Antimicrobial Efficacy of Acacia nilotica, Murraya koenigii L. Sprengel, Eucalyptus hybrid, Psidium guajava extracts and their combinations on Fusobacterium nucleatum and Porphyromonas gingivalis

Abstract

Background: The herbal extracts have been effectively tried in the treatment and prevention of many oral diseases. Aim: The aim is to assess the antimicrobial efficacy of Acacia nilotica, Murraya koenigii L. Sprengel, Eucalyptus hybrid, Psidium guajava extracts and their combinations on Fusobacterium nucleatum (Fn) and Porphyromonas gingivalis (Pg). Materials and Methods: The extraction process was carried out by Soxhlet apparatus using ethanol as solvent. The combinations of the four plant extracts were prepared by combining an equal quantity of 10% solution of each of the four plant extracts. The antimicrobial efficacy testing of the plant extracts and their combinations on Fn and Pg was performed using agar well diffusion method. Columbia 5% of sheep blood agar plates were used for antimicrobial efficacy testing under anaerobic conditions. The qualitative assay was carried out to identify the various phytochemical constituents. Dimethyl sulfoxide and 0.2% chlorhexidine acted as negative and positive controls, respectively. The mean diameter of inhibition zone between different categories was compared using one-way analysis of variance. Results: All the individual plant extracts and their double, triple, and quadruple combinations were effective in inhibiting the growth of these bacteria. However, 0.2% chlorhexidine produced the highest mean diameter of inhibition zone. Conclusion: The plant extracts in combinations offer enhanced antimicrobial efficacy due to their synergistic action besides slowing the development of bacterial resistance. Hence, these extracts in combinations could be used tried as effective alternates to chlorhexidine.

Keywords: Acacia nilotica, Eucalyptus hybrid, Fusobacterium nucleatum, Murraya koenigii L. Sprengel, Porphyromonas gingivalis, Psidium guajava

Introduction

Dental plaque is defined clinically as a structured, resilient, yellow-grayish material that adheres tenaciously to the intraoral hard surfaces, including removable and fixed prosthesis. Dental plaque as a potential risk factor in the etiology of periodontal diseases has been well documented in medical literature. The complete removal of plaque from the dento-gingival region is an established method of preventing gingivitis and periodontitis.

Although over 500 bacterial species comprise of plaque, colonization follows a regimented pattern with adhesion of initial colonizers to the enamel salivary pellicle followed by secondary colonization through inter-bacterial adhesion. A variety of adhesins and molecular interactions underlie these adhesive interactions and contribute to plaque development. This can eventually lead to diseases such as caries and periodontal disease.

“Home care” with tooth brushing and other mechanical cleansing procedures is the most dependable means of controlling plaque. However, mechanical plaque control is not properly practiced by most individuals. Adjunctive chemical plaque control using chlorhexidine and antibiotics have also been recommended in addition to mechanical plaque control. However, the additional effects of adjunctive antibiotic therapy are small, and topical chlorhexidine therapy is not without side effects. This necessitates the development of strategies that can act against the plaque bacteria.

The herbal extracts have been effectively tried in the treatment and prevention of many
oral diseases. The previous studies have demonstrated the antimicrobial efficacy of Acacia nilotica, Murraya koenigii L. Sprengel, Eucalyptus hybrid and Psidium guajava extracts on dental caries and primary plaque colonizers. The assessment of the antimicrobial efficacy of these extracts on secondary and tertiary plaque colonizers will offer an effective strategy to prevent periodontal diseases. In this background, the present study was undertaken to assess the antimicrobial efficacy of A. nilotica, M. koenigii L. Sprengel, E. hybrid, P. guajava extracts and their combinations on Fusobacterium nucleatum (Fn) and Porphyromonas gingivalis (Pg).

Materials and Methods

Study design and setting

This in vitro study was conducted over a period of 6 months from December 2013 to May 2014 at Center for Scientific Research and Development, People’s University, Bhopal. The study protocol was approved by the Research Advisory Committee.

