Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of \textit{Acacia nilotica} extract

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Oral diseases are one of the major public health issues. Due to acquisition of pathogenic resistance over conventional antimicrobials, the search for natural alternatives continues. In the present study, thirty two methanol and ethyl acetate extracts prepared from 14 different plant species were screened against oral pathogens. Principal Component Analysis indicated that methanol extract of \textit{Acacia nilotica} twig was the most influential with highest F1 score and showed almost 2 fold higher antimicrobial activity in comparison to others. GC-MS analysis of \textit{Acacia nilotica} twig revealed the presence of various bioactive such as limonene, stigmasterol, linoleic acid, ricinoleic acid, santalol, undecylenic acid. Evaluation of antimicrobial potential of medicinal plants may thrive a safe, inexpensive and efficient therapeutic in developing formulation for oral care products.

Keywords: Antimicrobial activity, GC-MS, Medicinal plants, Oral pathogens, Principal Component Analysis (PCA)

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Introduction

Oral health and hygiene includes every aspect of keeping the mouth clean and germ free. Tongue scraping removes the white or yellow coating of bad breath-generating pathogens\textsuperscript{1}. Poor oral hygiene may lead to many diseases such as dental caries, tooth sensitivity, toothache, bad breath, oral sores, bleeding gums etc\textsuperscript{2}. Oral infections may show the symptoms of many systemic diseases earlier in life including diabetes, high BP, heart disease, cancer, gastrointestinal infections and autoimmune diseases\textsuperscript{3}.

A number of factors can be responsible for poor oral health such as having too hot and cold beverages, sweet eatables, smoking, hormonal changes, genetic susceptibility, prolonged illnesses and their medications, acidity, dry mouth, overgrowth of pathogenic microbes etc. Pathogens such as \textit{S. mutans} are the most common causal agent of dental caries\textsuperscript{4}. \textit{E. faecalis} was found the most commonly isolated species from the root canals of teeth\textsuperscript{5} whereas \textit{S. aureus} was most prevalent in gingivitis suffering patients\textsuperscript{6}. Overgrowth of fungus \textit{C. albicans} causes oral candidiasis or thrush especially in immuno-compromised individuals, which in turn may lead to bad breath and biofilm formation\textsuperscript{7}. Many antimicrobial agents including chlorhexidine, fluorides, sodium lauryl sulfate etc. are used for prevention and cure of oral diseases. There are reports on their side effects such as hypersensitivity reactions, toxicity, tooth staining and desquamation of oral mucosa etc\textsuperscript{8-9}.

India ranks the second largest exporter of medicinal plants after China. Many herbs and spices are conventionally used for oral care in different regions of the country such as cloves oil for dental caries\textsuperscript{10}, turmeric for pyorrhea\textsuperscript{11} and cardamom is used as mouth freshener. \textit{Aloe vera} mouthwashes are used to reduce the risk of dental plaque and gingivitis\textsuperscript{12}. A decoction triphala ingredients namely harad, baheda and amla can be effectively used to manage dental caries, gingival and periodontal diseases\textsuperscript{13}. Even the use of neem, miswak and babul datun is a proficient way of dental care\textsuperscript{14}. For identification of compounds gas chromatography with mass spectrometry is widely used in quality control of active pharmaceutical ingredients, bulk drugs and their formulations\textsuperscript{15}. Therefore, the main objective of the present study was to screen the antimicrobial potential of different medicinal plants against the selected oral pathogens and the GC-MS analysis of most effective extract.
Methodology

Collection of plant samples
Locally available plant samples were collected from Rohtak district of Haryana, India (Table 1). The plant material was identified and authenticated by comparing the herbarium specimen available in Department of Genetics, M. D. University, Rohtak (India).

Preparation of extracts
Thirty two plant extracts of methanol and ethyl acetate were prepared by cold percolation method. 10 gram of dried extract was dissolved in 100 mL solvent and rotated in incubator shaker for 3 days. After that it was filtered, lyophilized and stored for further use.

Antibacterial activity
Antibacterial activity of 40 µl of sample solution was added separately to each well of agar plate i.e. (2 mg/well) from prepared plant extracts of 50 mg/mL (stock solution) was evaluated by agar well diffusion method. Pathogenic strains namely; Streptococcus mutans (MTCC 497), Enterococcus faecalis (ATCC 29212), Staphylococcus aureus (ATCC 259323) and Candida albicans (ATCC 10231) were used for the study. Pure Dimethyl Sulfoxide (DMSO) and 2% Chlorhexidine diacetate were used as negative and positive control respectively.

Minimum Inhibitory Concentration
The most effective plant extracts which exhibited strong antimicrobial activity at 10 mg/mL was used to determine their MIC using 96 well plate methods.

Statistical analysis
Experiment was done in triplicates. The data from zone of inhibition of different extracts were represented as mean ± standard error. Principal Component Analysis (PCA) was performed using statistical software XLSTAT version 2018.1.

GC-MS conditions
Methanol extract of Acacia nilotica twig which showed the most significant activity were identified using GC-MS at AIRF centre, JNU, New Delhi. GC conditions are shown in Table 2. All data were obtained by collecting the full scan mass spectra with scan speed of 3333 within the scan range 40 to 650 m/z. Various components were identified by the software libraries viz. WILEY8, NIST11.

