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Ehretia laevis leaves: Potential herbal remedy for mouth microflora

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The advantages and uses of folklore herbs have been acclaimed and executed from ancient times in India. The use of these Folklore remedial herbs for therapeutic applications is contributing to maintenance of human health. The ancient literature, i.e., Ayurveda and Unani, also describes the global usage of herbal medicine for treatment, and its formulation's concoction for prevention of various diseases. *Ehretia laevis* is a conventional therapeutic herb from ancient times, frequently designated as Khandu Chakka by local people in Maharashtra. *E. laevis* leaves are used in the treatment of skin infections, fungal infections, mouth blisters, eczema, cuts and wounds, diabetes, asthma, fever and joint pain etc. The leaf of this plant contains abundant therapeutically beneficial secondary metabolites besides primary metabolites.

This paper describes antimicrobial sensitivity of *E. laevis* leaf (fresh and dried) acetone and ethyl alcohol (95%) soxhlet extract and dried leaf dimethyl sulphoxide (DMSO) extract (prepared after from evaporation of acetone in dried leaf acetone extract) against isolated oral microbial flora i.e., *Streptococcus* spp*, Staphylococcus aureus* and *Candida* spp*.* Amoxicillin in dimethyl sulphoxide was also tested for antimicrobial sensitivity. The result revealed that *E. laevis* fresh leaf ethyl alcohol and acetone extract and dried leaf acetone and DMSO extract was efficacious against isolated oral microbial flora. The extracts showed positive results for flavonoids and tannins. The results showed the antibacterial and antifungal potential of this folklore plant, particularly against *S. aureus* and *Candida* spp*.,* which are microorganisms that are becoming resistant against most therapeutic drugs. This use of this folklore herb requires further study on pharmacological drug formulations. And it can also be used in herbal products i.e., toothpastes, mouthwash etc.

Keywords: Antimicrobial activity, *Candida* spp*, Ehretia laevis*, Phytochemical*, Streptococcus* spp*, Staphylococcus aureus*
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For thousands of years folklore therapies have been comprehensive healing preventive traditional system used for nurturing the health of human beings[1]. In India, conventional remedial treatment is the basis of several methodologies i.e., Ayurveda, Siddha and Unani[2]. To encourage the correct use of herbal therapy, and to find out their prospective for sources of new medicine, it is crucial to investigate medicinal plants which have folklore recognition in a more intensified way[3-8].

Ehretia laevis is a medium height flourishing herb belonging to the family Boraginaceous that includes approximately 150 species[9]. This herb is primarily dispersed all around tropical and subtropical regions of Asia, Africa and Australia[10]. This plant is used from ancient period and has many medicinally useful chemicals components[11]. In India, in Wardha taluka (District Maharashtra), *Ehretia laevis* Roxb. herb is used by tribals to heal injuries and fractures, often known as Khandu Chakka[11]. In ancient literature of Ayurveda and Unani, this herb is reported for its medicinal value to treat respiratory ailment, as well as to treat jaundice, ulcers, liver diseases, diabetes mellitus, and microbial infections i.e., syphilis, toothache, stomach and venereal diseases[12]. Li *et al*. reported the presence of phenolic acids, flavonoids, triterpenoids, steroids etc. in the genus *Ehretia* with antimicrobial, antidiabetic and anti-inflammatory activities[13].

Salivary microorganisms are mainly responsible for oral health problems i.e., caries[14], which disturbs the normal microflora in the mouth cavities[15]. In the remote areas of Pakistan and Rajasthan, *E. lavies* are used for dental caries and mouth ulcers, respectively,[16,17]. There are reports regarding antimicrobial activity of *E. lavies* extracts against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Escherichia coli* using ethanol, chloroform and water-based solvents[18-21]. In one of the studies on *E. laevis* leaves, acetone extract was studied against *P. aeruginosa, E. coli and S. aureus*[22,23]. It is reported that the methanolic and

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ethanolic extract of *E. laevis* inhibits the microflora of saliva[24].