Preparation of plant extracts

The branches of four plants were collected from the surrounding areas, identified and authenticated by a taxonomist. The branches were thoroughly washed using water treated with reverse osmosis. Healthy leaves were separated from these branches and shade dried over a period of 3–4 weeks at room temperature. The dried leaves were hand crushed separately to obtain coarse powder, and subsequently, the fine powder was prepared using mixer grinder. The fine powder was stored in airtight plastic bottles, labeled, and stored in refrigerator at 4°C till further use.

The extraction process was carried out by Soxhlet apparatus using ethanol as solvent. The dried leaf extract was added to a required quantity of dimethyl sulfoxide (DMSO) to obtain the working concentration of the extract (i.e., 100 mg/ml). The combinations of the four plant extracts were prepared by combining an equal quantity of 10% solution of each of the four plant extracts.

Antimicrobial efficacy testing

The antimicrobial efficacy testing of the plant extracts and their combinations on Fn and Pg was done using agar well diffusion method. Columbia 5% sheep blood agar plates were used for antimicrobial efficacy testing under anaerobic conditions. The diameter of the inhibition zone was measured at three different planes on the undersurface of the agar plate using a transparent scale. DMSO and 0.2% chlorhexidine acted as negative and positive controls, respectively. The experiment was carried out in duplicate sets. The six readings were used to compute the mean diameter of inhibition zones for each extract and their combinations against these bacteria.

Photochemical constituents assay

The assessment of various phytochemical constituents such as alkaloids (using Mayer’s reagent and Dragendorff’s reagent), anthraquinones (Bornträger’s test), terpenoids (Salkowski’s test), saponins (Froth and emulsion test), flavonoids (Shinoda and alkaline reagent tests), tannins (Ferre chlorde and lead acetate tests), and cardiac glycosides (Legal test and Keller–Kiliani test) was done using a qualitative assay.

Statistical analysis

The data were entered into a personal computer, and statistical analysis was performed using SPSS version 20 (IBM, Chicago USA). The mean diameter of inhibition zone between different categories was compared using one-way analysis of variance and Tukey’s post hoc test. The statistical significance was fixed at 0.05.

Results

The details of the four plants used in the present study are denoted in Table 1. The details of bacteria (Fn and Pg) used in this study are denoted in Table 2. The mean diameter of inhibition zone produced by each plant extract and their double, triple, and quadruple combinations against Fn and Pg are denoted in Table 3.

All the individual plant extracts and their double triple and quadruple combinations were effective in inhibiting the growth of these bacteria. However, 0.2% chlorhexidine produced the highest mean diameter of inhibition zone compared to plant extracts and their combinations against both of these bacteria. DMSO failed to inhibit the growth of both of these bacteria.

Among the various plant extracts and their combinations, the double combination of A. nilotica and E. hybrid produced the maximum zone of inhibition against Fn (14.42 ± 0.67), whereas M. koenigii L. Sprengel produced the highest zone of inhibition on Pg (15.33 ± 0.82). However, the post hoc test revealed a significant difference between 0.2% chlorhexidine and all other plant extracts as well as their combinations with no significant difference between other categories.

The qualitative assay of phytochemical constituents in A. nilotica, revealed the presence of anthraquinones, flavonoids, tannins, and cardiac glycosides. M. koenigii L. Sprengel contained tannins and cardiac glycosides. E. hybrid demonstrated the presence of terpenoids, saponins, flavonoids, tannins, and cardiac glycosides. P. guajava contained anthraquinones, terpenoids, flavonoids, tannins, and cardiac glycosides [Table 4].

Discussion

Dental plaque is the principal etiologic factor in periodontal disease. The accumulation of dental plaque with no
Table 1: Details of four plants used in the present study

| Plant          | Botanical name | Family          | Weight of dried extract (g) | Yield (%) |
|----------------|---------------|-----------------|----------------------------|-----------|
| Babul          | A. nilotica   | Leguminosae     | 9.5                        | 19        |
| Curry          | M. koenigii L. Sprengel | Rutaceae | 5.4                        | 10.8      |
| Eucalyptus     | E. hybrid (E. camaldulensis × E. ovata) | Myrataceae | 15.8                        | 31.5      |
| Guava          | P. Guajava    | Myrataceae      | 12.6                        | 25.1      |

