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Evaluation and Comparison of Antimicrobial Efficacy of Leaves and Roots Extract of Moringa Oleifera Plant against Periodontal Pathogens - An Invitro Study.

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KEYWORDS

Moringa oleifera, antibacterial, anti-inflammatory, Invitro, periodontal microbes

ABSTRACT:

Introduction: Periodontitis is one of the main causes of tooth loss and is caused by various species of microorganisms Due to the increased incidence of resistance to conventional drugs used and their side effects, herbal medicines have turned out to be a popular form of therapy for both prophylaxis and treatment of various ailments. *Moringa oleifera* plant is a medicinal plant with multifarious ethnomedicinal uses to treat various ailments.*Moringa* has been proven effective as antibacterial and anti-inflammatory agent.

Objectives: This in vitro study was carried out to evaluate and compare the antimicrobial efficacy of leaf and root extract of *Moringa oleifera* against four strains of periodontal pathobionts namely *T.forsythia, F.nucleatum, P.gingivalis,P.intermedia.*

Method: The root and leaves extract were prepared using 95%ethanol by Maceration method of extraction. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts were determined against each bacterium using thioglycolate broth through serial dilution method.

Result: All the test microorganisms were found to be sensitive to both leaf and root extracts. Ethanolic leaf extracts exhibited bactericidal activity against all except *P.intermedia* to which it was bacteriostatic. Root extract inhibited growth of the test microorganisms *F.nucleatum*. *P.gingivalis* and *P.intermedia* and was bactericidal to *T.forsythia*.

Conclusion: Extracts of this easily available plant could be a possible source to obtain a new and effective herbal medicine in the treatment of periodontal diseases. The combination potentials of its extracts with commercially available antibiotics in the treatment of periodontitis holds a promising approach in future.

INTRODUCTION-

Periodontal disease is defined as a multifactorial inflammatory disease that is marked by destruction of the supporting tissues around teeth including periodontal ligament,cementum, alveolar bone, and it is the major cause of tooth loss, if left untreated [1,2].Gingival and periodontal diseases have afflicted humans since the dawn of history. Periodontal disease is a chronic infectious disease of the oral cavity and one of the principal causes of tooth loss in humans. This chronic inflammatory disease that affects the supportive tissues

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of the teeth has a multifactorial etiology[3]. Gingivitis can progress to periodontitis which is characterized by alveolar bone loss, attachment loss and pocket formation. Periodontitis is the 11th most prevailing disease globally and is associated with systemic diseases like Diabetes mellitus, respiratory diseases, cancers etc.

One of the major etiological factors for periodontal disease is the dental plaque biofilm on the teeth surfaces .[3]Effective biofilm destruction plays a key role in oral health. The noxious products produced by the bacteria in dental plaque trigger the inflammatory process in the periodontal tissues. *Fusobacterium nucleatum* in human dental plaque is one of the most commonly implicated micro-organisms in the causation of periodontal disease[3,4] The "Red complex" is an aggregate of three oral bacteria namely-*Tannerella forsythia, Porphyromonas gingivalis* and *Treponema denticola* which are responsible for severe clinical manifestation of chronic periodontal disease. Hence, reducing their levels in the oral cavity is one of the rationales for the prevention and control of periodontal disease. The elimination of the subgingival biofilm and calculus consists of subgingival debridement and the use of adjunctive agents (physical, chemical, host-modulating) and systemic or locally delivered antimicrobials [5-9]. 8–12% of patients within a population exhibit residual periodontal pockets that do not respond favorably to SRP alone [10]. This could be attributed to inadequate control of periodontal biofilm and poor oral hygiene [11].

