Research Article

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ANTI-MICROBIAL EFFICACY OF AN ETHANOLIC EXTRACT OF *MURRAYA KOENIGII* AGAINST PERIODONTAL PATHOGENS LIKE *PORPHYROMONAS GINGIVALIS* AND *AGGREGATIBACTER ACTINOMYCETEMCOMITANS*: *IN VITRO* ANALYSIS

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ABSTRACT

Periodontitis is primarily an infectious disease with *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* present in plaque biofilm as the key pathogens, causing destruction of supporting periodontal tissues. Herbal formulations are widely substituting synthetic anti-microbials due to their minimal adverse effects and cost-effectiveness. *Murraya koenigii* (*M. koenigii*) is a well-known anti-microbial agent used in treating systemic infections. Hence its anti-microbial efficacy against key periodontal pathogens also needs to be tested. The aim of this study is to determine Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and zone of inhibition of *M. koenigii* ethanolic extract against *P. gingivalis* and *A. actinomycetemcomitans.* Thioglycollate agar was used for culturing both *P. gingivalis* and *A. actinomycetemcomitans.* For determination of MIC and MBC broth dilution technique was used. For MIC, serial dilutions of extract were made and culture tubes were incubated in an anaerobic jar and observed for their turbidity. For MBC determination, dilution tubes sensitive to MIC were plated and incubated for the next 24 hours to monitor growth, and colony count was taken. MIC results showed *A. actinomycetemcomitans* was more sensitive to *M. koenigii* extract than *P. gingivalis* and got inhibited at 0.8 µg/ml. Also, MBC results showed extract has strong bactericidal activity towards *A. actinomycetemcomitans*. Disk diffusion test results showed bactericidal activity against both *A. actinomycetemcomitans* and *P. gingivalis* with a larger inhibition zone (15 mm) towards *P. gingivalis* at a concentration of 75 µl/ml. *M. koenigii* ethanolic extract is bactericidal against periodontal pathogens like *A. actinomycetemcomitans* and *P. gingivalis* and can be used as a safe and effective alternative for synthetic chemotherapeutic agents in the future.

Keywords: *M. koenigii, P. gingivalis, A.actinomycetemcomitans.*

INTRODUCTION

Periodontal disease is a poly microbial, multifactorial disease characterized by complex host-parasite interactions, microbial composition changes, altered immune-inflammatory response, leading to gingival inflammation, loss of connective tissue attachment, periodontal ligament destruction, and alveolar bone resorption. The primary etiological factor responsible for periodontal disease is dental plaque.[1] It consists of microorganisms of various strains and species embedded in an extracellular matrix composed of bacterial metabolic products and substances from serum, saliva, and blood. *P. gingivalis* and *A. actinomycetemcomitans* present in plaque biofilm are considered key periodontal pathogens for causing periodontal destruction.[2] Hence; plaque control is regarded as the cornerstone of good oral hygiene practice.

Mechanical measures of plaque control include home care measures like tooth brushing, flossing, and professional procedures like scaling and root planning (SRP), which is considered as the "gold standard" treatment for periodontal diseases. Various chemical measures include the use of mouthwash, topical gels, and local drug[3] delivery system. However, in an attempt to overcome certain limitations of mechanical procedures and ill-effects of synthetic chemotherapeutic agents like the emergence of drug resistance, hypersensitivity reactions, pigmentation of teeth, research continues to find new and better alternatives to conventional treatment protocols.[4] In this regard, there is a continuous ongoing hunt for potent molecules from natural herbal medicines.[5]

Extracts obtained from natural resources can be utilized as therapeutic agents in treating human diseases and health-promoting agents in prophylactic therapy. These extracts are secondary metabolites of plants such as alkaloids, flavonoids, terpenoids, vitamins, tannins, etc., which act as an active constituent and physiologically affect the body at different stages of body development and helps to make the body disease-free.[6] Till date, about only 1% of available plant species have been phytochemically investigated. Therefore, there is great potential for discovering novel bioactive compounds which can be used therapeutically with minimal or virtually no side effects.[7] According to the World Health Organization, about 80% of the population, primarily in developing and industrialized countries, still relies on herbal medicines for their primary health care.[8]

