Recent Antimicrobial and Pharmacological studies in *Ficus religiosa* Linn

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**Abstract**

*Ficus religiosa* L., commonly known as ‘Peepal tree’ is a medicinally important tree species belonging to the Moraceae family. It is considered a sacred tree in India and is respected by followers of many religions. It is extremely popular in indigenous system of medicine like Ayurveda, Siddha, Unani and Homeopathy. Studies have been carried out in the past that validate the antimicrobial property of *Ficus religiosa* and have been documented. Recent pharmacological reports show the potential of this tree as a source of many bio medicinally active compounds/molecules that could be used for future drug synthesis. These reports have focused on investigating the antidiabetic, anticancer, antiulcer, anticonvulsant, antioxidant, nootropic and wound healing properties of different parts of *Ficus religiosa*. While working on antimicrobial property studies with different solvent extracts of various parts of this tree, recent research articles have been surveyed. Most of the recent reports have used Disc Diffusion, Well Diffusion, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bacterial Concentration) methods for determination of antimicrobial potential. The present review compiles findings of all recent studies dealing with both, antibacterial and antifungal properties of *Ficus religiosa*.

**Keywords**

*Ficus religiosa*; antimicrobial; pharmacological; Disc Diffusion; Well Diffusion; MIC; MBC; antibacterial; antifungal.

**Introduction**

*Ficus religiosa* L. is an important medicinal tree species belonging to the family Moraceae. It is commonly known as the Peepal tree and is one of the most revered trees in Asia due to its mythological and traditional background. It is also known as, “the sacred fig tree” or “Bo tree” and is the most planted tree species near religious or spiritual places in Indian cities and villages. It grows up to elevations of 5,000 feet (Starr *et al*., 2003). The specific term ‘religiosa’ is related to the religious significance attached to this tree. The prince “Siddhartha” is believed to have sat and meditated under the Peepal tree and found enlightenment from which time he became “Buddha”. This is probably why many people use its decoction as a brain tonic (Devi *et al*., 2011). The tree is therefore sacred to Buddhists not just in India, but also in other East Asian countries like China, Japan and even Sri Lanka. Scientifically, the enlightenment can be due to the effect
of this tree on the central nervous system (CNS). It is said that the Peepal Tree’s infrared sauna produces radiant energy, which is the same as the sun, but without the harmful ultraviolet rays. Unlike traditional saunas which heat the body indirectly via air or steam, the infrared sauna of the Peepal tree is absorbed directly into the human body and provides a large number of benefits including better circulation and increased energy as the sauna’s radiant energy penetrates deeply into joints, muscles and tissues, speeding oxygen flow, increasing circulation and improving metabolism. (http://www.bodhitreeyJogacentre.ca/Bodhi%20Tree%20Yoga%20Kemptville.swf).

The effect on the CNS is also scientifically studied and is reported as the nootropic (Vinutha et al., 2007; Kaur et al., 2010; Devi et al., 2011), anti-convulsant (Vyawahare et al., 2007; Patil et al., 2011; Singh et al., 2012, 2013) and memory enhancing properties (Devi et al., 2011; Rao et al., 2011) of various parts of the tree. The botanical classification of *Ficus religiosa* (modified from Chandrasekar et al., 2010) is as follows:

<table>
<thead>
<tr>
<th>Domain</th>
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<tr>
<td>Kingdom</td>
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<td><em>Ficus</em> (FY-kus) Linnaeus</td>
</tr>
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<td>Specific epithet</td>
<td><em>religiosa</em> L.</td>
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</table>

