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# ANTIMICROBIAL ACTIVITY OF ALTERNATE NATURAL RESOURCES ON THE ORAL PATHOGENS OF DIABETIC PATIENTS

Health Science

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## ABSTRACT

Oral diseases continue to pose a significant health challenge worldwide in diabetic patients. Among these, periodontal diseases stand out as one of the most critical oral health issues on a global scale. A total of 50 mouth swabs were collected from the diabetic patients admitted in a Tertiary care hospital, Chennai. A total of 47 bacterial and 3 fungal pathogens were identified from 50 samples of diabetic patients. The most prevalent pathogens were *Streptococcus mutans, Staphylococcus aureus, Escherichia coli* and *Candida albicans*. These organisms were subjected to well diffusion test to test the antimicrobial activity of 3 natural resources (*Azadirachta indica*, *Mentha piperita* and *Mangifera indica*). 38 mm of highest inhibition zone in *Streptococcus mutans* was produced against 75 µl concentration of *Mangifera indica* aqueous extract. All the plant extracts had a significant effect on the bacterial isolates compared to the control antibiotic. Phytochemical analysis had shown the presence of most of the phytochemical constituents in all the extracts. The comparison was made between the *Mangifera indica* and *Azadirachta indica* of both aqueous and ethanol extracts by using the biostatistics analysis in that mean and standard deviation 0.05 of P value showed the significant results.

## KEYWORDS

*Streptococcus mutans, Staphylococcus aureus, Escherichia coli* and *Candida albicans, Azadirachta indica, Mentha piperita and Magnifera indica*

## INTRODUCTION

The coexistence of oral diseases and diabetes mellitus necessitates a comprehensive approach to address these health challenges and their potential interplay. By understanding and tackling these issues collectively, healthcare professionals and policymakers can work towards improving global oral health and diabetes management, thus enhancing the overall well-being of affected populations (1). The prevalence of periodontal disease in individuals with diabetes exhibits considerable variability. Studies have shown that all diabetic patients, on average, have periodontal disease compared to only 50% of control individuals without diabetes (2). For those with type 2 diabetes, the most common form of diabetes accounting for 95% of the diabetic population, the risk of periodontal disease is three times higher than that in the general population (3). The oral flora, along with the gastrointestinal microbiome, constitutes one of the most diverse collections of microorganisms in the human body. In healthy individuals, the most common pathogens in the oral cavity include *Streptococcus spp*. (4), which can also cause dental and oral maxillofacial infections under certain conditions like caries, periodontitis, endodontic infections and tonsillitis in diabetes patients. Alongside these commensals, other bacterial pathogens are associated with oral cavity infections, such as *Staphylococcus spp* and *Candida spp* (5).

## MATERIALS & METHODS:

A total of 50 mouth swabs were collected from the diabetic patients admitted in a Tertiary care hospital, Chennai.All the media used in this study were prepared according to the devised written procedure of Manufacture company (Himedia). Preliminary identification of pathogen by straining methods and biochemical characterization. Fungi were identified by lactophenol cotton blue method and Germ tube test.Stock cultures were maintained at $4^0$C on slopes of nutrients agar. Antimicrobial sensitivity test down by Kirby- Bauer method (6). The plant samples were washed under tap water, rinsed with distilled water, and dried in the shade. Subsequently, they were ground into powder form, and each plant sample was weighed and soaked in the respective solvents (distilled water, ethanol, and acetone) (7). For each extract, 300 mg was weighed and dissolved in 5 ml of acetone. The volume was then made up to 300 ml by adding distilled water in a standard flask, creating a stock solution. The obtained plant extracts were stored at 4°C until further use for phytochemical and antimicrobial activity screening (7). The confirmatory qualitative phytochemical screening of plant extracts was performed to identify the main classes of compounds (tanins, saponins, terpenoids, alkaloids etc) (8). At last to find out the minimum inhibitory concentration of effective extract of medicinal plants. Data was analyzed by using Analysis of Variance (ANOVA) with drug treatment as independent factor with p value < 0.05.

