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Antimicrobial activity of phytochemicals from *Ficus religiosa*

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ABSTRACT

The present study focuses on the antimicrobial activity of plant extracts, which have been used since ancient times in India for treating various diseases. This experiment was performed to determine the antimicrobial effect of aqueous and alcoholic extracts of different parts of the peepal tree against the gram-positive culture of Staphylococcus aureus and gram-negative cultures of Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae by disc diffusion method and broth technique. The disc diffusion technique using alcoholic extract of bark showed inhibition to the pathogen Pseudomonas aeruginosa. It was found that alcoholic extract of bark showed highest antibacterial activity followed by alcoholic extract of leaves.

Keywords: *Ficus religiosa*, phytochemicals, aqueous extracts, alcohol extracts, antimicrobial.

INTRODUCTION

Since ancient times plant-derived herbal extracts have been used in medicine because of their ability to treat various diseases. Phytochemicals are compounds that are produced by plants (phyto means "plant") and these substances have biological activity which make them effective against various microbial infections (Mendoza and Silva, 2018). Therefore, these chemical compounds which are present in the plants may also provide desirable health benefits due to their natural antimicrobial property. These compounds kill or slow the spread of microorganisms like bacteria, viruses, fungi and protozoans. *Ficus religiosa* commonly known as 'peepal tree' belongs to the family Moraceae, is an important and popular medicinal plant in the indigenous systems of Ayurveda, Siddha and Unani (Chavan *et al.*, 2019). It is traditionally used as antiulcer, antibacterial, antidiabetic, in the treatment of gonorrhea and various skin diseases (Chandrasekar *et al.*, 2010). Peepal tree is known to have a long-life span indicating that there are phytochemicals present in it which gives it resistance against many microbial infections.

The broad–spectrum antibacterial and antifungal properties of ethanolic extracts of 22 traditionally used Indian medicinal plants were studied against seven bacteria and five filamentous fungi, where of the 22 plants tested, the leaves of *Ficus religiosa* demonstrated highest antibacterial activity and to a lesser extent antifungal activity. And, it was found that ethanolic extracts of *Ficus religiosa* leaves (25 mg/ml) was found effective against four bacteria- *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 9027) but comparatively less effective against two fungi- *Candida albicans* (IMI 349010), *Aspergillus niger* (IMI 076837). An interesting study showed that 70% aqueous- ethanol extracts of *Ficus religiosa* completely inhibited the growth of *Helicobacter pylori* at 500 microgram/ml (Sonawane *et al.*, 2015). Also, a study on the antimicrobial activity of diethyl ether and methanol extracts of barks and leaves against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Aspergillus niger* showed that methanolic extracts of bark and leaves from the peepal tree exhibited more antimicrobial activity than diethyl ether extracts (Ramakrishnaiah and Hariprasad, 2012). Therefore in the present study, *Ficus religiosa* (Peepal tree) was selected and different parts of the peepal tree along with a medicinal herbal peepal tree oil were used for testing the antimicrobial activity.

MATERIAL AND METHODS

1) ***Test Sample:*** Different parts of peepal tree (aerial roots, fruits, leaves, bark), traditional herbal peepal tree oil.
2) ***Cultures:*** Overnight culture suspensions of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*
3) ***Media:*** Sterile Nutrient broth tubes, Sterile Nutrient Agar slants, Sterile Nutrient agar plates.
4) *Glasswares:* Sterile 10 ml pipette, Sterile 1 ml pipette, sterile dilution tubes, sterile suspension tubes.
5) ***Miscellaneous:*** Sterile distilled water, 99% ethanol, sterile saline, colorimeter, sterile filter paper, sterile conical funnel, analytical balance, sterile mortar and pestle, alcohol jar, sterile filter paper disc, sterile cotton swab.

2.1) Preparation of extracts from different parts of the peepal tree-

- Grind all the required parts of the peepal tree thoroughly using mortar & pestle one by one.
- Weigh 500 mg of the grounded powder in an analytical balance.
- For preparation of alcoholic extracts, add 10 ml of 99% ethanol in the 250 mg of powder and filter in a test tube.
- For preparation of aqueous extracts, add 10 ml of distilled water in the 250 mg of powder and filter in a test tube.