*Ficus religiosa* L. is the most popular member of the genus *Ficus*, and is known by more than 150 names (Figure. 1). Even in one language, the tree is referred to in multiple ways across the world. Consider the examples of Sanskrit where it is known by 33 names namely Achyutavas, Ashvatha, Bodhidru, Bodhidruma, Chaityadr, Chaityavriksha, Chaladala, Chalapatra, Devatma, Dhanurvriksha, Gajabhakshaka, Gajapatra, Gajashana, Guhyapushpa, Kapitana, Kesavalaya, Krishnavass, Kshiradruma, Kunjarashana, Mahadruma, Mangalya, Nagabandhu, Pavitraka, Pippala, Seyva, Shrimana, Shubhada, Shuchidruma, Shymala, Vipra, Vishala, Vriksharaja, Yajnika. In Tamil, people call it Aswartham, Achuvattam, Arasu, Ati, Arayal, Asuvattam, Attiru, Attugamani, Ilanai, Kanavam, Kunjarasam, Magadurumam, Mere, Narayanan, Padorogam, Panai, Pittalam, Saladalam, Saranam, Suvalai, Tanavam, Tiru or Vanagandi. And even in French, it has around 6 names that are Allemaron, Aoa, Arbre des conseils, Arbre du diable, Arbre des pagodes, Figueur des pagodes (Kirtikar and Basu, 1993; Panda, 2005; Kunwar and Bussmann, 2006; Pullaiah, 2006; Khare, 2007; Singh et al., 2011).

The present review has been undertaken during the antimicrobial activity studies conducted and the literature survey carried out with different solvent extracts of *Ficus religiosa* using both *in vivo* plant parts and *in vitro* regenerated tissues. There are numerous pharmacological, phytochemical and ethnomedicinal studies carried out on this tree and many recent reviews have mentioned these studies (Chandrasekar et al., 2010; Makhija et al., 2010; Singh et al., 2011; Panchavat, 2012). Recently, many reports have been published on the antimicrobial activity of this medicinally
important tree species (Ramakrishnaiah and Hariprasad, 2013; Supriya and Harshita, 2013; Tambekar et al., 2013; Salem et al., 2013; Manimozhi et al., 2012; Rajiv and Sivaraj, 2012). We have done a thorough literature survey of these studies that have been published till date. There is a noticeable change in the area of research in this field since last two to three years. More attention is being drawn to the various antimicrobial properties of the tree, compared with pharmacological studies using animal models that used to be the main focus till then.

Pharmacological studies for different diseases

Since time immemorial, different parts of this tree like bark, leaves, fruits etc. have been used as medicine by human and animals alike. It has proven to be a cure for over more than 50 diseases and more than 65 pharmacological studies have been reported. The therapeutic utilities of F. religiosa have been indicated in traditional systems of medicine like Ayurveda, Unani, Siddha, etc. (Singh et al., 2011). A large number of reports have been published till date dealing with the pharmacological studies done on animal models using different extracts of various parts of the medicinally important tree species. These include Ficus religiosa as a source of bioactive molecules that have antidiabetic properties (Elavarasi et al., 2013; Khan et al., 2012; Shukla et al., 2012; Verma et al., 2012; Choudhary et al., 2011; Pandit et al., 2010; Kirana et al., 2009; Deshmukh et al., 2007; Ambika and Rao, 1967; Brahmacari and Augusti, 1962). Also for the first time an antidiabetic biomolecule named stigmasterol was isolated from Ficus religiosa leaves (Vedulla et al., 2013). In the similar way, research is being carried out on the isolation of other bioactive molecules active against different diseases, which can, in future, be used as constituents of drugs. Different parts of the tree also are known to promote wound healing (Bairy et al., 2011; Murthi et al., 2011; Charde et al., 2010; Jain et al., 2009; Roy et al., 2009; Nayem et al., 2009; Choudhary et al., 2006). Ficus religiosa was also found to have anticancer (Gulecha et al., 2011; Lansky et al., 2008; Mousa et al., 1994), antiulcer (Gregory et al., 2013; Thorat et al., 2013; Bairy et al., 2011; Khan et al., 2011; Saha et al., 2010), anti-asthmatic (Kapoor et al., 2011), dermatoprotective (Waheed et al., 2013), hepatoprotective (Chourasia et al., 2013), antihelmenthic (Iqbal et al., 2001) and antioxidant properties (Yadav et al., 2011; Gupta et al., 2011; Pandit et al., 2010; Preethi et al., 2010; Kirana et al., 2009; Sultana et al., 2009; Sreeleekshmi et al., 2007; Sharma and Gupta, 2007).

