Full Length Research Paper

Antimicrobial and phytochemical screening of *Olea europaea* Linn. extracts against dental pathogens

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Dental caries is a common disease in human population which has a multifactorial etiology. The present study aimed to investigate the antimicrobial efficacy of various extracts of *Olea europaea* against six bacterial pathogens [Staphylococcus aureus, Streptococcus mutans, Staphylococcus sanguinis, Staphylococcus sobrinus, Staphylococcus salivarius, Lactobacillus acidophilus and one fungi (Candida albicans)]. Plant material was crushed and extracted in petroleum ether, acetone, methanol and aqueous through successive method by using Soxhlet apparatus. The antimicrobial activity of extracts was examined by agar well diffusion method at 200 mg/ml sample concentration and minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) by two fold serial dilution method. Ofloxacin was used as positive control to determine the sensitivity of the strains. The results show that methanol extract was more active than other extracts in its antimicrobial activity. The zone of inhibition exhibited by methanol extract against tested microorganisms ranged between 13.6±0.28 to 20.6±0.28 mm, respectively. Phytoconstituents analysis of plant extract revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins. The results validate the traditional uses of *O. europaea* in treatment of dental diseases.

Key words: Antimicrobial activity, agar well diffusion method, dental pathogens, *Olea europaea*.

INTRODUCTION

Human mouth contains a variety of bacteria but few are specifically involved in dental infections. Bacterial invasion causes demineralization and destruction of hard tissues of teeth. The acid production by bacteria causes accumulation of tooth surface, finally producing dental caries. Several bacteria are responsible for dental caries and periodontal infections that is *Streptococcus mutans*, *S. sobrinus*, *Lactobacillus acidophilus*, *Actinomyces* spp., *Nocardia* spp., *Campylobacter*, *Fusobacterium*, *Haemophilus*, *Prevotella*, *Porphyromonas*, *Veillonella* (Kononen et al., 1992, 1994; Marsh, 1992; Schupbach et al., 1995). Some of these organisms produce high level of lactic acid causing fermentation of dietary sugars and are resistant to the adverse effect of low pH (Hardie, 1982).

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According to World Health Organization (WHO) more than 80% of the world’s population relies on traditional medicine for their primary healthcare need. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Diallo et al., 1999). Various plants possess broad spectrum of synthetic activity and have been the source of many useful compounds (Sofowora, 1982).

*Olea europaea* (Oleaceae) is commonly known as olive tree. It is a tree bearing silvery green leaves and small white, feathery flowers. It is globally distributed especially in the tropical region. *O. europaea* possess broad spectrum of antimicrobial properties and extensively used to treat various diseases. This herb is used orally for sore throat, kidney problems and backache. Leaf infusions are used elsewhere as a lotion to treat eye infections or a gargle to relieve sore throat (Ross, 2005).

The objective of present study was to investigate the antimicrobial properties of *O. europaea* to cure dental diseases.

**MATERIALS AND METHODS**

**Plant material**

*O. europaea* was collected from G.B. Pant Herbal Nursery, Herbertpur, Dehradun, Uttarakhand and authenticated at Botanical Survey of India, Northern regional center, Dehradun. Stem and bark are included in this study. The plant materials were shade dried at room temperature and crushed to powder with a help of an electric grinder. The powder was sieved through a 1 mm mesh and was stored in an air-tight container for future use.

**Preparation of extract**

Plant extracts were prepared by immersing separately 200 g of dried powder in 600 ml of four different solvents that is petroleum ether (PET), acetone (ACE), methanol (MeOH) and aqueous (H2O) by soxhlet assembly and extracted for 72 h through successive methods (Ahmad et al., 1998). Plant extracts were filtered through Whatman No.1 filter paper and crude extracts obtained by removing or soxhlet assembly and extracted for 72 h through successive methods (Ahmad et al., 1998). Plant extracts were filtered through Whatman No.1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30-60°C and stored in sterile bottles at 4°C until further use. The yield of PET extract was 5.8 g, ACE extract 6.9 g, MeOH extract 8.6 g and H2O extract 9.3 g respectively. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/ml for agar well diffusion method.