2.2) Preparation of medicinal herbal peepal tree oil:

Medicinal herbal peepal tree oil made using a traditional method from the bark of the peepal tree.

2.3) For agar disc diffusion method:

- Swab the fourtest culture suspension on sterile nutrient agar plate.
- Place sterile filter paper discs soaked with the respective extracts on the plates with the help of sterile forceps.
- Incubate for 24 hours at 37º C.
- Observe for zone of inhibition and note down the results.

2.4) For Broth technique:

- Take sterile nutrient broth tubes (10 ml) + 0.1 ml culture suspension + 0.5 ml extract
- Incubate for 24 hours at 37º C.
- The turbidity is observed visually and spectrophotometrically at 600nm.

RESULTS

The alcoholic and aqueous extracts of different parts of the peepal tree were made. As seen in figure 1, these extracts had different colours. This difference in colour indicates that different phytochemicals, which are present in various parts of the peepal tree were extracted in different types of solvents.

Agar disc diffusion method:

The extracts had a higher antibacterial effect against *Pseudomonas aeruginosa* and Staphylococcus *aureus* as compared to all the cultures. *Pseudomonas aeruginosa* (Figure 2, A) and *Staphylococcus aureus* (Figure 2, D) exhibited a zone of inhibition with the medicinal herbal peepal tree oil also. For *Pseudomonas aeruginosa* (Figure 2, A) alcoholic extracts of fruits, leaves and bark showed a bigger zone of inhibition followed by alcoholic extract of roots. For *Escherichia coli* (Figure 2, C) alcoholic extracts of leaves and roots showed higher zone of inhibition

followed by alcoholic extracts of bark. For *Klebsiella pneumoniae* (Figure 2, B) alcoholic extract of bark showed more inhibition followed by alcoholic extract of leaves and roots. For *Staphylococcus aureus* (Figure 2, E) alcoholic extracts of leaves and bark showed more inhibition followed by alcoholic extract of fruits.

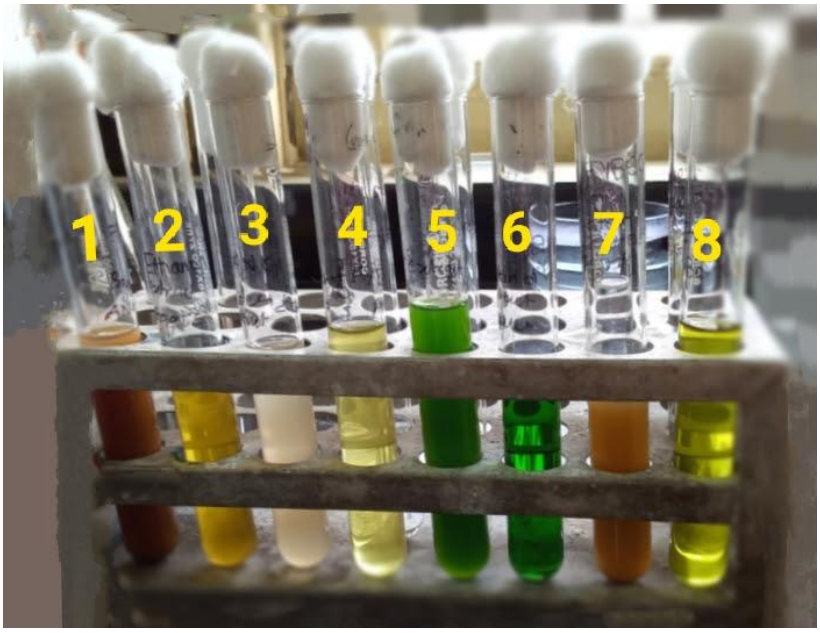


Figure 1: Alcoholic and aqueous extracts of different parts of peepal tree. Tube 1-aqueous extract of roots, Tube 2-alcoholic extract of roots, Tube 3-aqueous extract of fruits, Tube 4-alcoholic extract of fruits, Tube 5-aqueous extract of leaves, Tube 6-alcoholic extract of leaves, Tube 7-aqueous extract of bark, Tube 8-alcoholic extract of bark.