Murraya koenigii is one of the plant species with proven potential medicinal properties. Different parts of *M. koenigii*, such as its seeds, roots, bark, leaves, and fruits, promote various biological activities and possess multiple pharmacological properties to treat many human ailments. Phytoconstituents of the plant predominantly include carotenoids, carbazole alkaloids, coumarin, carbazole carboxylic acid, lipids, vitamins, proteins, and essential oils.[9] *M. koenigii* leaves also contain a wide range of crystalline glycosides; carbazole alkaloids such as koenigii, girinimbin, isomahanimbin, koenine, koenimbine, O-methyl murrayamine, O-methyl mahanine, isomahanine, bismahanine,

bispyrayafoline, murrayazoline, murrayazolidine and essential oils that consists of sesquiterpenes, monoterpenoids and its oxygenated derivatives.[10] Fresh young leaves contain yellow-colored volatile oil rich in vitamin A, calcium, and carbazole alkaloids and are also a source of proteins, carbohydrates, fiber, minerals, carotene, nicotinic acid, vitamin C and oxalic acid.[9]

M. koenigii is known for its anti-microbial, anti-oxidative, anti-inflammatory, antiulcer, analgesic, anti-diabetic, anti-osteoporotic, cytotoxic, phagocytic and immunomodulatory activity.[11] It also contains chlorophyll that is proposed as an anti-cariogenic agent and reduces halitosis.[12] Carbazole alkaloids, essential oils, and ethanolic extracts of *M. koenigii* leaves have shown antibacterial activity against gram-positive, gram-negative and antibiotic-resistant bacteria with a larger zone of a diameter of inhibition, indicating the presence of a broad spectrum of antibacterial activity. Hence, *M. koenigii* leaves could be efficiently used as a natural remedy in everyday meals for the prevention of several bacterial infections.[13]

To evaluate the anti-microbial efficacy of a drug and bacterial sensitivity to that drug, it is necessary to test the specific isolated bacteria against an appropriate anti-microbial agent. The lowest concentration of an antibiotic that will inhibit the growth of the organism being tested is known as the minimal inhibitory concentration (MIC). It determines the concentration of antibiotics needed to inhibit the pathogen. The minimal bactericidal concentration (MBC) is defined as the lowest concentration of an antibiotic, killing the majority (99.9%) of a bacterial inoculum. Zone of inhibition determines the area of media where bacteria cannot grow due to the drug that impedes their growth. The larger the zone of inhibition, the more significant is the antibacterial activity.[14]

Considering all the above findings, the present study aimed to evaluate anti-microbial efficacy of *M. koenigii* ethanolic extract by determining Minimum Inhibitory Concentration (MIC), Minimum Bactericidal concentration (MBC) and zone of inhibition against periodontal pathogens like *P. gingivalis* and *A. actinomycetemcomitans.*

MATERIAL AND METHODS

The present *in vitro* study was conducted at Maratha Mandal's Central Research Laboratory, Karnataka, India over two months, from October to December 2020. The *M. koenigii* ethanolic leaf extract used in this study was obtained from a herbal extract dealer under aseptic conditions.

Determination of MIC

Determination of MIC and MBC is traditionally done with the broth dilution technique. Serial dilutions of antibiotics are incorporated into the broth media in either the wells of micro titer plates or in culture tubes. For determination of MIC and MBC, for both *P. gingivalis*, *A. actinomycetemcomitans* media used was Thioglycollate broth. In the initial tube, 20 µl of the extract were added into 380 µl of Thioglycollate broth. 200 µl of Thioglycollate broth was added into the following nine tubes separately for dilutions. Two hundred µl were then transferred from the initial tube to the first tube containing 200 µl of Thioglycollate broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube, 200 µl were transferred to the second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution. From the maintained stock cultures of *P. gingivalis*, *A. actinomycetemcomitans*, 5 microliter, was taken and added into 2 ml of Thioglycollate broth. In each serially diluted tube, 200 µl of the above culture suspension were added. The tubes were incubated for 48-72 hours in an anaerobic jar at 37°C and observed for turbidity.[15]

Determination of MBC

MBC was done to check whether the bacteriostatic or bactericidal effect of the extract (Drug) was against the organism. For determination of MBC, MIC dilutions tubes, which were sensitive in MIC, were plated and incubated for 24 hours, and colony count was taken on the next day. If growth is seen, it has a bacteriostatic effect, and if no growth is seen, it has a bactericidal effect.[16]