**Test microorganisms**

The microorganisms were selected for the study on the basis of their clinical pharmaceutical importance as well as for their potential to cause infection in dental region. *S. mutans* (MTCC 1144), *S. sobrinus* (MTCC 890), *S. sanguinis* (ATCC 10556), *S. salivarius* (ATCC 33478), *S. salivarius* (MTCC 1938), *L. acidophilus* (MTCC 10307) and *Candida albicans* (MTCC 227) were purchased from IMTECH, Chandigarh and National Chemical Lab (NCL), Pune. *S. aureus*, *S. mutans*, *S. sanguinis*, *S. sobrinus*, *S. salivarius* and *L. acidophilus* were isolated from patient’s sample collected from Aggarwal Dental Clinic, Haridwar. The isolated pathogens were identified according to published guidelines (Burneti et al., 1994).

**Preparation of inoculum**

Stock cultures were maintained at 4°C on slopes of nutrient agar. Slants for experiment were prepared by transferring a loopful culture from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37°C.

**Antimicrobial activity**

The antimicrobial activity of different extracts was determined by agar well diffusion method (Perez et al., 1990). In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) medium no. 173 (Hi media Pvt Ltd., Mumbai, India). 0.1 ml of 12-16 h incubated cultures of pathogens were mixed in molten medium and poured in pre-sterilized petri plates. Plates were allowed to solidify for 5-10 min. A cork borer (6 mm diameter) was used to punch wells in medium and filled with extracts of 45 μl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. Efficacies of extracts against pathogens were compared with broad spectrum antibiotic Ofloxacin (positive control). Ofloxacin was dissolved into double distilled water. Plates were incubated at 37°C for 24 h in BOD incubator. At the end of incubation, inhibition zones formed around the well were measured with transparent ruler in millimetre. Each sample was assayed in triplicate and mean values were observed. The antimicrobial activity was interpreted from size of diameter of zone of inhibition measured to the nearest millimeter (mm) as observed from clear zones surrounding the wells.

**Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

Two-fold serial dilution method (Aboaba et al., 2006) was used to determine the minimum inhibitory concentration (MIC). MeOH extract was diluted double fold (2:2) with nutrient broth in a series of six test tubes. Concentration of 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml of crude MeOH extract were prepared separately and dissolved in 1 ml of DMSO. An aliquot of 1 ml of microorganism suspension (1.5×10^6) was inoculated into each tube (Figure 1). Control tubes were inoculated with same quantity of sterile distilled water. All tubes were incubated at 37°C for 24 h. The lowest concentration that did not permit any visible growth when compared with control was considered as the minimum inhibitory concentration. The minimum bacteriocidal concentration was considered as the lowest concentration that could not produce a single bacterial colony. The contents of all tubes that showed no visible growth were cultured on MHA medium incubated at 37°C for 24 h.

**Phytochemical screening**

The phytochemical analysis of plant extracts were carried out by standard qualitative methods (Trease and Evans, 1987; Scalbert, 1991).

**Test for alkaloids**

The test solution was acidified with acetic acid and a drop of Mayer’s reagent was added. A white precipitate indicated the presence of alkaloid.
Test for flavonoids

On addition of conc. HCl in MeOH extract of the material, a red colour appeared which indicated the presence of flavonoids.

Test for glycosides

The extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with Ba(OH)₂. The remaining extract contained the glycosides. The hydrolysis of the solution was done with conc. H₂SO₄ and after the hydrolysis the presence of sugar was determined with the help of Fehling's solution.

Test for steroids

The extract was mixed with 3 ml CHCl₃ and 2 ml conc. H₂SO₄ was poured from the side of the test tube and the colour of the ring at the junction of two layers was noted. A red colour showed the presence of steroids.

Test for Tannin

Extract was added in 1% ferric chloride and the colour was observed. Bluish black colour appeared which disappeared on addition of dilute H₂SO₄; a yellow brown precipitate showed the presence of tannins.