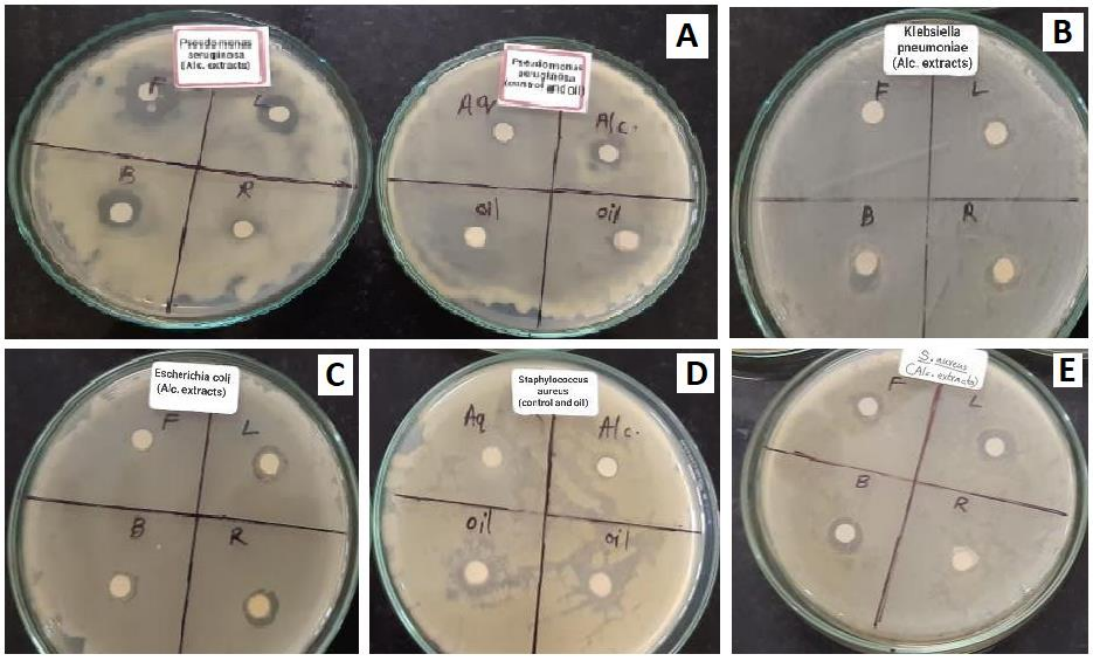
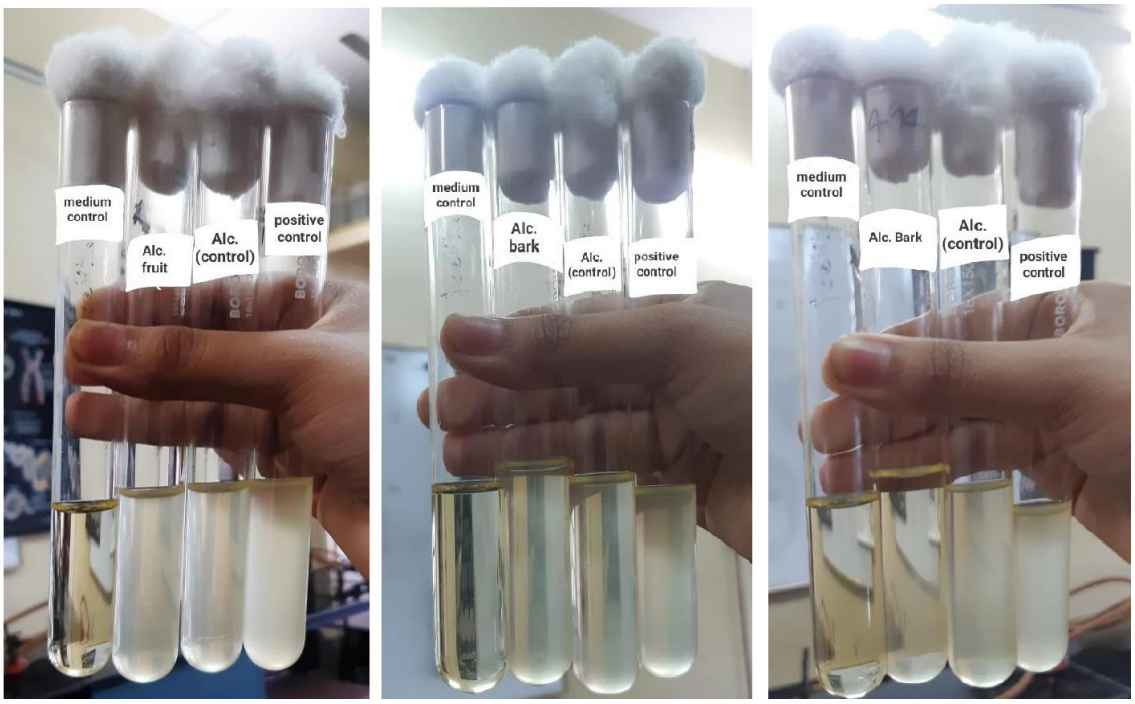