Presently, there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases (Makhija et al., 2010). Preliminary phytochemical analysis of various parts of Ficus religiosa have shown the presence of a large number of phytochemicals including tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (Babu et al., 2010; Jiwala et al., 2008). Its bark showed the presence of bergapten, bergaphtol, lanosterol, ß-sitosterol, stigmasterol, leucoanthocyanin, leucoanthocyanidin etc. (Swami and Bisht, 1996; Swami et al., 1989; Ambike and Rao, 1967; Hussain et al., 1992). Leaves were found to yield campesterol, stigmasterol, isofucosterol etc (Panda et al., 1976; Verma and Bhatia, 1986; Behari
et al., 1984). The fruits were found to be rich in amino acids like asparagines and tyrosine (Grison et al., 2002). Alanine, threonine, tyrosine have been reported in seeds of *F. religiosa* (Ali and Qadry, 1987). The crude latex showed the presence of a serine protease, named “religiosin”, an acidic protein which plays an important role in cheese production and has detergent activity (Kumari et al., 2010).

Figure. 2 shows the popularity of *Ficus religiosa* on the basis of published papers upto the year 2006 (http://www.newcrops.uq.edu.au/listing/species_pages_F/Ficus_religiosa.htm).

**Antibacterial and antifungal activities**

Medicinal plants are rich source of a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids. These phytochemicals are responsible for the numerous pharmacological and antimicrobial activities shown by the plant. There is found to be an increasing antibiotic resistance exhibited by various pathogens to commonly used synthetic antibiotics. This has led to the search and screening of several medicinal plants for their potential antimicrobial activity so as to overcome various diseases caused by these resistant pathogens. Consider the example of diarrhoea which is a common cause of morbidity and mortality among infants and children in developing countries. Most of the intestinal flora of humans is non-pathogenic. However, certain strains like Enterotoxigenic *E. coli* (ETEC) are highly pathogenic. ETEC is the most prevalent among the various types of diarrhoeagenic *E. coli* in India (Uma et al., 2009). Hence, there is a great search for medicinal plants which contain various natural drug molecules that have an inhibitory effect on the growth of such pathogens.

Owing to some of these bioactive molecules, various parts of *Ficus religiosa* are found to exhibit antimicrobial activity both against various species of bacteria and fungi. A large number of studies dealing with the microbial assay (which measures the activity of antibiotics that is the extent of ability to inhibit the growth of microorganism) have been done and numerous reports have been published till date (Table. 1).

Ramakrishnaiah and Hariprasad (2013) investigated the antimicrobial activity of *Ficus religiosa* by measuring the zone of inhibition (ZoI) produced by two types of solvent extracts namely methanol and diethyl ether extracts of bark and leaves, on three bacteria (two Gram negative bacteria (*E.coli* and *Pseudomonas aeruginosa*), one Gram positive bacteria (*Staphylococcus aureus*) and one fungus (*Aspergillus niger*). The different concentrations of methanol and diethyl ether extractions (100, 200, 300 and 400 mg/ml) of both bark and leaves of *F.religiosa* were used for the assay. ‘Disc diffusion method’ was used to carry out the assay. The methanol extracts of leaves and bark showed antimicrobial activity against three bacteria. At lower concentrations methanol extracts showed less antimicrobial activity and showed higher activity at 400 mg/ml concentration against the three tested bacteria. Both leaf and bark methanol extracts gave ZoI 2.8 and 2.2mm respectively in *S.aureus* and 2.4 and 1.8mm respectively in *E.coli*. *P.aeruginosa* gave a small ZoI (2.2 and 1.1mm) in methanol extracts of leaves and bark. But at lower concentrations no activity was observed whereas at higher concentrations (40mg/ml) very less
activity was observed against fungi (A.niger). The diethyl ether extracts of leaves and bark showed varied antimicrobial activity against tested three bacteria and no activity against on A.niger in all concentrations. In case of diethyl ether extract of bark E.coli ZoI was 1.0 and 0.9mm and S. aureus it was 1.4 and 1.2mm, bacteria showed inhibition and no activity on P. aeruginosa and A.niger. Diethyl ether extracts of leaves showed inhibitory activity on three tested bacteria and no activity on A.niger. The above results indicated that the diethyl ether extract of leaves and barks had no activity on A.niger in all tested concentrations. The results showed that the methanol extracts of leaves and bark extracts of Ficus religiosa had considerable inhibition activity on tested bacteria than diethyl ether extracts of leaves and bark of the Ficus religiosa.