Test for saponins

Extracts were diluted with water to 20 ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

RESULTS

The present study shows that medicinal plants possess antimicrobial properties that support their value in herbal medicine for the treatment of dental ailments. The results enumerate the effect of the different extracts of the plant against the pathogens tested at a concentration of 200 mg/ml of plant extracts which shows significant antimicrobial activity against all the pathogens (Table 1). MeOH extract showed the maximum antimicrobial activity against the S. sobrinus (20.6±0.28 mm) followed by L. acidophilus (20.0±0.50 mm), S. aureus (19.0±0.50 mm), S. sanguinis (18.3±0.28 mm), S. salivarius (17.6±0.57 mm), S. mutans (16.6±0.28 mm) and Candida albicans (13.6±0.28 mm). MeOH extract showed maximum activity followed by PET, ACE and H₂O extract. The results of MICs and MBCs show that they ranged from 3.12 to 25 mg/ml (Figure 1). O. europaea presented similar MICs against S. sanguinis and S. sobrinus (6.12 mg/ml) respectively. Moreover, MeOH extract of this plant manifested a better MIC against S. aureus (3.25 mg/ml) and least MIC recorded against C. albicans (25 mg/ml). The phytochemical analysis of plant extract disclosed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins which might be accountable for its antimicrobial potential (Table 2).
Table 1. The inhibition zone diameter of various extracts of *Olea europaea* against dental pathogens.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Microorganisms</th>
<th>PET</th>
<th>ACE</th>
<th>MeOH</th>
<th>H₂O</th>
<th>Positive Control (Ofloxacin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. aureus</em></td>
<td>10.6±0.28</td>
<td>13.0±0.50</td>
<td>17.6±0.28</td>
<td>14.3±0.28</td>
<td>33.6±0.28</td>
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<tr>
<td>2</td>
<td><em>S. aureus</em> MTCC 1144</td>
<td>11.6±0.28</td>
<td>11.6±0.28</td>
<td>19.0±0.50</td>
<td>14.0±0.50</td>
<td>34.0±0.50</td>
</tr>
<tr>
<td>3</td>
<td><em>S. mutans</em></td>
<td>10.3±0.28</td>
<td>14.3±0.28</td>
<td>16.3±0.76</td>
<td>17.6±0.28</td>
<td>31.6±0.28</td>
</tr>
<tr>
<td>4</td>
<td><em>S. mutans</em> MTCC 890</td>
<td>12.0±0.50</td>
<td>14.6±0.28</td>
<td>16.6±0.28</td>
<td>18.6±0.28</td>
<td>32.6±0.57</td>
</tr>
<tr>
<td>5</td>
<td><em>S. salivarius</em></td>
<td>11.3±0.28</td>
<td>13.0±0.50</td>
<td>16.3±0.28</td>
<td>16.6±0.28</td>
<td>29.3±0.28</td>
</tr>
<tr>
<td>6</td>
<td><em>S. salivarius</em> MTCC 1938</td>
<td>10.6±0.28</td>
<td>13.6±0.28</td>
<td>17.6±0.28</td>
<td>15.3±0.28</td>
<td>29.6±0.57</td>
</tr>
<tr>
<td>7</td>
<td><em>S. sanguinis</em></td>
<td>9.3±0.28</td>
<td>14.3±0.57</td>
<td>17.0±0.28</td>
<td>15.3±0.28</td>
<td>29.6±0.57</td>
</tr>
<tr>
<td>8</td>
<td><em>S. sanguinis</em> ATCC 10556</td>
<td>8.6±0.28</td>
<td>16.0±0.50</td>
<td>18.3±0.28</td>
<td>16.6±0.28</td>
<td>35.6±0.28</td>
</tr>
<tr>
<td>9</td>
<td><em>S. sobrinus</em></td>
<td>11.0±0.50</td>
<td>15.3±0.28</td>
<td>20.0±0.50</td>
<td>18.3±0.57</td>
<td>27.3±0.57</td>
</tr>
<tr>
<td>10</td>
<td><em>S. sobrinus</em> ATCC 33478</td>
<td>12.0±0.76</td>
<td>15.3±0.28</td>
<td>20.0±0.50</td>
<td>19.6±0.28</td>
<td>27.6±0.28</td>
</tr>
<tr>
<td>11</td>
<td><em>L. acidophilus</em></td>
<td>12.0±0.50</td>
<td>15.6±0.28</td>
<td>19.3±0.76</td>
<td>16.0±0.50</td>
<td>29.6±0.28</td>
</tr>
<tr>
<td>12</td>
<td><em>L. acidophilus</em> MTCC 10307</td>
<td>11.3±0.28</td>
<td>15.3±0.28</td>
<td>20.0±0.50</td>
<td>17.3±0.28</td>
<td>30.0±0.50</td>
</tr>
<tr>
<td>13</td>
<td><em>C. albicans</em></td>
<td>7.6±0.57</td>
<td>11.6±0.28</td>
<td>13.6±0.28</td>
<td>9.6±0.28</td>
<td>22.3±0.28</td>
</tr>
</tbody>
</table>

