An *in vitro* study on the anti-microbial efficacy of ten herbal extracts on primary plaque colonizers

Byalakere Rudraiah Chandrashekar[1]*, Ramesh Nagarajappa[2], Rupal Singh[3], Roopesh Thakur[3]

[1]Ph.D Scholar, Faculty of Dental Sciences, Pacifi c Academy of Higher Education and Research University, Udaipur, Rajasthan, India, [2]Department of Public Health Dentistry, Rama Dental College, Khanpur, Uttar Pradesh, India, [3]Center for Scientific Research and Development, People's University, Bhanpur, Bhopal, Madhya Pradesh, India

ABSTRACT

Introduction: To assess and compare the anti-microbial efficacy of ten herbal extracts against primary plaque colonizers. **Materials and Methods:** The branches of 10 plants with ethno-pharmacological background were collected and healthy leaves separated to prepare the fine powder. The extraction procedure was carried out by cold infusion method using ethanol. The working concentration of the extract (100 mg/ml) was prepared by mixing dried leaf extract with dimethyl sulfoxide (DMSO). 0.2% chlorhexidine and DMSO were used as positive and negative controls, respectively. Agar well diffusion method was used for anti-microbial efficacy testing. The statistical analysis was done using SPSS 16. The mean diameter of inhibition zone between the categories was compared using one-way analysis of variance and Tukey's *post-hoc* test. **Results:** The highest mean diameter of inhibition zone against *Streptococcus mutans* was produced by *Acacia nilotica* followed by *Psidium guajava*, *Eucalyptus* hybrid and *Murraya koenigii* L.S extracts in the descending order. The highest mean inhibition zone against *Streptococcus sanguinis* was produced by chlorhexidine followed by *A. nilotica*, *Eucalyptus*, *P. guajava*, and *M. koenigii* L.S extracts in the descending order. The highest mean inhibition zone against *Streptococcus salivarius* was produced by *P. guajava*, followed by chlorhexidine, *A. nilotica*, *Eucalyptus*, *M. koenigii* L.S and *Hibiscus sabdariffa L* extracts in the descending order. **Conclusion:** *P. guajava*, *Eucalyptus*, *A. nilotica*, *M. koenigii* L.S and *H. sabdariffa L* have the potential to inhibit primary plaque colonizers, and they could be used as anti-plaque agents.

Key words: *Acacia nilotica*, *Eucalyptus* hybrid, *Murraya koenigii* L. sprengel, *Psidium guajava*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*

INTRODUCTION

Oral health is an inseparable part of general health. Oral health has an impact on general health and well-being as the oral diseases cause pain and suffering, alter the eating pattern, speech, and affect the quality of life as a whole. Oral health also has an effect on other chronic diseases. Oral diseases are one among the most common chronic diseases affecting mankind. In view of their high

*Address for correspondence:
Dr. Byalakere Rudraiah Chandrashekar, Faculty of Dental Sciences, Pacific Academy of Higher Education and Research University, Udaipur - 313 003, Rajasthan, India. E-mail: drchandrubr@yahoo.com

prevalence, the impact on individual, family, and society as a whole as well as the high cost of treating them, oral diseases are considered an important public health problem. The literature suggests oral diseases to be the fourth most expensive diseases to treat in some countries.[1]

Although the World Health Organization report indicates a decline in the dental caries prevalence in most developed countries in the recent times, it can still be considered a major public health problem in many developing countries as the disease affects 60-90% of their school going children.[2] Loss of teeth due to periodontitis often causes discomfort, compromises the esthetics and function. Moreover, the recent studies suggest an association between chronic low-grade infections like periodontitis with systemic health problems[3] including cardiovascular diseases, pre-term low birth weight, diabetes mellitus, and chronic obstructive pulmonary disease.[3] The treatment of established dental diseases is very expensive and can never be considered an option for the poor. The literature indicates that the cost of treating dental caries alone which is estimated at US$ 3513/1000 children may exceed the total health budget for children in the majority of the low-income countries.[1] There is an immediate need for promoting preventive measures that are acceptable, cost-effective, and easily available. The robust, effective, feasible, and innovative strategies need to be evolved to combat these diseases.