Determination of Zone of Inhibition

Zone of inhibition was determined using disk diffusion test. The media used was Brain Heart Infusion agar for *A. actinomycetemcomitans* and blood agar for *P. gingivalis.* Inoculum preparation was done using a loop or swab colonies that were transferred to the plates. Turbidity was visually adjusted with broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed. Also, the suspension was standardized with a photometric device. Inoculation of agar plate was done within 15 min of adjusting the inoculum. The excess inoculum was removed and the inoculated plate was allowed to stand before making wells for at least 3 minutes but no longer than 15 minutes. The stock solution was prepared by dissolving 10 mg of the compound in 1 ml of Dimethyl Sulfoxide (DMSO). Five wells were made by a heating tube of 5 mm diameter and pressing on the agar plate on each plate. The compound was added with the help of a micropipette in each well, and culture plates were incubated for 18-24 hours, within 15 minutes of compound application. For anaerobic organisms, culture plates were incubated in the anaerobic jar at 37ºC. Culture plates were read-only if the lawn of growth was confluent or nearly confluent. The inhibition zone diameter was measured to the nearest whole millimeter by holding the measuring device.

RESULT

MIC results showed that *A. actinomycetemcomitans* was inhibited at 0.8 µg/ml and *P. gingivalis* was inhibited at 25 µg/ml; indicating *A. actinomycetemcomitans* is much more sensitive to *M. koenigii* extract than *P. gingivalis*. Table 1, MBC results from Table 2, Figure 1, depicted *M. koenigii* ethanolic extract was bactericidal against both *A. actinomycetemcomitans* and *P. gingivalis*, at a concentration of 0.8 µg/ml and 25 µg/ml, respectively, indicating its strong cidal activity against *A. actinomycetemcomitans*. Table 3, Figure 2 depicts ethanolic extract of *M. koenigii* at a concentration of 75 µl/ml showed 13 mm zone of inhibition against *A. actinomycetemcomitans* and 15 mm zone of inhibition against *P. gingivalis*, indicating its bactericidal activity against both organisms, with relatively higher cidal action against *P. gingivalis*.

DISCUSSION

Nature has an answer to cure every disease. Combating microbial infections with the least possible side effects is a challenging task. Hence, there has been tremendous growth in the field of herbal medicine as therapeutic and prophylactic agents and is gaining popularity across the globe because of negligible side effects, low costs, and easy availability. India is well known for its enormous biodiversity of medicinal herbs and is used to treat various ailments for ages. Herbal drugs are the secondary metabolites of plants known as phytochemicals which, in contrast to primary metabolites, are required only indirectly to enable plants to survive and reproduce in a given competitive ecosystem.[17]

Table 1: MIC Results

Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
Curry leaves										
Aa	S	S	S	S	S	S	S	S	R	R
Pg	S	S	S	R	R	R	R	R	R	R

Aa: *Aggregatibacter actinomycetemcomitans*, *Pg: Porphyromonas gingivalis,* µl: microliter, ml: milliliter, S: sensitive, R: resistant

Table 2: MBC results

Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
Curry leaves										
Aa	NG	NG	NG	NG	NG	NG	NG	NG	83	94
Pg	NG	NG	NG	16	24	40	56	62	98	102

Aa: *Aggregatibacter actinomycetemcomitans*, *Pg: Porphyromonas gingivalis*, µl: microliter, ml: milliliter, NG: no growth

Table 3: Disc Diffusion Results

Samples	75 µl/ml	50 µl/ml	25 µl/ml	10 µl/ml	5 µl/ml
Curry Leaves					
Aa	13 mm	12 mm	R	R	R
Pg	15 mm	13 mm	R	R	R

Aa: *Aggregatibacter actinomycetemcomitans*, *Pg: Porphyromonas gingivalis*, µl: microliter, mm: millimeter, R: resistant