P. Guajava = Psidium Guajava, M. koenigii = Murraya koenigii, A. nilotica = Acacia nilotica, E. camaldulensis = Eucalyptus camaldulensis, E. ovata = Eucalyptus ovata, E. hybrid = Eucalyptus hybrid

Table 2: Details of bacteria used in the present study

| Bacteria             | ATCC number | Selective media used for revival | Type of haemolysis on blood agar plate | Media for antimicrobial efficacy testing |
|----------------------|-------------|---------------------------------|---------------------------------------|----------------------------------------|
| F. nucleatum         | 25586       | Brain heart infusion agar with 5% sheep blood | Beta haemolysis | Columbia 5% sheep blood agar |
| P. gingivalis        | 32777       | Brain heart infusion agar with 5% sheep blood | Beta haemolysis | Columbia 5% sheep blood agar |

F. nucleatum = Fusobacterium nucleatum, P. gingivalis = Porphyromonas gingivalis

Table 3: Antimicrobial efficacy of plant extracts and their combinations on Fusobacterium nucleatum and Porphyromonas gingivalis

| Plant extract | Mean diameter of inhibition zone in mm (SD) |
|---------------|---------------------------------------------|
|               | F. nucleatum | P. gingivalis |
| A. nilotica   | 11.75 (0.61) | 12.50 (0.55) |
| M. koenigii L. Sprengel | 13.67 (0.88) | 15.33 (0.82) |
| E. hybrid     | 12.17 (0.26) | 13.00 (0.63) |
| P. Guajava    | 11.17 (0.68) | 12.33 (1.21) |
| A. nilotica + M. koenigii L. Sprengel | 11.00 (0.45) | 12.33 (1.21) |
| A. nilotica + P. Guajava | 14.00 (0.84) | 13.33 (1.51) |
| A. nilotica + E. hybrid | 14.42 (0.67) | 11.83 (0.75) |
| M. koenigii L. Sprengel + P. Guajava | 11.67 (0.75) | 10.67 (0.82) |
| M. koenigii L. Sprengel + E. hybrid | 13.50 (0.45) | 14.25 (1.23) |
| E. hybrid + P. Guajava | 14.17 (0.68) | 11.33 (0.41) |
| A. nilotica + M. koenigii L. Sprengel + P. Guajava | 12.25 (2.12) | 11.92 (1.43) |
| A. nilotica + M. koenigii L. Sprengel + E. hybrid | 12.17 (0.26) | 12.83 (0.93) |
| A. nilotica + E. hybrid + P. Guajava | 13.50 (0.45) | 14.25 (0.42) |
| M. koenigii L. Sprengel + E. hybrid + P. Guajava | 12.58 (1.11) | 12.17 (0.26) |
| A. nilotica + M. koenigii L. Sprengel + E. hybrid + P. Guajava | 13.25 (1.23) | 12.50 (1.00) |

Chlorhexidine

18.08 (1.02)
19.50 (0.55)

Statistical inference

F: 21.880, df: 15, P: <0.001
F: 29.058, df: 15, P: <0.001

A. nilotica = Acacia nilotica, P. Guajava = Psidium Guajava, M. koenigii = Murraya koenigii, F. nucleatum = Fusobacterium nucleatum, P. gingivalis = Porphyromonas gingivalis, SD = Standard deviation, E. hybrid = Eucalyptus hybrid

Table 4: Results of qualitative assay of phytochemical constituents in plant extracts

| Phytochemical constituents and test used | A. nilotica | M. koenigii L. Sprengel | E. hybrid | P. Guajava |
|----------------------------------------|------------|------------------------|----------|-----------|
| Alkaloids (using Mayer’s reagent)      | -          | -                      | -        | -         |
| Alkaloids (using Dragendorff’s reagent) | -          | -                      | -        | -         |
| Anthraquinones (Borntrager’s test)     | +          | -                      | -        | +         |
| Terpenoids (Salkowski’s test)          | -          | -                      | +        | +         |
| Saponins (froth and emulsion test)     | -          | -                      | +        | -         |
| Flavonoids (Shinoda test)              | +          | -                      | -        | +         |
| Flavonoids (alkaline chloride test)    | +          | -                      | +        | +         |
| Tannins (ferric chloride test)         | +          | +                      | +        | -         |
| Tannins (lead acetate test)            | +          | -                      | +        | -         |
| Cardiac glycosides (legal test)        | +          | +                      | +        | +         |
| Cardiac glycosoids (Killer –kiliani test) | +        | +                      | +        | +         |