In the present study, *E. laevis* leaf (fresh and dried) acetone, ethyl alcohol (95%) soxhlet extract, dried leaf dimethyl sulphoxide extract, amoxicillin dimethyl sulphoxide extract was tested against isolated and selected oral microflora *Streptococcus* spp, *Staphylococcus aureus* and *Candida spp*. The qualitative analysis of flavonoids and tannins was done. The antimicrobial sensitivity was compared with each other.

Material and Methods

Microorganisms

Streptococcus spp and *Staphylococcus aureus* were isolated from a sample collected from dental caries and the mouth of the patient. The collection of *Candida* spp was done from Indira Gandhi Medical College, Nagpur (Maharashtra), India.

Isolation of oral microflora

The microbial sample collected on sterile cotton swabs was transferred immediately into 500 µL phosphate-buffered saline (PBS) buffer (0.12 M NaCl, 0.01 M Na_2HPO_4, 5 mM KH_2PO_4 (pH 7.5)[24]. The microorganisms were streaked on a nutrient agar medium with 0.4% sucrose (alternate medium for mitis-salivarius agar)[15] and mannitol salt agar (MSA) for the isolation of *Streptococcus* spp and *Staphylococcus aureus,* respectively. All the streaked plates were incubated at 37°C for 24 h. Based on colony characteristics, selected colonies were subcultured on a selected selective medium. The morphological characteristics of the isolated cultures were studied by gram staining and motility. Further confirmation was done by studying the biochemical characteristics. *Candida* spp. was subcultured and maintained on Sabouraud dextrose agar (SDA).

Collection and pretreatment of *E. laevis* leaves

The leaf (obtuse mature) of *E. laevis* from a medium hight plant was collected from a home garden in the Nagpur area (Maharashtra, India). It was rinsed with distilled water to make a paste and also dried in the shed for powder.

Preparation of extract

Acetone and ethyl alcohol (95%) with Soxhlet apparatus

Dried leaf powder and fresh leaf paste of *E. laevis herb* were extracted separately with acetone as well as ethyl alcohol (95%) using Soxhlet apparatus (leaf: solvent ratio 1:10) for 6-8 h. The volume of all the extracts was reduced to 50 mL by recovering the solvent and stored in screw cap bottles at room temperature for further use. The number of extracts was as following: [A] Dried leaf acetone extract, [B] fresh leaf acetone extract, [C] dried lead ethyl alcohol extract, [D] Fresh leaf ethyl alcohol extract, [E] Dried leaf dimethyl sulfoxide (DMSO)extracts of 50 mg/mL and 100 mg/mL concentrations (DMSO was added after evaporation of acetone in dried leaf acetone extract) and [F] Amoxicillin antibiotic was dissolved in dimethyl sulphoxide to make the concentration of 50 mg/mL and 100 mg/mL.

Antimicrobial effect of extract

The antimicrobial effect of each extract was tested against isolated oral microorganisms *Streptococcus* spp, *Staphylococcus aureus* and *Candida* spp by Kirby-Bauer method[25] using Mueller Hinton agar (MHA). Twenty milliliters (20 mL) inoculums of *Streptococcus* spp (nutrient broth with 0.4% sucrose, 37°C, 24 h), *Staphylococcus aureus* (nutrient broth, 37°C, 24 h) and *Candida* spp (Saubouard dextrose broth, 28-30°C for 24-48 days) was prepared. The broth culture (0.1 mL) of each organism was spread on separate Muller Hinton Agar (MHA) plate by using a sterile spreader. Plates were punctured in 2 mm size wells (2-3) with a sterile borer. Extracts (0.1 mL) were added to the individual wells. Control was set by using the respective solvent. The plates with bacterial and fungal lawn were incubated at 37°C for 24 h and 28-30°C for 24-48 days, respectively.

Qualitative phytochemical tests for phenolic contents

Ferric chloride test for tannins

E. laevis leaf extract (1 mL) was mixed with a few drops of ferric chloride ($FeCl_3$) solution. It develops an intense green to a purple-blue colour, indicating the presence of tannins[26].

Ferric chloride test for flavonoid

Leaf extract (0.5 mL) was boiled with distilled water and filtered. The filtrate (2 mL) was mixed with a few drops of 10% $FeCl_3$ solution. It develops a greenish-blue colour, which indicates the presence of a phenolic hydroxyl group[26].