Another study was carried out by Supriya and Harshita (2013) in which extracts of dried powdered leaves of Ficus religiosa in petroleum ether, chloroform, methanol and water was made. These extracts were then subjected for in vivo antimicrobial activity against E.coli and S.aureus by cup plate diffusion technique in which wells were bore in the agar plates that were flooded with the bacterial culture and the extract was filled into these wells. Different dilutions of the four extracts were made and the dried extracts were dissolved in 5% DMSO to a concentration of 200 mg/ml. Chloramphenicol (30 mcg/disc) was used as standard antibiotic. The plates were incubated and the antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (Zone Size Interpretative Scale). It was found that chloroform extract showed good activity giving a zone of 16 mm with E.coli and 16 mm with S.aureus which is more compared to methanol and water extracts. The petroleum ether extract did not show any activity (Supriya and Harshita, 2013).

Salem and his colleagues reviewed the different antimicrobial activities and phytochemical composition of extracts of Ficus spp. To study the antimicrobial activities, different antimicrobial methods such as disc and well diffusion, minimum inhibitory concentration (MIC), minimum bacterial concentrations (MBC) were used for the evaluation of different extracts. This review gives the idea of which solvent works potentially against different pathogenic microorganisms. Aqueous extracts showed high antimicrobial activity against B.subtilis and multi drug resistant P.aeruginosa. Ethanolic leaves extract was successful to inhibit wide range of microorganisms which mainly includes B.subtilis, S.aureus, P.aeruginosa, E.coli but failed to show any action against C.albicans and A.niger. The fruit extracts have high potential towards antibacterial activity but no antifungal activity. The 70% (v/v) Aqueous-ethanolic extracts was successful to inhibit Helicobactor pyroli completely at the concentration 500 µg/ml in all strains. Chloroform extracts showed high antibacterial activity against infectious S.typhi, S.typhimurium, P.vulgaris at the concentration 20 µg/ml. Different extracts of bark (MeOH, Aqueous, Chloroform) showed maximum inhibitory activity against 3 potential enteroxigenic E.coli which were isolated from the patients with diarrhoea, but bark extracts with solvent system such as acetone, methanol, ethyl acetate showed moderate antibacterial activity against P. aeruginosa, E. coli, P. vulgaris , B. subtilis and S. aureus (Salem et al., 2013).
Tambekar and his colleague carried out the studies on antimicrobial potential and phytochemical analysis of medicinal plants from Lonar Lake. Lonar lake is situated in Buldhana district in Maharashtra (India) which is surrounded by dense forest of many medicinally important plants including Syzigium cumini, Ficus bengalensis, Cynodon dactylon, Justicia adhatoda, Calotropis gigantea, Ficus racemosa, Tinospora cardifolia, Achyranthes aspera, Ficus religiosa, Santalum ovatum and Ziziphus oenoplia which have shown antibacterial activity against some enteric bacteria Escherichia coli, Staphylococcus aureus, Salmonella typhi, Salmonella enterica, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa and Klebsiella pneumonia. Because of high alkalinity and diverse atmospheric conditions Lonar lake was selected to study antibacterial potential and phytochemical analysis of these medicinal plants. The antibacterial study was carried out by agar disc diffusion method. Leaves extracts were prepared with water, acetone, ethanol, and methanol. In case of Ficus religiosa aqueous and ethanolic leaves extracts showed antibacterial activity against E.coli and P.vulgaris (Tambekar et al., 2013).