The practices established for the prevention of dental caries and periodontal diseases are use of fluorides in various forms and mechanical oral hygiene along with proper professional care respectively. In reality, however, the motivation and dexterity anticipated for optimum oral hygiene are beyond the ability of the majority of the population especially in developing countries owing to the low priority given to oral health. Anti-microbial mouth rinses have also been recommended as adjuncts to mechanical plaque control methods. The most commonly used anti-plaque agent is chlorhexidine gluconate. The use of chlorhexidine, however, has some established side effects such as alteration in taste sensation, staining of teeth, and development of resistant microorganisms which debilitate their long term use.[4] There exists a need to develop some innovative strategies that act against both dental caries and periodontal diseases simultaneously. One such innovation would be to explore the anti-bacterial potential of medicinal plants that have some ethno-pharmacological background on dental caries and plaque bacteria. The need for an herbal alternative that can inhibit both dental caries and plaque microorganisms led us to undertake the present study to assess and compare the anti-microbial efficacy of 10 herbal extracts against primary plaque colonizers.

MATERIALS AND METHODS

Plant material

This was an *in vitro* study conducted over a period of 6 months from July to December 2013 at the research laboratory, Center for Scientific Research and Development, People's University, Bhopal. The branches of ten plants with ethno-pharmacological background which are easily available in central India were collected from the surrounding areas. The plants were identified and authenticated by a taxonomist. The branches were thoroughly washed in running water and then the healthy leaves were separated from these branches. The leaves were again rinsed in water treated with reverse osmosis and shade-dried over a period of 3-4 weeks at room temperature. The dried leaves were hand-crushed separately to obtain coarse powder of the 10 leaves. Subsequently, the fine powder was prepared using a mixer grinder and stored in airtight plastic bottles. The bottles were labeled and stored in the refrigerator at 4°C till further use.

Preparation of the herbal extracts using cold infusion method

Ethanol was used as a solvent for the extraction process in the present study. Two g of fine powder was weighed into a bottle and mixed with 10 ml of ethanol. The mixture was subjected to gentle and intermittent stirring for 15 min every 2 h for 48 h on a rotary shaker. The mixture was then filtered using Whatman filter paper. The filtrate was allowed to dry completely. The weight difference between the empty bottle and the bottle containing extract was used to determine the yield of each herbal extract. The dried leaf extract was mixed with the required quantity of dimethyl sulfoxide (DMSO) to obtain the working concentration of the extract (100 mg/ml).

Anti-microbial efficacy testing

Bacterial strains

The American Type Culture Collection (ATCC) strains of three primary plaque colonizers viz., *Streptococcus mutans* (ATCC), *Streptococcus sanguis* (ATCC) and *Streptococcus salivarius* (ATCC) were imported from USA and revived at the research laboratory for further microbiological assay. The three bacterial cultures were maintained on brain heart infusion agar slants with periodical subculturing and stored at 4°C.

The anti-microbial efficacy of the 10 herbal extracts at 10% concentration (50 μl volume) was assessed using agar well diffusion method. The diameter of the inhibition zone was measured at three different planes on the under surface of the agar plate using a transparent scale. 0.2% chlorhexidine was used as a positive control while DMSO acted as a negative control. The anti-microbial efficacy testing on these bacteria was done in duplicate, and the mean diameter of inhibition zones for each extract against the bacteria was computed.

Data entry and statistical analysis

The data were entered onto a personal computer, and statistical analysis was done using SPSS version 20 (IBM, Chicago, USA). The diameter of the inhibition zone against bacteria was expressed as mean and standard deviation (SD). The mean diameter of inhibition zone between different categories was compared using one-way analysis of variance (ANOVA). Tukey's *post hoc* test was used for multiple pair-wise comparisons wherever ANOVA yielded a significant result. The statistical significance was fixed at 0.05.

RESULTS

The details of the 10 plant extracts used in the present study, their yield using ethanol as solvent and cold infusion method for the extraction process is denoted in Table 1. The details of bacteria used for anti-microbial efficacy testing are denoted in Table 2.