Results

Streptococcus spp showed colorless colonies on nutrient agar (with 0.4% sucrose). It was gram-positive cocci present in straight chains, non-motile and fermented glucose, sucrose and lactose sugar. In this microorganism catalase, urease, H_2S and methyl

red test (MR) were negative, whereas Voges-Proskauer (VP) test was positive. *Staphylococcus aureus* is a mannitol fermenting microorganism that shows yellow-colored colonies on Mannitol salt agar. It was Gram-positive cocci present in grape-like bunches, non-motile, fermented glucose, lactose and sucrose sugar. This microorganism was catalase, urease, H_2S and MR, VP positive.

E. laevis fresh leaf ethyl alcohol extract has shown more inhibitory action against *Candida* spp (20 mm). It was compared with the efficacy of all extracts against isolated microorganisms. And the percentage reduction in antimicrobial sensitivity of *Staphylococcus aureus* (15 mm) and *Streptococcus* spp (10 mm) was 75% and 50%, respectively (Fig. 1a,b,c and Fig. 2). While, on the, in *E. laevis* fresh leaf acetone extract, the antimicrobial action was 75% for *Staphylococcus aureus* (15 mm), 60% for *Candida* spp (12 mm) and negligible action for *Streptococcus* spp. The dried leaf acetone extract was also effective on isolated and selected mouth microflora. The effectivity of extract on S*taphylococcus aureus*, *Streptococcus* spp and *Candida* spp was 50% (10 mm), 40% and 35%, respectively (Fig. 1 d, e, f and Fig. 2) when it was compared with a zone of inhibition of *Candida* spp of fresh leaf ethyl alcohol extract. *E. laevis* dried leaf ethyl alcohol extract was ineffective against all the microorganisms (Fig. 1a, b, c and Fig. 2).

The antimicrobial sensitivity of *E. laevis* dried leaf DMSO extract was increased with the increase in concentration. Dried leaf *E. laevis* DMSO extract (50 mg/mL and 100 mg/mL) has shown greater mode of action against *Streptococcus* species (12 mm; 60% and 25 mm; 125%) (Fig. 3a and Fig. 4), than

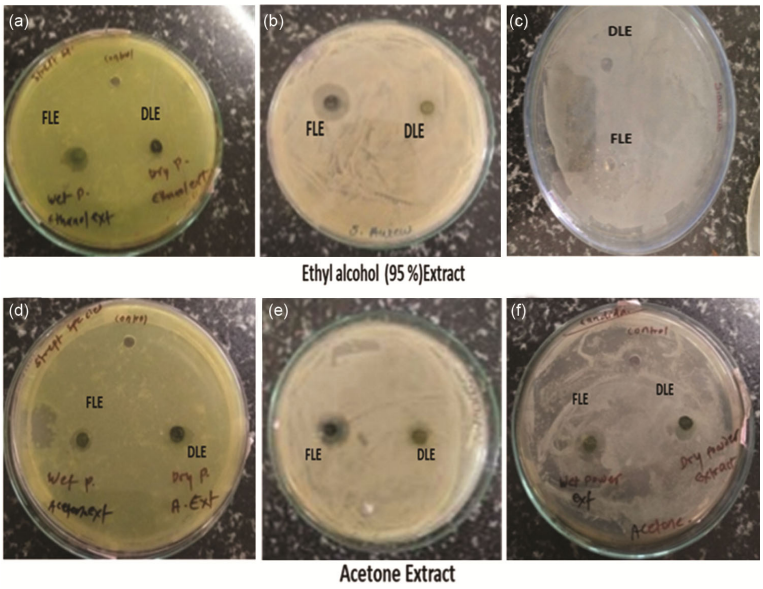

Fig. 1 — Antimicrobial activity of *Ehretia laevi*s leaves ethyl alcohol and acetone extract against test organisms (a) & (d) *Streptococcus* spp, (b) & (e) *Staphylococcus aureus* and (c) & (f) *Candida* spp [FLE: Fresh leaf Extract, DLE: Dried Leaf Extract]