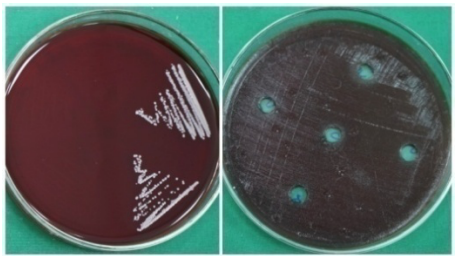

Figure 1: *A. actinomycetemcomitans*

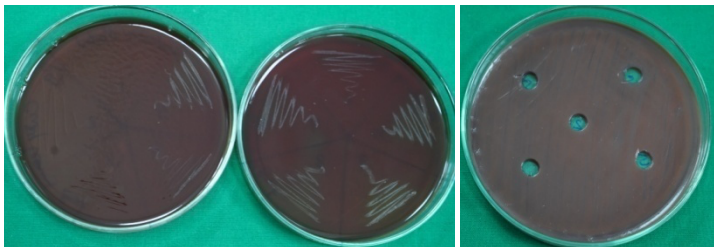

Figure 2: *P. gingivalis*

M. koenigii, one such plant of Indian origin, is used as a spice in Indian food for its characteristic flavor and aroma and is used for therapeutic purposes for centuries in the Ayurvedic medicinal system. However, its implications in dentistry are still not thoroughly explored. Leaves of *M. koenigii* are a rich source of minerals like calcium, phosphorus, iron, zinc, magnesium, vitamins like vitamin A, vitamin B, vitamin C; and vitamin E, carbohydrates, proteins, oxalic acid, and phytochemicals present includes crystalline glycosides; carbazole alkaloids, flavonoids, phenols, tannins and essential oils which have anti-microbial, antioxidant, analgesic, anti-inflammatory properties, cytotoxic and immunomodulatory activities.[10,18]

Dental plaque essentially harboring various microorganisms is the primary etiological factor for periodontal diseases. Amongst the poly-microbial micro biota, bacterial species like *A. actinomycetemcomitans* and *P. gingivalis* form the late colonizers of dental plaque and are considered vital for the body periodontal destruction. Chandrashekhar BR *et al*.[19] demonstrated antibacterial efficacy *M. koenigii* extract on primary plaque colonizers like *S. mutans*, *S. sanguinis*, and *S. salivarius* with greater antibacterial activity towards *S. salivarius*. However, there is not much literature available on the antibacterial efficacy of *M. koenigii* against late plaque colonizers like *A. actinomycetemcomitans* and *P. gingivalis*. Hence this study was undertaken to evaluate the anti-microbial efficiency of ethanolic extract of *M. koenigii* by determining MIC, MBC and zone of inhibition against *A. actinomycetemcomitans* and *P. gingivalis* using disk diffusion test.

In the present study, results of minimum inhibitory concentration tests of *M. koenigii* ethanolic extract showed *A. actinomycetemcomitans* was inhibited at a much lower concentration of 0.8 µg/ml than *P. gingivalis* 25 µg/ml, indicating *A. actinomycetemcomitans* to be more sensitive to the extract than *P. gingivalis*. Also, the extract showed bactericidal activity against both *A. actinomycetemcomitans* and *P. gingivalis*, with a strong cidal activity against *A. actinomycetemcomitans* (0.8 µg/ml). A similar *in vitro* study by Nithya *et al*. showed inhibitory effects of *M. koenigii* ethanolic extract against the most

predominant periodontal pathogens such as *Porphyromonas gingivalis* and *Prevotella intermedia* with MIC of 0.8 mg/ml. Also, the sub MIC concentration of extract suppressed the adherence and co-aggregation of this microorganisms.[11] Disk diffusion test showed *M. koenigii* ethanolic extract exhibits bactericidal activity against both *P. gingivalis* and *A. actinomycetemcomitans* with a slightly greater inhibition zone (15 mm), indicating greater bactericidal activity against *P. gingivalis* at a concentration of 75 µl/ml. This was in accordance with a study by Chandrashekhar BR *et al*.[20] who evaluated anti-microbial efficacy of herbal extracts and Chlorhexidine on *F. nucleatum* and *P. gingivalis* which showed a zone of inhibition of 15.33 mm against *P. gingivalis* and 13.67 mm against *F. nucleatum* by *M. koenigii* ethanolic extract, which was fairly comparable to Chlorhexidine that showed zone of inhibition of 18.8 mm against *F. nucleatum* and 19.5 mm against *P. gingivalis*. This clearly explains the bactericidal activity of *M. koenigii* ethanolic extract against the periodontal pathogens. There are no contrasting studies available in the previously published literature to the above results. Our present study is the first of its kind assessing the anti-microbial efficacy of *M. koenigii* ethanolic extract against *A. actinomycetemcomitans* and *P. gingivalis.*

Another study by Chandrashekhar *et al*.[21] demonstrated the antibacterial activity of *M. koenigii* against *Streptococcus mutans* and *Lactobacillus acidophilus* also proves their anti-cariogenic activity.