S. mutans

The extracts of *Azadiractha indica*, *Hibiscus sabdariffa L*, *Acacia nilotica*, *Psidium Guajava*, *Eucalyptus* hybrid and *Murraya koenigii* L. sprengel inhibited the growth of *S. mutans* while extracts of *Magnifera indica*, *Ocimum sanctum* Linn, *Rosa rubiginosa* and *Aloe barbadensis* failed to inhibit

the growth. The extracts of *A. nilotica* produced the highest zone of inhibition (21.83 mm ± 0.41, mean ± SD) followed by *P. guajava* (21.17 mm ± 1.08), *Eucalyptus* hybrid (20.83 ± 1.08), *M. koenigii* L.S (9.75 ± 1.17), *H. sabdariffa* L (7.42 ± 0.20) and *A. indica* (7.25 ± 0.28). The mean zone of inhibition produced by chlorhexidine (positive control) was 14.25 mm (0.52) while DMSO failed to inhibit the growth of *S. mutans*. The difference in the mean zone of inhibition between the various groups was statistically significant ($P = 0.001$, Table 3). The multiple pair-wise comparisons revealed that the extracts of *A. nilotica*, *P. guajava*, and *Eucalyptus* hybrid produced a significantly higher zone of inhibition compared to chlorhexidine, while the extracts of *M. koenigii* L. sprengel and *H. sabdariffa* produced lesser zone of inhibition ($P = 0.001$, Table 4).

S. sanguis

The extracts of *A. nilotica* (21.33 ± 0.26), *Eucalyptus* hybrid (19.17 ± 0.82), *P. guajava* (18.58 ± 0.59), *M. koenigii* L.S (10.42 ± 0.92), and *H. sabdariffa* L (6.50 ± 0.63) inhibited the growth of *S. sanguis*. The extracts of *A. indica*, *M. indica*, *O. sanctum* Linn, *R. rubiginosa*, and *A. barbadensis* along with DMSO failed to inhibit the growth of *S. sanguis*. However, chlorhexidine (21.83 ± 0.61) produced significantly higher inhibition zone compared to all the plant extracts ($P = 0.001$, Table 4) except *A. nilotica* which produced almost similar efficacy.

S. salivarius

Among the various effective plant extracts against *S. salivarius*, *P. Guajava* (23.00 ± 0.48) produced the highest zone of inhibition followed by *A. nilotica* (21.17 ± 1.1), *Eucalyptus* hybrid (20.00 ± 0.45), *M. koenigii* L.S (13.25 ± 0.69), and *H. sabdariffa* L extract (9.75 ± 0.27). The extracts derived from the leaves of *A. indica*, *M. indica*, *O. sanctum* Linn, *R. rubiginosa*, and *A. barbadensis* as well as DMSO failed to

Table 1: The plant profile and yield of the ten herbal extracts assessed in the present study

Plant name	Botanical name	Family	Weight of dried extract (g)	Yield (%)
Neem	*A. indica*	Meliaceae	0.053	2.65
Guava	*P. guajava*	Myrataceae	0.139	6.95
Eucalyptus	Eucalyptus hybrid (*E. canaldulensis*×*E. ovate*)	Myrataceae	0.198	9.9
Babul	*A. nilotica*	Leguminosae	0.091	4.55
Curry	*M. koenigii* L.S	Rutaceae	0.101	5.05
Rose	*R. rubiginosa*	Rosaceae	0.079	3.95
Hibiscus	*H. sabdariffa* L	Malvaceae	0.104	5.2
Mango	*M. indica*	Anacardaceae	0.047	2.35
Tulsi	*O. sanctum Linn*	Lamiaceae	0.017	0.85
Aloe vera	*A. barbadensis*	Asphodelaceae	0.011	0.55

A. indica: Azadiractha indica, *P. guajava*: Psidium guajava, *E. canaldulensis*: Eucalyptus canaldulensis, *E. ovate*: Eucalyptus ovate, *A. nilotica*: Acacia nilotica, *M. koenigii L.S*: Muraya koenigii L. Sprengel, *R. rubiginosa*: Rosa rubiginosa, *H. sabdariffa L*: Hibiscus sabdariffa L, *M. indica*: Magnifera indica, *O. sanctum* Linn: Ocimum sanctum Linn, *A. barbadensis*: Aloe barbadensis, *A. vera*: Aloe vera