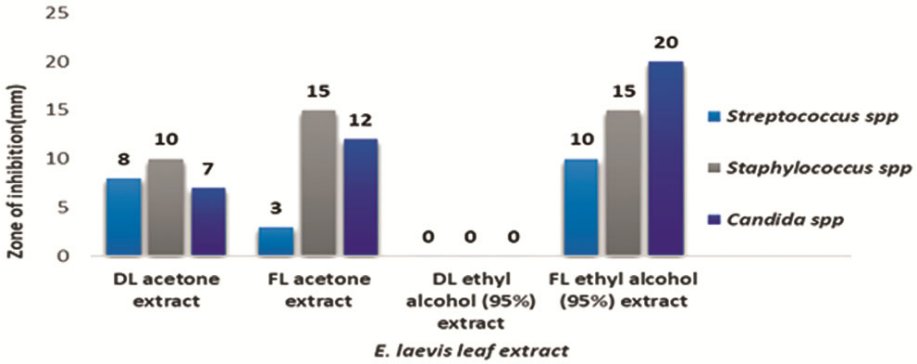

Fig. 2 — Antimicrobial activity of *Ehretia laevi*s leaves ethyl alcohol and acetone extract against oral test pathogens

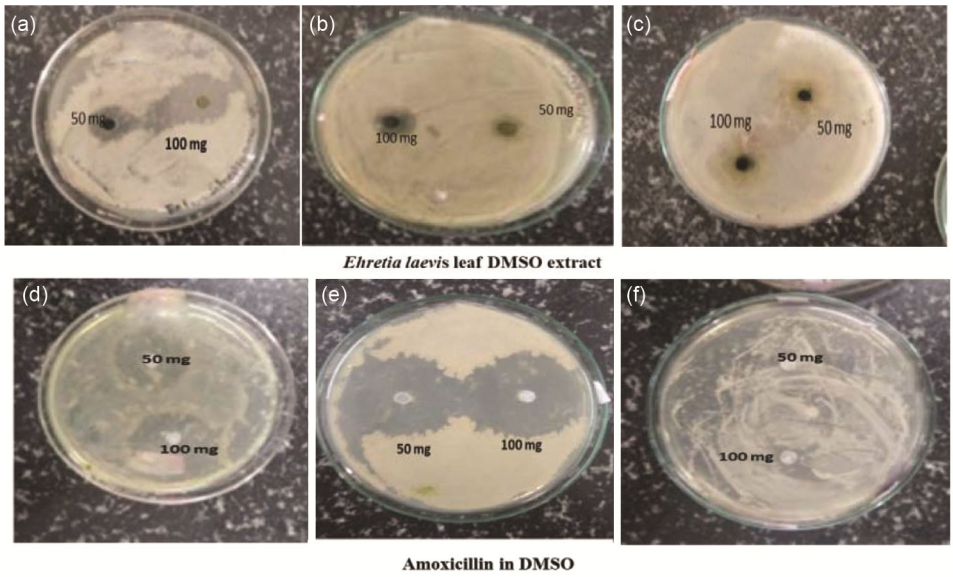

Fig. 3 — Antimicrobial activity of *E. laevis* leaf DMSO extract and amoxicillin DMSO extract against test organisms (a) & (d) *Streptococcus* spp, (b) & (e) *Staphylococcus aureus* and (c) & (f) *Candida* spp

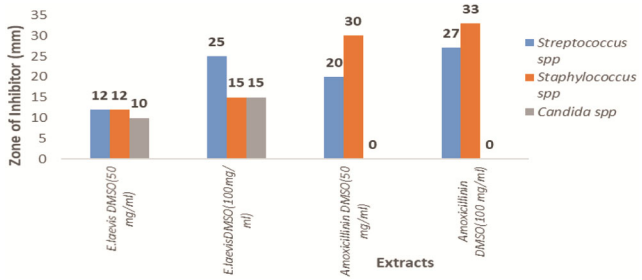

Fig. 4 — Antimicrobial activity of *E. laevis* leaf DMSO extract and amoxicillin DMSO extract against oral test pathogens