Recently, Gupta A *et al*.[22] demonstrated the effectiveness of *M. koenigii* mouthwash in maintaining the salivary pH and tongue coating pH, which inhibits oral pathogenic bacterial growth. Ramesh G *et al*. [23] found increased pH of saliva and tongue coating after chewing curry leaves. It was concluded that it could be used as a home remedy as it creates an oral environment that is unfavorable for microbes. Ningappa *et al*.[24] found *M. koenigii* extract to exhibit a broad spectrum of antibacterial activity against human pathogenic bacteria, like *Escherichia coli*, *Salmonella typhi*, *Vibrio cholera*, *Klebsiella pneumonia*, *Bacillus subtilis* which was as good as commercial antibiotics. Considering the facts mentioned above, we can state that *M. koenigii* ethanolic extract exhibits a wide spectrum of antibacterial action and can be used as a potential antiplaque agent, ultimately helping in the prevention and treatment of periodontal diseases. However, the present study can be extended to evaluate this extract's effect against other pathogenic organisms present in the dental plaque. This study's *in vitro* nature calls for further clinical research using *M. koenigii* extract. A combination of other herbal extracts with *M. koenigii* extract can also be tested to evaluate anti-microbial activity for better results.

CONCLUSION

Herbal medicines over conventional synthetic drugs provide an edge over the latter when supported by proper scientific evidence. The present study offers appropriate scientific data to conclude the anti-microbial activity of *M. koenigii* ethanolic extract against key periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis* in dental plaque. The values of MIC and MBC obtained from this study can be used for formulating different antiplaque agents using *M. koenigii* ethanolic extract. Thus, in this era of herbal medicines, innovative formulations using the *M. koenigii* extract can serve humankind by preventing as well as treating periodontal diseases, replacing the conventional synthetic chemotherapeutic agents, in turn reducing their side effects. However, further studies evaluating the efficacy and safety of the *M. koenigii* ethanolic extract are required for establishing enough scientific data before prescribing this herbal medicine as an alternative to a regular chemotherapeutic agent.