Table 2: Details of the bacteria used for antimicrobial efficacy testing

Bacteria	ATCC Number	Selective media used for revival	Type of hemolysis on blood agar plate	Media for antimicrobial efficacy testing
S. mutans	25175	Brain heart infusion agar with 5% sheep blood	Gamma hemolysis	Brain heart infusion agar
S. sanguis	10556	Brain heart infusion agar with 5% sheep blood	Alpha hemolysis	Brain heart infusion agar
S. salivarius	13419	Brain heart infusion agar with 5% sheep blood	Gamma haemolysis	Brain heart infusion agar

ATCC: American Type Culture Collection, *S. mutans*: *Streptococcus mutans*, *S. sanguis*: *Streptococcus sanguinis*, *S. salivarius*: *Streptococcus salivarius*

Table 3: Antimicrobial efficacy of various herbal extracts on S. mutans, *S. sanguis* and *S. salivarius*

Herbal extract	Mean diameter of inhibition zone in mm (SD)		
	S. mutans	*S. sanguis*	*S. salivarius*
A. indica	7.25 (0.28)	No activity	No activity
P. guajava	21.17 (1.08)	18.58 (0.59)	23.00 (0.48)
Eucalyptus hybrid (*E. canaldulensis* × *E. ovate*)	20.83 (1.08)	19.17 (0.82)	20.00 (0.45)
A. nilotica	21.83 (0.41)	21.33 (0.26)	21.17 (1.1)
M. koenigii L.S	9.75 (1.17)	10.42 (0.92)	13.25 (0.69)
R. rubiginosa	No activity	No activity	No activity
H. sabdariffa L	7.42 (0.20)	6.50 (0.63)	9.75 (0.27)
M. indica	No activity	No activity	No activity
O. sanctum Linn	No activity	No activity	No activity
A. barbadensis	No activity	No activity	No activity
0.2% Chlorhexidine	14.25 (0.52)	21.83 (0.61)	22.08 (0.38)
DMSO	No activity	No activity	No activity
Statistical inference	*F* value: 1363.69 df: 11 *P* value: 0.001	*F* value: 2433.569 df: 11 *P* value: 0.001	*F* value: 3037.169 df: 11 *P* value: 0.001

S. mutans: *Streptococcus mutans*, *S. sanguis*: *Streptococcus sanguinis*, *S. salivarius*: *Streptococcus salivarius*, *A. indica*: *Azadiractha indica*, *P. guajava*: *Psidium guajava*, *E. canaldulensis*: *Eucalyptus canaldulensis*, *E. ovate*: *Eucalyptus ovate*, *A. nilotica*: *Acacia nilotica*, *M. koenigii* L.S: *Muraya koenigii* L. Sprengel, *R. rubiginosa*: *Rosa rubiginosa*, *H. sabdariffa* L: *Hibiscus sabdariffa* L, *M. indica*: *Magnifera indica*, *O. sanctum* Linn: *Ocimum sanctum* Linn, *A. barbadensis*: *Aloe barbadensis*, DMSO: Dimethyl sulfoxide, SD: Standard deviation

Table 4: Multiple pair-wise comparisons between different categories on antimicrobial efficacy against *S. mutans, S. sanguis,* and *S. salivarius*

Herbal extract*	Mean diameter of inhibition zone in mm (SD)		
	S. mutans	*S. sanguis*	*S. salivarius*
A versus B	0.001	0.001	0.001
A versus C	0.001	0.001	0.001
A versus D	0.001	0.001	0.001
A versus E	0.001	0.001	0.001
A versus F	1.000	0.001	0.001
A versus G	0.001	0.001	0.001
A versus H	0.001	1.000	1.000
B versus C	0.998	0.600	0.001
B versus D	0.732	0.001	0.001
B versus E	0.001	0.001	0.001
B versus F	0.001	0.001	0.001
B versus G	0.001	0.001	0.037
B versus H	0.001	0.001	0.001
C versus D	0.166	0.001	0.002
C versus E	0.001	0.001	0.001
C versus F	0.001	0.001	0.001
C versus G	0.001	0.001	0.001
C versus H	0.001	0.001	0.001
D versus E	0.001	0.001	0.001
D versus F	0.001	0.001	0.001
D versus G	0.001	0.794	0.037
D versus H	0.001	0.001	0.001
E versus F	0.001	0.001	0.001
E versus G	0.001	0.001	0.001
E versus H	0.001	0.001	0.001
F versus G	0.001	0.001	0.001
F versus H	0.001	0.001	0.001
G versus H	0.001	0.001	0.001