Rajiv and Sivaraj studied different parts of the Ficus religiosa mainly bark, fruit, leaves, stem by making aqueous extracts. The aqueous extracts of selected parts were used to screen for their phytochemical and antimicrobial activity and showed presence of many important phytochemicals such as alkaloids, phenols, sugar, terpenoids, glycosides, flavonoids and tannins. For antimicrobial activity disc diffusion method was used to screen many common pathogens such as E.coli, P.aeruginosa, Aeromonas hydrophila, S.aureus, S. pyogenes, A. niger, Candida albicans with different concentrations. Results obtained showed that methanol has higher activity than chloroform and aqueous extract. Hexane extract did not show any antibacterial activity. Ethanolic leaves extracts have more antibacterial activity with less antifungal activity. This work also showed that 70% percent aqueous-ethanol extracts are very sensitive to Helicobacter pyroli at 500 µg/ml in all strains. The chloroform extracts were found to be successful to inhibit the growth of S.typhi, S. typhimurium and P. vulgaris at different concentrations. Aqueous extracts showed good antimicrobial activity against food borne pathogens with high activity on B.subtilis with 24 mm inhibition zone (Rajiv and Sivaraj, 2012).

The acetone, methanol and the ethyl acetate extracts of the bark powder of Ficus religiosa were checked for antibacterial activity against some medically important bacteria Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Bacillus subtilis and Staphylococcus aureus. The antimicrobial assay was performed by agar disc diffusion assay. It was observed that methanol extracts had activity against B.subtilis, E.coli, P.vulgaris, S.aureus whereas acetone extracts showed more antibacterial activity only against B.subtilis, E.coli. The ethyl acetate extracts were found to have no activity against any of the tested bacteria (Manimozhi et al., 2012).

A lot of research has been done on the antimicrobial and antiviral properties of various parts of Ficus religiosa since 1994. In the past, several studies have been carried out that validated the antimicrobial and antiviral potential of F.religiosa. These studies up to 2010 have
been compiled and reviewed earlier (Singh et al., 2011). Only the very recent reports have been mentioned above in the present review.

Older reports include the investigation of the inhibitory effect of aqueous, ethanol and methanolic extracts of leaves against four bacterial species and it was found that the aqueous extract had maximum effect followed by methanolic and ethanolic extract (Preethi et al., 2010). 70% ethanolic bark extract was tested against different *H.pylori* strains that were obtained from gastric antral biopsy of infected patients (Zaidi et al., 2009). Uma and Prabhakar (2009) investigated the antimicrobial activity of aqueous, methanol, chloroform, petroleum ether extracts of the bark of *Ficus religiosa* ‘enterotoxigenic’ *E. coli*, which is a highly pathogenic strain of bacteria found in the human intestine that is responsible for causing diarrhoea in humans. The inhibitory effect of the extracts was checked against three enterotoxigenic *E. coli*, which were isolated from patients suffering from diarrhoea. The chloroform, methanol and water extracts of leaves were observed to have activity against a range of bacteria and fungus namely *S.typhi*, *S.typhimurium*, *P.vulgaris*, *K.pneumoniae*, *P.aeroginosa*, *A.niger*, *P.chrysogenum* using methanol extracts, *S.typhi*, *P.aeroginosa*, *K.pneumoniae*, *P.vulgaris*, *A.niger*, *P.chrysogenum* using chloroform extracts and *S.typhi*, *A.niger*, *P.chrysogenum* using water extracts. The method of well diffusion was used for the assay (Hemaiswarya et al., 2009). Aqil and Ahmad (2007) investigated the activity of 70% ethanol extracts of leaf against nine bacterial species and found an inhibitory effect against six of those strains including *S.aureus* and *E.coli*. Nair and Chanda (2007) conducted studies and found that the aqueous and ethanol extract of bark had activity against *P.mirabilis*, *S.aureus*, *A.fecalis* and *S. typhimurium*. The same team also conducted studies in 2006 to show the activity of the ethanolic leaf extract against two other strains namely *B.cereus* and *S. agalactiae*.The activity of chloroform extract of fruits was investigated against *P.aureus*, *A.chroococcum*, *K.pneumonia*, *S.lactic* and *B.megaterium* (Mousa et al., 1994). The activity of aqueous and methanol extracts of bark was shown against HIV-1 strain (Kusumoto et al., 1995). Swami and Bisht (1996) proved that the furanocoumarins (bergapten and bergaptole) isolated from the bark of *F.religiosa* had activity against *S.aureus*, *E.coli*, *Penicillium glaucum* and even a protozoan namely *Paramecium*.