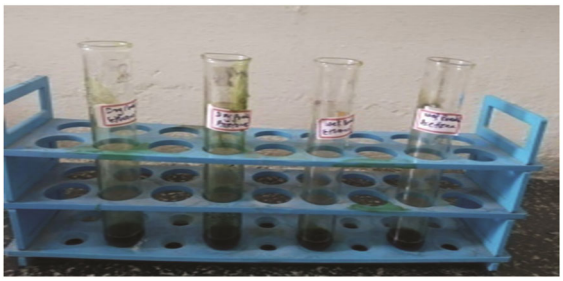

Fig. 5 — Qualitative test of tannins and flavonoids of *E. laevis* leaf extract

*Staphylococcus aureus (*12 mm; 60% and 15 mm; 75%) (Fig. 3b and Fig. 4) and *Candida* spp (10 mm; 50% and 15 mm; 75%) (Fig. 3c and Fig. 4). Amoxicillin dissolved in DMSO solvent (50 mg/ mL and 100 mg/mL) was effective only against *Streptococcus* spp and *Staphylococcus aureus* and its zone of inhibition was increased with an increase in the concentration of the drug (Fig. 3d, e and Fig. 4). This drug has shown greater sensitivity against *Staphylococcus aureus* (30 mm and 33 mm) than in *Streptococcus* spp (20 mm and 27 mm) (Fig. 4). Phytochemical analysis of tannins and flavonoids has shown positive results (Fig. 5, Table 1).

Table 1 — Qualitative ferric chloride test for detection of tannins and flavonoids in *E. laevis* leaf extracts:

Phytochemical test	Color change	Present/Absent
For Tannins	Purple blue colour	Present of tannins
For flavonoids	Greenish-blue colour	Presence of phenolic hydroxyl group

Discussion

The comparative data of antimicrobial sensitivity indicates greater effectivity of *E. laevis* fresh leaf ethyl alcohol extract against *Candida* spp followed by *Staphylococcus aureus* and *Streptococcus* spp. The percentage zone of inhibition against *Candida* spp, *S. aureus* and *Streptococcus* spp in *E. laevis* fresh leaf acetone extract was greater than dried leaf acetone extract. but it was less than fresh leaf ethyl alcohol extract. This antimicrobial effect was increased in dried leaf DMSO extract (50 mg/mL and 100 mg/mL). This may be due to the solubilization power of DMSO with polar and non-polar components[27] and also the diffusion capacity of extract in agar medium. Torne *et al*. reported the presence of a high quantity of tannin and flavonoids in *E. laevis* plant extracts[2]. The positive experimental

results by $FeCl_3$ test for tannins and flavonoids also supported the antimicrobial activity of *E. laevis* leaf extracts. The antimicrobial activity is attributed to the active constituents of secondary metabolites i.e., phenolic compounds and tannins[28,29] in the leaf of this plant. It is also reported in the literature that the presence of phenolic acids in plants is responsible for antimicrobial characteristics[30]. This polyphenolic compound tannin is a remedy for many diseases[31] which also inhibits the growth of many fungi, yeasts, and bacteria[32]. Bele *et.al.* published the medicinally important antibacterial properties of tannins[33] and Kumar *et al*.[34] mentioned the antimycobacterial activity properties of flavonoids.

Conclusion

The antimicrobial sensitivity data exhibit the efficacy of *E. laevis* leaf extracts against isolated and selected oral microflora i.e., *Streptococcus* spp, *Staphylococcus aureus* and *Candida* spp. This folklore herb *E. laevis* has great potential as an alternative antibacterial and antifungal drug. Nowadays, the problem of drug resistance in *S. aureus* and *Candida* spp is increasing. So, it can be an alternative therapeutic agent for the oral antimicrobial treatment or control of oral microflora. This study has shown promising results for antimicrobial activity of *E. laevis* against isolated and selected oral pathogens. The results revealed the presence of medicinally important constituents in these extracts. Many shreds of evidence gathered in earlier studies also confirm the bioactivity of *E. laevis* leaves. Therefore, it can be a good source for pharmaceutical drug formulation research, and for the preparation of herbal products such as oral ointments, mouthwash and toothpaste.

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Conflicts of Interest

The authors have no conflict of interest.

Authors' Contributions

Conceptualized the present work, designed all the experiments, analyzed and interpreted the data of experiments. Prepared and drafted the original manuscript, reviewed and edited the manuscript.

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