A: *Azadiractha indica*, B: *Psidium guajava*, C: *Eucalyptus hybrid*, D: *Acacia nilotica*, E: *Murraya koenigii* L. Sprengel, F: *Hibiscus sabdariffa* L, G: 0.2% chlorhexidine, H: Dimethyl sulfoxide, *S. mutans*: *Streptococcus mutans*, *S. sanguis*: *Streptococcus sanguinis*, *S. salivarius*: *Streptococcus salivarius*, SD: Standard deviation

inhibit the growth of *S. salivarius*. Although a significant difference in the mean diameter of inhibition zone was noted between various categories, the multiple pair-wise comparisons revealed that the extracts of *P. guajava* produced a significantly higher zone of inhibition against *S. salivarius* compared chlorhexidine and other extracts (*P* = 0.001, Table 4). The mean diameter of inhibition zone produced by chlorhexidine was 22.08 ± 0.38).

It can be inferred from the above results that the leaf extracts derived from *A. nilotica*, *P. guajava*, *Eucalyptus* hybrid, and *M. koenigii* L.S possess anti-microbial efficacy

against all these bacteria. The inhibitory effect of *P. guajava*, *A. nilotica*, and *Eucalyptus* hybrid extracts on *S. mutans* were significantly higher than that produced by chlorhexidine.

DISCUSSION

The increasing trends in dental caries and gingival periodontal diseases among the population in developing countries, the increasing costs of treating these diseases, and the potential side effects associated with conventional anti-plaque agents call for alternate strategies. The extracts derived from medicinal plants are staging a comeback, and herbal "renaissance" is occurring all over the globe. The plant medicines, now a day, symbolize safety, in contrast to the synthetics that are shown to be unsafe to humans and the environment to some extent. Plant medicines are the foremost remedial agents used in traditional medicine for

several 1000 years. The literature demonstrates that around 2500 plant species have been used by traditional healers in India and 100 of these are used as regular sources of medicine. The last few decades have witnessed a surge in the research concerning the traditional use, and efficacy of medicinal plants in different parts of the world.[5] The extracts of medicinal plants have been shown to possess anti-plaque, anti-oxidant, anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, and analgesic effects. The present study was undertaken to screen ten plant extracts that are easily available in central India for their anti-microbial efficacy against three bacteria that are regarded as primary plaque colonizers.

The extract of *A. indica* has been found to possess anti-microbial and anti-viral properties.[6] Prashant *et al.*[7] (2007) found that the extracts derived from the twigs of *A. indica* inhibited *S. mutans*, *S. sanguis*, and *S. salivarius*. The mean diameter of the inhibition zone produced against these bacteria at 10% concentration at the end of 24 h was 1.1 mm, 1.0 mm, and 0.8 mm respectively. The mean diameter of the inhibition zone by the leaf extract of *A. indica* against *S. mutans* was much higher in our study compared to these results. The anti-microbial efficacy of extracts derived from *A. indica* could be attributed to the presence of alkaloid margosine, resins, tannins, oils, saponins, flavonoids, sterols, gum, chloride, fluoride, silica, sulfur, and calcium.[8] However, the leaf extract of *A. indica* in our study failed to inhibit *S. sanguis* and *S. salivarius*. The difference in the part of the plant, the bacterial strains, the solvent, and the extraction process employed for anti-microbial efficacy testing may explain these differences observed in our study and others.

The leaf of *P. guajava* has been widely used in the management of gastroenteritis and child diarrhea by the rural folk who cannot afford antibiotics. The leaf extracts of *P. guajava* inhibited the growth of all the three bacteria in our study, and its efficacy was comparable to chlorhexidine. The anti-microbial efficacy of *P. guajava* leaf extract could be due to the presence of guajaverin, essential oil constituents (monoterpenes, 1.8-cineol, ϱ-cimen, and acetate of α-terpenil), and psidiolic acid.[9] Hema *et al.* (2009)[10] in their study found *P. guajava* to be effective against *Pseudomonas lundensis*, *Aspergillus niger*, and *Aspergillus flavus*. Gonçalves *et al.* (2008)[11] found the essential oils, methanol, hexane, ethyl acetate extracts of *P. guajava* leaves to be effective against *Staphylococcus aureus*, *Salmonella* spp. and *Escherichia coli*. Hence, the extract could be considered important potential sources for anti-microbial compounds. Ismail *et al.* (2012)[12] in their study found Guava to be effective against *S. aureus*, and *E. coli*.

