened for both samples of the 16 organisms investigation, streptococcus pneumoniae, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Enterobacter faecalis, Shigella flexneri, Micrococcus species and fungal organisms. *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Microsporium gypseum*, *Trichophyton rubrum* and *Mucor* species are found to be sensitive to life extracts. The fungi organisms are found to be more sensitive than Gram – Positive bacteria and Gram-Negative bacteria. The inhibition is found to be more in ethanol and chloroform extract. This research proves that *Bulphyllum kaitense*. would be the herbal medicine and stem can be used as herbal and scientific medicine throughout in treating microbial infections in humans (Kalaiarasan., 2012). Singh, 2009, our knowledge the usage of plant products in nanobiotechnology has grown tremendously in the recent years. *Ficus religiosa*, is a widely branched with

long-tipped, leathery and heart shaped leaves and purple fruits growing in pairs. It has got mythological, religious and medicinal importance in Indian culture since ancient times. *Ficus racemosa* noticed that the used in Indian folk medicine for the treatment of various diseases/disorders including jaundice, dysentery, diabetes, diarrhea and inflammatory conditions (Ahmed., 2010). Leaves against *Actinomyces viscosus*. The hydro alcoholic extract of *Ficus racemosa* Linn was found effective against *Actinomyces viscosus*. The minimum inhibitory concentration was determined using Broth dilution technique and found to be 0.08mg/ml. The zone of inhibition was measured using cup plate diffusion technique (Tanvir Shaikh *et al.*, 2010). Goyal, 2012 early reported analysis of the antimicrobial effect of *F. racemosa* roots involved a comparison with commercially available antibiotics. We demonstrated the different extract of leaves were tested for antibacterial potential against *E. coli*, *B. pumilis*, *B. subtilis*, *P. aeruginosa* and *S. aureus*. Out of all extracts tested petroleum ether extract was the most effective extract against the tested microorganisms (Mandal *et al.*, 2000). Jagtap supriya *et al.*, 2012 Similar report investigated the antimicrobial activity of *Ficus glomerata* bark extract tested in vitro against two different bacterial species *Bacillus subtilis* and *Escherichia coli* have results of antimicrobial activity revealed that methanolic extract found good activity as compared to petroleum ether. Methanolic extract is more potent towards gram positive bacteria. Obtained that hydro alcoholic extract of leaves was found effective against *Actinomyces viscosus*. The minimum inhibitory to be 0.08mg/ml of concentration was found in *Ficus racemosa* leaves extract (Shaikh *et al.*, 2010). Noticed that the plant possess potent inhibitory activity against six organisms of fungi. *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton soundanense*, *Candida albicans*, *Candida krusei* and *Torulopsis glabrata* (Deraniyagala *et al.*, 1998). Anti-inflammatory evaluation of *Ficus racemosa* leaf extract at a dose of 400 mg/kg exhibited maximum anti-inflammatory effects in the plant extract (Mandal *et al.*, 2000). Rao *et al.*, 2008 The *Ficus glomerata* leaf ethanol fruits extract in the different gastric ulcer models, pylorus ligation, ethanol and cold restraint stress induced ulcers in rats. The extract showed dose depend inhibition of ulcer. Ethanol extract of stem bark found potent wound healing in excised and incised wound model in rat (Biswas, 2003).

Conclusion

These present investigations provide scientific evidence for the traditional of *Ficus macemosa* as well as good antibacterial and antifungal nanoparticle synthesized agents.

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