*Zone of inhibition in millimetre (mm) in triplicate expressed as means and standard error of means.

Table 2. The phytochemical screening of crude extracts of *Olea europaea*.

<table>
<thead>
<tr>
<th>Phytoconstituent</th>
<th>Solvents</th>
<th>PET</th>
<th>ACE</th>
<th>MeOH</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Present, - = Absent.

DISCUSSION

Based on results, the antimicrobial potential of *O. europaea* extracts showed broad spectrum activity against selected pathogens. Our results are significantly correlated with other workers regarding this plant. Literature survey showed that major parts of *O. europaea* that is flowers, stems, leaves and fruits had good antimicrobial activity. The fruit extract of *O. europaea* exhibited antibacterial activity against *S. aureus* (18 mm) at concentration 800 µg/ml (Gupta et al., 2008). *O. europaea* aqueous extracts were screened for their antimicrobial activity against six bacteria that is, *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and two fungi that is, *C. albicans* and *C. neoformans* (Pereira et al., 2007). According to Anesini and Perez (1993), aqueous extract of dried fruit was inactive against *E. coli* and *S. aureus* at a concentration of 62.5 mg/ml. The isolated phenolic components of *O. europaea* showed inhibitory effect against some foodborne pathogens such as *Campylobacter jejuni*, *Helicobacter pylori* and *S. aureus* (Ahmed et al., 2014). Ziad et al. (2011) documented a MIC with ethyl acetate fraction of *O. europaea* at 10 µg/µl for *E. coli* and at 5.5 µg/µl for *K. pneumoniae*.

The phytochemical study showed that the presence of glycosides, alkaloids, flavonoids and amino acids in ethanolic, hydro-alcoholic and aqueous extract (Kaskoos, 2013). Khan et al. (2007) reported that phytoconstituents of flowers of *O. europaea* had shown the presence of flavonoids, steroids, glycoside, tannins and fatty acids. *O. europaea* leave extract showed major antibacterial activity due to the presence of phenolic compounds. The HPLC-DAD analysis of *O. europaea* leaves showed the presence of seven phenolic compounds that is caffeic acid, verbascoside, oleuropein, luteolin 7-O-glucoside, rutin, apigenin 7-O-glucoside and luteolin 4’-O-glucoside (Pereira et al., 2007).

Therefore, the activity observed for *O. europaea* provides a rationale for its use in treatment of dental infection diseases. The presence of phytomedicine in *O. europaea*
would be responsible for the demonstrated antimicrobial activity of the extracts.

**Conclusion**

*O. europaea* stems extracts possess a broad spectrum of activity against a panel of microorganisms responsible for the most dental diseases. This study can boost a new possibility for finding novel clinically effective antimicrobial compounds.

**Conflict of Interests**

The author(s) have not declared any conflict of interest.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