Our study demonstrated the anti-microbial efficacy of the leaf extracts of *P. guajava* against primary plaque colonizers similar to these studies. However, we could not compare our findings with other studies as this study is the first of its kind assessing the anti-microbial efficacy of *P. guajava* leaf extract on oral bacteria.

Eucalyptus was found to inhibit all the three bacteria in our study, and the mean inhibition zone was comparable to chlorhexidine. Nagata *et al.* (2006)[13] demonstrated anti-bacterial activity of macrocarpals which are phloroglucinol derivatives from *Eucalyptus* leaves against periodontopathic bacteria. *Porphyromonas gingivalis* exhibited the highest sensitivity to macrocarpals among the various tested bacteria. Takarada *et al.* (2004)[14] in their study found the leaf extracts of *Eucalyptus* to be effective against *S. mutans*, *Streptococcus sobrinus*, *Actinobacillus actinomycetemcomitans*, *P. gingivalis*, and *Fusobacterium nucleatum*. The study also found that the *Eucalyptus* oil was successful in preventing the adhesion of *S. mutans*. The results of our study clearly demonstrated the usefulness of the leaf extract of *Eucalyptus* hybrid as an anti-bacterial agent similar to these studies.

A. nilotica exhibited an inhibitory effect against all the three bacteria tested in the present study. The inhibitory effect was comparable to chlorhexidine. Deshpande Kadam (2013)[15] found *A. nilotica* extracts to inhibit the growth of *S. mutans* (mean diameter of the inhibition zone was 31 mm ± 0.7). An *in vitro* study by Dabur *et al.* (2007)[16] found the extracts of *A. nilotica* to possess anti-microbial and anti-fungal properties. Pai *et al.* (2010)[17] in their *in vitro* study found *A. nilotica* to be effective against *Candida albicans*. All these studies demonstrated the anti-microbial potential of *A. nilotica* against oral microbes similar to the findings in our study. The anti-microbial potential of *A. nilotica* could be due to the presence of tannins, alkaloids, flavonoids, saponins, anthraquinones, and cardiac glycosides.[18]

M. koenigii L. used as a spice for its characteristic flavor and aroma is an important herb of Indian Origin. This herb has been extensively used for centuries in Ayurvedic medicine.[19,20] The herb is reported to possess a variety of medicinal benefits that include stimulant, anti-microbial, anti-diabetic, anti-oxidant, anti-dysenteric, anti-carcinogenic properties. The carbazole alkaloids, b-carotene, tocopherol, and lutein in *M. koenigii* L. are reported to possess anti-microbial properties.[19] The leaf extract of *M. koenigii* L. in our study inhibited all the three bacteria thus, demonstrating the anti-bacterial efficacy similar to these studies. However, the mean diameter of

the inhibition zone produced by the plant extract was significantly lesser than chlorhexidine. Ningappa *et al*. (2010)[19] found *M. koenigii* to exhibit a broad spectrum of anti-bacterial activity against human pathogenic bacteria, which was as good as commercial antibiotics. The authors recommended the extracts of *M. koenigii* L. *to be a* nontoxic, anti-oxidant antibiotic. Sunitha *et al*. (2012)[21] found the alcoholic extracts of *M. koenigii* L. to exhibit the anti-bacterial activity on dental caries bacteria. Ramesh *et al*. (2012)[22] found the chewing of curry leaves to increase the salivary pH. The authors concluded that the plant extract could be used as home remedy as it creates an oral environment that is unfavorable for microbes. The results of our study and all the studies cited demonstrate that the leaf extract of *M. koenigii* L. could be tried as a potential anti-microbial agent that is effective against dental caries and plaque microorganisms.