REFERENCES

1. Nagasri M, Madhulatha M, Musalaiah SV, Kumar PA, Krishna CH, Kumar PM. Efficacy of *Curcumin* as an adjunct to scaling and root planning in chronic periodontitis patients: A clinical and microbiological study. J Pharm Bio allied Sci 2015; 7 Suppl 2: S554-8.
2. Popova C, Dosseva-Panova V, Panov V. Microbiology of Periodontal Diseases. A Review. Biotechnology & Biotechnological Equipment 2013; 27(3): 3754-59.
3. Cobb CM. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planning. J Clin Periodontol 2002; 29 Suppl 2: 6–16.
4. Lecic J, Cakic S, Janjic Pavlovic O, Cicmil A, Vukotic O, Petrovic V, *et al*. Different methods for sub gingival application of Chlorhexidine in the treatment of patients with chronic periodontitis. Acta Odontol Scand 2016; 74(6): 502-7.
5. Gunjan M, Naing T.W, Saini RS, Dr. Ahmad A, Dr. Naidu J.R., Kumar I. Marketing Trends & Future Prospects of Herbal Medicine in the treatment of Various Disease. World Journal of Pharmaceutical Research 2015; 4(9): 132-155.
6. Cosmas UO., Okafor O.E., Agha N.C., Nwaogu LA., Igwe KO, Igwe CU. Phytochemical and chemical composition of *Combretum zenkeri* leaves. J. Med. Plants Res 2010; 4(10): 965–8.
7. Palombo EA. Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. Evid Based Complement Alternat Med 2011; 2011. Article ID: 680354.1-15
8. Balakrishnan R, Vijayraja D, Jo SH, Ganesan P, Su-Kim I, Choi DK. Medicinal Profile, Phytochemistry and Pharmacological Activities of *Murraya koenigii* and its Primary Bioactive Compounds. Antioxidants (Basel) 2020; 9(2): 101.
9. Gahlawat DK, Jakhar S and Dahiya P. *Murraya koenigii* (L.) Spreng: An ethnobotanical, phytochemical and pharmacological review. Journal of Pharmacognosy and Phytochemistry 2014; 3(3): 109-119.
10. Roop JK. *Murraya koenigii* (Linn.) A plant with potential therapeutic properties. Int J Biol Med Res 2018; 9(3): 6466-672.
11. Varghese A, Babu HM, Kukkera PN. Comparative evaluation of efficacy of *Murraya koenigii* and Chlorhexidine gluconate in the treatment of gingivitis: A randomized controlled clinical trial. J Indian Soc Periodontol 2018; 22(5): 427-32.
12. Pandita V, Patthi B, Singla A, Singh S, Malhi R, Vashishtha V. Dentistry meets nature-role of herbs in periodontal care: A systematic review. J Indian Assoc Public Health Dent 2014; 12 (3): 148-56.
13. Nagappan T, Ramasamy P, Wahid ME, Segaran TC, Vairappan CS. Biological activity of carbazole alkaloids and essential oil of *Murraya koenigii* against antibiotic resistant microbes and cancer cell lines. Molecules 2011; 16(11): 9651-64.
14. Munro S. Disk Diffusion Test. In: Isenberg HD, Garcia SL, editors. Clinical Microbiology Procedures Handbook. 2nd ed. Vol. 2. Washington, DC: ASM Press; 2007. p. 5.1.1-15.
15. Roe-Carpenter D. Broth Micro dilution MIC Test for Anaerobic Bacteria. In: Isenberg HD, Garcia SL, editors. Clinical Microbiology Procedures Handbook. 2nd ed. Vol. 2. Washington, DC: ASM Press; 2007. p. 5.7.1-8.
16. Moody J, Knapp C. Tests to Assess Bactericidal Activity. In: Isenberg HD, Garcia SL, editors. Clinical Microbiology Procedures Handbook. 2nd ed. Vol. 2. Washington, DC: ASM Press; 2007. p. 5.10.1.1-17.

17. Mandal S. Curry plant, *Murraya koenigii* L. An indigenous spice plant with versatile medicinal property: A mini review. Int J Clin Exp Physiol 2016; 3(2): 59-65.
18. Ameh SJ, Obodozie OO, Inyang US, Abubakar MS and Garba M. Current phytotherapy: A perspective on the science and regulation of herbal medicine. Journal of Medicinal Plants Research 2010; Vol. 4(2): 072-081.
19. Chandrashekar BR, Nagarajappa R, Singh R, Thakur R. An *in vitro* study on the anti-microbial efficacy of ten herbal extracts on primary plaque colonizers. Journal of Young Pharmacists 2014; 6(4): 33-9.
20. Chandrashekar BR, Nagarajappa R, Jain R, Singh R, Suma S, Thakur R. Antimicrobial Efficacy of *Acacia nilotica*, *Murraya koenigii* L. Sprengel, *Eucalyptus hybrid, Psidium guajava* extracts and their combinations on *Fusobacterium nucleatum* and *Porphyromonas gingivalis*. Indian J Dent Res 2018; 29(5): 641-5.
21. Chandrashekar BR, Nagarajappa R, Jain R, Singh R, Thakur R, Shekar S. Antimicrobial efficacy of *Acacia nilotica*, *Murraya koenigii* (L.) Sprengel, *Eucalyptus hybrid, Psidium guajava* extracts and their combination on *Streptococcus mutans* and *Lactobacillus acidophilus*. Dent Res J 2016; 13 (2): 168-73.
22. Gupta A and Purohit A. Effectiveness of Curry-Leaf Mouthwash in Maintaining Salivary and Tongue pH as Compared to Chlorhexidine Mouthwash: A Randomized Controlled Trial. J Nat Ayurvedic Med 2018; 2(1): 000114.
23. Ramesh G, Nagarajappa R, Madhusudan AS, Sandesh N, Batra M, Sharma A, Patel SA. Estimation of salivary and tongue coating pH on chewing household herbal leaves: A randomized controlled trial. Anc Sci Life 2012; 32(2): 69-75.
24. Ningappa MB., Dhananjaya BL, Dinesha R, Harsha R, Srinivas L. Potent antibacterial property of APC protein from curry leaves (*Murraya koenigii* L.). Food Chemistry 2009; 118(3): 747-50.

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