## OBSERVATIONS AND RESULTS

**Table 1: Pathogens isolated in the study**

| S. NO | MICROORGANISM | NO. OF PATIENTS |
|---|---|---|
| 1 | Streptococcus mutans | 20 |
| 2 | Staphylococcus aureus | 15 |
| 3 | Escherichia coli | 12 |
| 4 | Candida albicans | 3 |
| | TOTAL | 50 |

**Table 2: Phytochemical screening of medicinal plants**

| S. NO | Phytochemical constituent | Mentha piperita(mint) | | | Azadirachta indica(neem) | | | Mangifera indica(mango leaf) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Ethanol | Aqueous | Acetone | Ethanol | Aqueous | Acetone | Ethanol | Aqueous | Acetone |
| 1 | Terpenoids | + | + | + | - | + | + | + | + | + |
| 2 | Alkaloids | + | + | + | + | + | - | + | + | + |
| 3 | Carbohydrates | + | + | + | - | + | + | + | - | + |
| 4 | Flavonoid | - | + | + | + | + | - | + | - | + |
| 5 | Tannins | - | + | - | - | + | + | - | + | + |
| 6 | Phenols | + | + | + | - | + | + | + | + | + |
| 7 | Glycosides | + | + | + | - | + | + | + | + | + |
| 8 | Saponin | - | + | - | - | + | + | + | + | - |

**+ ---------Positive - ---------- Negative**

**Table 3: Statistical analysis of Antimicrobial activity of medicinal plants on isolated pathogens**

| Plant | Microorganism | Ethanol | | | | Aqueous | | | | Acetone | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 20µl | 50µl | 75µl | P value | 20µl | 50µl | 75µl | P value | 20µl | 50µl | 75µl | P value |
| Mint | *E. coli* | 10±3.6 | 18.3±3.8 | 18.5±3.5 | **0.04*** | 10±3.6 | 16.9±3.6 | 17.9±3.1 | **0.03*** | 5.03±3.6 | 9.9±3.6 | 12.8±3.6 | **0.03*** |
| | *S. aureus* | 10.0±2.9 | 30.2±3.5 | 11.7±3.06 | **0.03*** | 19.9±2.8 | 19.9±2.0 | 19.9±2.3 | **0.04*** | 11±3.6 | 10.0±3.33 | 10.6±2.6 | **0.04*** |
| | *S. mutans* | 13.8±2.3 | 0 | 0 | 0.16 | 19.6±2.3 | 25.8±2.3 | 21.3±1.8 | **0.03*** | 0 | 0 | 12.7±2.06 | **0.15** |
| | *C. albicans* | 10.0±1.9 | 9.7±2.06 | 12.1±2.5 | **0.03*** | 11.1±2.7 | 14.5±2.6 | 19.9±2.3 | **0.04*** | 10.5±1.7 | 10.1±1.7 | 15.03±2.6 | **0.04*** |
| Neem | *E. coli* | 21.6±2.6 | 30.2±2.1 | 16.3±2.9 | **0.04*** | 0 | 21.2±3.2 | 0 | 0.14 | 13.5±1.3 | 12.5±2.5 | 24.2±3.8 | **0.03*** |
| | *S. aureus* | 10.3±3.06 | 10.4±1.7 | 10.7±2.7 | **0.04*** | 11.8±3.6 | 11.6±2.6 | 20.7±2.8 | **0.03*** | 10.1±2.6 | 11.6±2.3 | 12.1±3.3 | **0.03*** |
| | *S. mutans* | 10.4±2.02 | 0 | 0 | 0.19 | 10.2±1.2 | 10.03±2.5 | 34.1±1.8 | **0.04*** | 10.5±1.8 | 0 | 10.03±1.1 | 0.06 |
| | *C. albicans* | 9.5±1.9 | 12.5±2.09 | 23.7±2.01 | **0.04*** | 20.4±2.6 | 0 | 30.4±1.8 | **0.08*** | 13.3±2.6 | 13.06±1.5 | 18.3±1.5 | **0.04*** |
| Mango leaf | *E. coli* | 0 | 8.5±2.9 | 0 | 0.17 | 2.6±27.6 | 26.9±2.6 | 38.6±2.4 | **0.03*** | 0 | 0 | 10.2±3.2 | 0.15 |
| | *S. aureus* | 14.9±2.6 | 29.2±1.6 | 12.9±20.3 | **0.04*** | 10.2±1.2 | 10.2±2.4 | 10.5±2.3 | **0.04*** | 10.2±1.4 | 9.03±2.3 | 0 | 0.07 |
| | *S. mutans* | 19.3±2.03 | 23.4±2.3 | 24.2±2.1 | **0.02*** | 0 | 0 | 0 | 0.29 | 13.4±2.0 | 11.3±1.6 | 16.1±2.0 | **0.03*** |
| | *C. albicans* | 14.9±2.6 | 29.2±1.6 | 12.9±20.3 | **0.03*** | 10.2±1.2 | 10.2±2.4 | 10.5±2.3 | **0.02*** | 10.2±1.4 | 9.03±2.3 | 0 | 0.08 |