H. sabdariffa L leaf extract inhibited the growth of all the three bacteria in the present study. However, the mean inhibition zone was significantly less compared to chlorhexidine. The anti-bacterial activity could be attributed to the presence of biterpenoids, alkaloids, phenolics, and flavonoids. Fullerton *et al*. (2011)[23] found the extracts of *H. sabdariffa* to be effective against *E. coli* and concluded that the extract proved to be a promising anti-microbial agent. Nagarajappa *et al*. (2013)[24] in their *in vitro* study found the leaves of *Hibiscus rosa-sinensis* to inhibit the growth of *S. mutans*. These results were consistent with the findings of our study.

The extracts of *O. sanctum* Linn, *M. indica*, *R. rubiginosa*, *A. barbadensis* failed to inhibit the growth of *S. mutans*, *S. sanguis* and *S. salivarius*. Tulsi (*O. sanctum*), is the holiest and most sacred herbs grown in India. In view of its therapeutic benefits, it is known as the queen of herbs or mother medicine of nature. Agarwal *et al*. (2010)[25] in their *in vitro* study found tulsi extract at 4% concentration to exhibit maximum anti-microbial efficacy against *S. mutans* (18 mm). The mean zone of inhibition at 10% concentration was 16 mm. *M. indica* contains bitter gum, tannins, and resins.[26] Prashant *et al*. (2007)[7] found the twig extract of Mango to inhibit the growth of *S. mutans*, *S. sanguis*, and *S. salivarius* at 10% concentration. *A. barbadensis* Miller is widely used as a convenient home grown remedy in the management of burns and also possesses many other medicinal benefits. Two *in vitro* studies on the efficacy of *Aloe vera* extracts on oral bacteria involved in dental caries and periodontitis found the product to be effective.[27,28] Kumar *et al*. (2012)[29] found the leaf, stem, and root extracts of *Rosa indica* to be effective against two Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa)* and two Gram-positive bacteria

(*Bacillus licheniformis* and *S. aureus)* The results of our study were contradictory to the findings of these studies. This contradiction in the efficacy of the extracts of *O. sanctum* Linn, *M. indica*, *A. barbadensis* and *R. rubiginosa* could be due to differences in the plant species, its part being used for extraction, the phytochemical constituents (that vary with the season, soil etc.), the extraction process, the bacteria, and the method of assessing the anti-microbial efficacy. The contradictory findings that may be due to differences in the phytochemical constituents of the herbal extracts in different regions highlight the need for mandatory and thorough investigation of plant extracts before their use in pharmacological industries.

SUMMARY AND CONCLUSION

The present study was undertaken to assess the anti-microbial efficacy of the leaf extracts of 10 plants that are easily available in Central India. The anti-microbial efficacy was assessed on three bacteria which are initial colonizers in the plaque formation. The study found the leaf extracts of *P. guajava*, *Eucalyptus* hybrid, *A. nilotica*, *M. koenigii* L.S and *H. sabdariffa* L to be effective against *S. mutans*, *S. sanguis* and *S. salivarius*. The mean inhibition zone produced by *P. guajava*, *Eucalyptus* hybrid, *A. nilotica* was comparable to 0.2% chlorhexidine. The mean diameter of the inhibition zone produced by *M. koenigii* L.S and *H. sabdariffa* L extracts was lesser in comparison to chlorhexidine. The leaf extract of *A. indica* though inhibited *S. mutans*, it failed to inhibit *S. sanguis* and *S. salivarius*. The leaf extracts of *M. indica*, *O. sanctum* Linn, *R. rubiginosa*, and *A. barbadensis* failed to inhibit the three bacteria at 10% concentration.

We can conclude from these results that the leaf extracts of *P. guajava*, *Eucalyptus* hybrid, *A. nilotica*, *M. koenigii* L.S, and *H. sabdariffa* L have the potential to inhibit primary plaque colonizers, and they could be used as anti-plaque agents. The *in vitro* nature of the study call for further research using these extracts. The present study was conducted using a working concentration of 100 mg/ml. The use of higher concentrations may yield significantly higher benefits and the anti-microbial efficacy testing using clinical isolates of these bacteria may yield different results. These extracts could also be further tested against *Lactobacillus acidophilus*, secondary and tertiary plaque colonizers. The further studies using the combination of these plant extracts could yield a formulation that is either as effective as chlorhexidine or superior to it. The development of such an innovative formulation is the need of the hour as it could help in preventing two most common dental diseases afflicting mankind.

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