*: Positive, -: Negative. P. Guajava = Psidium Guajava, M. koenigii = Murraya koenigii, A. nilotica = Acacia nilotica, E. hybrid = Eucalyptus hybrid
intervention or oral hygiene methods causes gingivitis which on further progression becomes periodontitis. Mechanical plaque control and use of chemotherapeutic agents are established methods to treat periodontal disease. The major limitation of these chemotherapeutic agents is the toxicity and development of resistant microorganisms. The search for newer and safer chemotherapeutic agents continues in an attempt to overcome the limitations of mechanical debridement and adverse effects of chemotherapeutic agents. Natural phytochemical isolated from plants are considered an effective alternative to synthetic chemicals. This study assessed the antimicrobial efficacy of A. nilotica, M. koenigii L. Sprengel, E. hybrid, P. guajava extracts and their combinations on Fn (secondary plaque colonizer) and Pg (tertiary plaque colonizer).

Clark et al. in their study while assessing the efficacy of Acacia arabica gum on the growth and protease activities of periodontopathic bacteria found the extract to inhibit the growth of Pg. The authors concluded that the extract could be of clinical value to inhibit these bacteria similar to the results of the present study. The phytochemical constituents such as alkakoids, saponins, cardiac glycosides, tannins, flavonoids, and anthraquinones in A. nilotica may be responsible for the antimicrobial action of plant extract.

Nagata et al. in their study on the inhibitory effects of macrocarpals on the biological activity of Pg and other periodontopathic bacteria found macrocarpals A, B, C derived from eucalyptus demonstrated the antibacterial activity against periodontopathic bacteria. Among tested bacteria, Pg displayed the greatest sensitivity to macrocarpals. The study concluded that eucalyptus leaf extracts may be useful as a potent preventative of periodontal disease. Takarada et al., while assessing the antibacterial efficacies of essential oils against oral pathogens found eucalyptus oil to exert an inhibitory effect on various oral bacteria that included Pg. Actinobacillus actinomycetemcomitans, Fn, Streptococcus mutans, and Streptococcus sobrinus. These results were similar to the results of the present study. M. koenigii L. Sprengel contains sterols, alkakoids, and flavonoids, which may be responsible for the antimicrobial action. Ramesh et al. in their study on the salivary and tongue coating pH on chewing household herbal leaves found curry leaf extracts as home remedies that create an oral environment unfavorable for microbes. Ravi and Divyashree in their review on P. guajava as an adjunct in the treatment of periodontal disease concluded that the extract has an excellent antibacterial and antiplaque action and hence, may be considered a good adjunct to the mainstream periodontal treatment. Quercetin derived from P. guajava was shown to exhibit excellent antibacterial actions against periodontal pathogens such as Aggregatibacter actinomycetemcomitans, Pg, Prevotella intermedia, Fn. Guava extract has demonstrated in vitro antiplaque actions by inhibiting growth, adherence, and co-aggregation of dental plaque bacteria. Guava extracts may inhibit plaque development without disrupting homeostasis of the oral cavity. The combinations of plants extracts used in the present study also inhibited the growth of Fn and Pg. The present study is the first of its kind assessing the antimicrobial efficacy of the combinations of A. nilotica, M. koenigii L. Sprengel, E. hybrid, P. guajava extracts on Fn and Pg. Hence, our results could not be compared with previously published literature.

Conclusions

The individual plant extracts and their combinations were effective in inhibiting the growth of Fn and Pg. Although 0.2% chlorhexidine produced a significantly higher zone of inhibition compared to all the plant extracts and their combinations, these extracts could be considered as potential antiplaque and anticaries alternates. The plant extracts in combinations offer enhanced antimicrobial efficacy due to their synergistic action besides slowing the development of bacterial resistance. Hence, these extracts in combinations could be used tried as effective alternates to chlorhexidine. However, the in vitro studies on humans are to be conducted to validate these in vitro results.

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Conflicts of interest

There are no conflicts of interest.

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