**Table 4: Comparative Analysis On Antimicrobial Activity Of Control Antibiotic And Effective Plant Extracts**

| organism | *Mentha piperita* | | | *Azadirachta indica* | | | *Mangifera indica* | | | Antibiotic |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dilution | extract | Mean ±SD | Dilution | extract | Mean ±SD | Dilution | Extract | Mean ±SD | |
| *Streptococcus mutans* | 75 µl | Ethanol | 16.5±3.5 | 75 µl | Acetone | 24.2±3.8 | 75 µl | Aqueous | 38.6±2.4 | 19.9±2.1 |
| *Staphylococcus aureus* | 75 µl | Aqueous | 19.9±2.3 | 75 µl | Aqueous | 20.7±2.8 | 75 µl | Ethanol | 36.6±2.6 | 10.5±1.9 |
| *Escherichia coli* | 75 µl | Aqueous | 25.8±2.3 | 75 µl | Aqueous | 34.1±1.8 | 75 µl | Ethanol | 24.2±2.1 | 31.8±1.6 |
| *Candida albicans* | 75 µl | Aqueous | 19.9±2.3 | 75 µl | Aqueous | 30.4±1.8 | 50 µl | Ethanol | 29.2±1.6 | 39.6±2.1 |

The study investigated the antimicrobial activity of extracts from *Mentha piperita*, *Azadirachta indica* and *Mangifera indica* against oral pathogens in diabetic patients. A total of 47 bacterial and 3 fungal pathogens were identified from 50 samples of diabetic patients. The most prevalent pathogens were *Streptococcus mutans, Staphylococcus aureus, Escherichia coli* and *Candida albicans* (Table 1). The three plant extracts (aqueous, ethanol and acetone) were subjected to phytochemical screening, revealed the presence of various bioactive compounds, including terpenoids, alkaloids, phenols, glycosides, flavonoids, tannins and saponins. These phytochemicals are known for their potential antimicrobial properties (Table 2).

According to the mean value observed for the antimicrobial activity of the phytochemical extracts, *Mentha piperita* has shown the highest mean 30.2 for 50µl ethanolic extract on *Staphylococcus aureus* and the the least mean 5.03 is observed for 20µl of acetone extract of *Escherichia coli*. *Azadirachta indica* has shown 30.4 for 75µl aqueous extract on *Candida albicans* and the least mean 10.2 is observed for 20 µl of aqueous extract of *Streptococcus mutans*. *Mangifera indica* has shown the highest mean value 38.6 for 75µl aqueous extract on *Escherichia. coli* and the least mean 8.5 is for 50µl of ethanolic extract of *Escherichia coli*(Table 3). The level of confidence for the hypothesis was set as 95% with a significant level of **< 0.05.** According to the observed values obtained, it was found that almost all the plant extracts had a significant effect on the bacterial isolates compared to the control antibiotic(Table 4). Hence, as per the study the hypothesis was proved.

## DISCUSSION

The prevalence of periodontal disease in diabetes is highly variable. It has been shown that 100% of diabetic patients had periodontal disease compared with 50% of control individuals. Antimicrobial property is considered as one of the key hallmarks of oral care agents, in view of the involvement of pathogenic bacteria and fungal species in oral infections;(7).*Mentha piperita*, *Azadirachta indica* and *Mangifera indica* extracts were subjected to synthetic phytochemical screening with aqueous, acetone and ethanol. In this study, three (aqueous, ethanol, and acetone) extract fractions of *Mentha piperita*, *Azadirachta indica* and *Mangifera indica* produced concentration-dependent inhibition of growth of the oral pathogens; in particular, the aqueous extract fraction demonstrated significant antimicrobial activity compared to the other extract fractions (8).

## CONCLUSION

Hence, this study concludes that apart from the use of synthetic antimicrobial drugs, medicinal plants such as *Mangifera indica, Mentha piperita* and *Azadirachta indica* also effective against oral pathogens of diabetic patients proved based on their antibacterial and antifungal activity.

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