Results

Antimicrobial activity
In the present study, zone of inhibition of all plant extracts against oral pathogens was ranged between 10-40 mm (Fig. 1 and Table 3). DMSO (-ve control) showed negligible antimicrobial activity while 2% Chlorhexidine diacetate (+ve control) have zone of inhibition range from 17-25 mm against the tested oral pathogens. In Principal Component Analysis, F1
represents horizontal axis which is positively correlated with activity against *S. aureus* followed by *E. faecalis, S. mutans* and *C. albicans*. F2 represents vertical axis that is positively linked with activity against *C. albicans* only (Fig. 2). Twig of *A. nilotica* extract is most influential extract with highest F1 score. Extract of *E. cardamomum* is positively correlated with a very high activity against *E. faecalis* and *S. aureus* but negatively correlated against *C. albicans* as F2 is negative (Table 4).

### Minimum Inhibitory Concentration

The MIC of the effective plant extracts viz. *A. nilotica, E. cardamomum, P. guajava* and *G. glabra* were employed against specific oral pathogens by 96 well plate method (Fig. 3). Lowest MIC was shown...
by *A. nilotica* extract against *C. albicans* and *E. cardamomum* against *E. faecalis* at concentration of 0.19 mg/ml respectively.

**GC-MS analysis**

The results pertaining to GC-MS analysis of the methanol extract of *Acacia nilotica* twig lead to the identification of a number of compounds. A total of 46 peaks were observed revealing 25 possible phytochemicals with their retention time and area percentage (Fig. 4 and Table 5).

**Discussion**

Numerous studies have been done on antimicrobial potential of different parts of *A. nilotica* including its leaves, bark, pod but very less studies are on the antimicrobial potential of its twig. Many bioactives found in the *A. nilotica* extract already have been reported in other medicinal plants and proved to exhibit antimicrobial activities such as Ricinoleic acid against *S. aureus*24, Santalol against *C. albicans* and *S. aureus*25, Undecylenic acid inhibits the biofilm formation in *C. albicans*26, Limonene against *E. faecalis* and *S. aureus*27, Stigmasterol against *S. aureus*28 and *C. albicans*29. The most abundant phytochemical found in the study is a polyunsaturated fatty acid i.e. 9, 12-Octadecadienoic acid (linoleic acid) with an area 42.79%. The fatty acid has evidenced to inhibit significant growth of *S. mutans* and *C. albicans* by penetrating and disrupting normal function of the cellular membranes30. Compounds such as mome inositol31, lanceol32 exhibits anti-proliferatory activity, neophytadiene possesses larvicidal property33, cis-9-octadecenamide is a natural sleep inducing lipid34, N-Hexadecanoic acid and Octadecanoic acid anti-inflammatory activity35.

Polar extract of small cardamom also showed very high antimicrobial activity in comparison to its nonpolar extract but most of the pharmacological analysis reports are on the role of its essential oil or nonpolar metabolites against oral microbes36. The spice is rich in terpenoids which can disrupt microbial membrane or alteration in the proton-motive force that could affect their biofilm formation37. *P. guajava* exhibited high activity against *C. albicans* followed by *T. chebula* and *P. granatum*. Due to the presence of phenolic compounds, they have an influence on virulence factors of Candida38.

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**Table 4 — Correlations between sample’s activity and factors**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Samples</th>
<th>Code</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. nilotica</em></td>
<td>S1</td>
<td>6.157</td>
<td>-0.266</td>
<td></td>
</tr>
<tr>
<td><em>E. cardamomum</em></td>
<td>S17</td>
<td>2.614</td>
<td>-1.736</td>
<td></td>
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<tr>
<td><em>G. glabra</em></td>
<td>S20</td>
<td>1.308</td>
<td>0.186</td>
<td></td>
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<tr>
<td><em>P. guajava</em></td>
<td>S23</td>
<td>-0.297</td>
<td>2.064</td>
<td></td>
</tr>
<tr>
<td><em>P. granatum</em></td>
<td>S25</td>
<td>0.365</td>
<td>0.948</td>
<td></td>
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<tr>
<td><em>T. chebula</em></td>
<td>S30</td>
<td>0.302</td>
<td>1.154</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 2 — Principal Component Analysis of antimicrobial activity of selected plant extracts**

**Fig. 3 — Minimum Inhibitory Concentration of effective plant extracts against oral pathogens**

**Fig. 4 — GC-MS Chromatogram for the methanol extract of *A. nilotica* twig.**
zeylanicum exhibited medium activity against S. mutans. Extracts of licorice root, cinnamon bark, harad fruit exhibited moderate activity against oral pathogens is also supported by other investigators\(^{39}\). Extracts of neem, turmeric, amla, large cardamom showed very weak or negligible activity against all the strains. However, these plants have very good antioxidant activity\(^{40}\) and may be helpful in holistic oral care.
Conclusion
Screening of plant extracts and GC-MS analysis will be beneficial in development of oral care products such as in tooth paste, endodontic irrigants, dental gel, mouthwash, mouth fresheners etc. Isolation of individual bioactive compounds and evaluation of their pharmacological potency will open a new area of investigation.

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