Figure.3 shows the preliminary results obtained in this laboratory against *E.coli* strain.

With this review, it has been found out that assay methods namely disc and well diffusion, MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bacterial Concentration) were used to study the antimicrobial activity of the various extracts from this tree. The findings and results from them have been illustrated here. Ethnopharmacology and natural product drug discovery remains a significant hope in the current scenario and the ethnopharmacology knowledge which is supported by an experimental base can serve as an innovative discovery system for affordable, safer and newer drugs (Patwardhan, 2005). With all the studies and reports made regarding *Ficus religiosa*, it can be rightly concluded that the numerous ethnomedicinal properties...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Part used</th>
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<th>Effective Conc. of extract</th>
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<td>$P._{vulgaris}$</td>
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<td>Acetone</td>
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<td>$B.<em>{subtilis}$, $E.</em>{coli}$</td>
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<td>Methanol</td>
<td>100 µg/ml</td>
<td>Disc Diffusion</td>
<td>$B.<em>{subtilis}$, $E.</em>{coli}$, $P.<em>{vulgaris}$, $S.</em>{aureus}$</td>
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|                               |                      | Ethyl Acetate     | --                        |                 | $S._{aureus}$, $S._{pyogens}$, $E._{coli}$,
<p>|                               |                      |                   |                           |                 | $A.<em>{hydophilica}$, $E.</em>{aerogens}$, $P.<em>{aeroginosa}$ |
| Rajiv and Sivaraj 2012        | Bark, Fruit, Leaves, Stem | Water           | 100 mg/ml                 | Disc Diffusion  | $S.</em>{aureus}$, $S.<em>{pyogens}$, $E.</em>{coli}$, $A.<em>{hydophilica}$, $E.</em>{aerogens}$, $P.<em>{aeroginosa}$ |
| Preethi et al. 2010           | Leaves               | Water, Ethanol, Methanol | Not specified | Disc Diffusion  | $P.</em>{aeroginosa}$, $B.<em>{subtilis}$, $E.</em>{coli}$, $S.<em>{typhi}$ |
| Zaidi et al. 2009             | Bark                 | 70% Ethanol       | 125- 250 µg/ml           | Pylori agar plates | $H.</em>{pyroli}$ |
| Uma et al. 2009               | Bark                 | Methanol, Water, Chloroform | 200 mg/ml      | Disc diffusion  | Three enteroxigenic $E.<em>{coli}$ |
| Hemaiswarya et al. 2009       | Leaves               | Chloroform        | Not specified             |                 | $S.</em>{typhi}$, $S.<em>{typhimurium}$, $P.</em>{vulgaris}$, $K.<em>{pnemoniae}$, $P.</em>{aeroginosa}$, $A.<em>{niger}$, $P.</em>{chrysogenum}$ |</p>
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<th>Concentration</th>
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<td>Aqil and Ahmad 2003</td>
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Figure 1 A) *Ficus religiosa* tree trunk with twigs appearing from it  
B) *Ficus religiosa* tree trunk with adventitious roots
Figure 2: Popularity of Ficus religiosa: showing papers published during the period from 1920s to 2006.


**Figure 3** *Ficus religiosa* antimicrobial studies with bark and leaf extract using Disc Diffusion and Well Diffusion Methods

A) Zone of inhibition of standard antibiotic (1) and methanol bark extract (2) against *E.coli* by Disc Diffusion method.

B) Zone of inhibition of standard antibiotic (1), methanol extract of bark (2) and leaf (4) against *E.coli* by Well Diffusion method
along with the antimicrobial activity which has been proven, shows that this tree has tremendous potential for isolating strong antimicrobial compounds or biomolecules which can be used in the near future in the area of drug synthesis.

For future studies, aqueous extracts should be used primarily for the above studies for antimicrobial activity against pathogenic microbes, so that direct bioactive antimicrobial molecules could be isolated and will be used for making antimicrobial drugs or ointments for patients suffering with these pathogen attacks.

Acknowledgement

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