Figure 2: Effect of alcoholic extracts of different parts of peepal tree on **(A)** *Pseudomonas aeruginosa,* **(B)** *Klebsiella pneumonia,* **(C)** *Escherichia coli,* **(D**) and **(E**) *Staphylococcus aureus* by"agar disc diffusion method".



Escherichia coli ***Klebsiella pneumoniae*** ***Staphylococcus aureus***

Figure 3: Effect of alcoholic extracts of different parts of peepal tree on *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* by "broth technique"

Table 1: Comparison of results by broth technique and disc diffusion method

Cultures	Results	Alc. extracts				Oil	Controls	
		Fruits	Leaves	Bark	Roots		Alc.	Positive
Escherichia coli	Absorbance	0.10	0.12	0.08	0.09	0.13	0.10	0.26
	Zone of inhibition	+	+++	++	+++	+	+	-
Klebsiella pneumoniae	Absorbance	0.11	0.09	0.11	0.11	0.15	0.03	0.11
	Zone of inhibition	+	++	+++	++	+	+	-
Staphylococcus aureus	Absorbance	0.08	0.04	0.01	0.06	0.05	0.06	0.12
	Zone of inhibition	++	+++	+++	+	+++	+	-

Key: "+" indicates diameter of zone of inhibition
"-" indicates no zone of inhibition
The results observed for disc diffusion technique were similar to that obtained for broth technique, suggesting the accuracy of the techniques used.

c) Broth technique

Visual turbidity and absorbance of the culture broth was taken as an indication of inhibition by the extracts. A lesser turbidity or absorbance indicated that the extract inhibited bacterial growth. For *Escherichia coli* the absorbance value was found less for alcoholic extract of roots compared to alcoholic control. As seen in figure 3 for *Staphylococcus aureus*, the alcoholic extract of bark showed less turbidity than the alcoholic control and the absorbance was found less for the alcoholic extract of bark followed by alcoholic extract of leaves followed by medicinal herbal peepal tree oil. The results clearly

indicate the inhibition of growth of bacteria by the extracts.

CONCLUSION

Both alcoholic and aqueous extracts of different parts of the peepal tree exhibited zones of inhibition using disc diffusion technique against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Alcoholic extracts gave more antibacterial activity than aqueous extracts and the highest antibacterial activity was observed with alcoholic extract of bark followed by alcoholic extract of leaves. Higher inhibition was observed with alcoholic extract of bark against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus,* whereas *Klebsiella pneumoniae* and *Escherichia coli* were not inhibited to large extent. Ayurvedic peepal tree oil was also effective to some extent against *Escherichia coli* and *Klebsiella pneumoniae* whereas it showed higher effectiveness against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Furthermore, combinations of aqueous and alcoholic extracts of various parts of the peepal tree, like roots, fruits, branches, leaves and bark may be done, as each part of the plant contains different phytochemicals, so it can be found out which combination is more effective. Such type of effect is called "Synergistic effect".

There is a worldwide increase in the rate of antibiotic resistance in *Pseudomonas aeruginosa* making it a multidrug-resistant pathogen of global concern. Therefore, in these cases, the use of these phytochemicals which are obtained from different parts of the peepal tree can be used for treating infections caused by these multi-drug resistant *Pseudomonas aeruginosa* and also other skin pathogens like *Staphylococcus aureus*. One of the major advantages of using these phytochemicals as medicine is that they can be used in remote parts of developing countries with few health facilities and it also has lesser side effects compared to other allopathic medicines.

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