A number of antiseptics such as chlorhexidine (CHX) have been found to be effective against a wide range of Gram-positive and Gram-negative species as well as capable of penetrating the plaque biofilm [12]. Currently, chlorhexidine is the most widely used chemotherapeutic agent against periodontal disease causing organisms[13]. However, it's widespread and prolonged use is limited by its side effects in the form of teeth staining, taste disturbances etc. Hence, there is a need to for an alternative and equally effective antimicrobial agent against periodontal disease-causing organisms. One such alternative strategy would be to ascertain the antimicrobial properties of medicinal plants. Traditional medicine has its roots, grounded deep in India and is being used here since times immemorial.[14] Ayurvedic medicine is a system of Hindu traditional medicine native to the Indian subcontinent and a form of alternative medicine. *Moringa* plant (drumstick tree) is a highly valued food plant. It has an impressive range of medicinal uses along with a high nutritional value. There is paucity of studies regarding the antimicrobial activity of different parts of *Moringa* plant against periodontal pathogens. Therefore, the purpose of this study was to investigate the antimicrobial efficacy of leaves and root extract of Moringa oleifera plant against four periodontal pathogens namely:

1. *Tannerella forsythia (Tf)*
2. *Fusobacterium nucleatum(Fn)*
3. *Porphyromonas gingivalis(Pg)*
4. *Prevotella intermedia. (Pi)*

2.OBJECTIVES:

The Study involves preparation of the leaves and roots extract of *Moringa oleifera* plant by cold maceration method using ethanol as extracting solvent. The extracts so obtained will be used to-

1. To Evaluate the antimicrobial efficacy of Moringa oleifera root extract using Minimum inhibitory concentration (MIC) against four periodontal pathogens.

2. To evaluate the antimicrobial efficacy of leaf extract of Moringa oleifera using MIC against four periodontal pathogens.

3. To compare the antimicrobial efficacy of leaves and roots extract using MIC against four periodontal pathogens.

3.MATERIALS AND METHODS

In this in vitro comparative study, the leaves and roots were obtained and the extract of *Moringa oleifera* was prepared in an Ayurvedic college in the city.

Preparation and extraction-

Cold maceration method-

First the leaves and roots were washed with tap water. The Roots were cut in small pieces of about 5cm. Then both the roots and leaves were dried in shade until they were completely dried (approx. 5 days) at room temperature and were ground into a coarse powder using an electric grinder.The coarse powder obtained was then stored in an airtight glass jar at room temperature.

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Ethanolic extraction-

The extract was prepared using 95%ethanol as the solvent by Maceration[15] method of extraction.

Two conical flasks were taken, one for leaves extract (fig 1-A) and other for root extract(fig1B). The coarse powder of the leaves and root was added to separate flasks. Ethanol was added to each flask until the coarse powder of plant was completely dissolved in each flask.This solution was kept for 7 days with intermittent stirring. After 7 days, the content of both beakers were filtered using Whatman filter paper.The supernatant collected in the beaker after filtration was the ethanolic extract and the residue in the filter paper was discarded .The supernatant thus collected from each flask was subjected to rotary vacuum evaporator separately where the ethanol was evaporated completely and a semi solid residue was left in each flask which is the ethanolic extract of respective plant part. This residue was then dried completely and placed into airtight vials of 5ml and stored in refrigerator until they were used for antimicrobial activity.

Antimicrobial activity was determined using MIC and MBC of the root and leaves extract against standard strains of 4 periodontal pathogens *viz.Tannerella forsythia, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia.*

A) MIC Test- **Minimum inhibitory concentration (MIC) Test[16]**

9 dilutions of each drug were done with Thioglycolate broth for MIC. In the initial tube 20microliter of drug was added into 380 microliters of Thioglycolate broth. For dilutions, 200microliter of Thioglycolate broth was added into the next 9 tubes separately. Then from the initial tube 200microliter was transferred to the first tube containing 200 microliters of Thioglycolate broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube, 200microliter was transferred to second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of Thioglycolate broth. In each serially diluted tube, 200microliter of above culture suspension was added. The tubes were incubated for 48-72 hours in anaerobic jar at 37°C and observed for turbidity.

B) MBC Test-**Minimum bactericidal concentrations (MBC) Test[16]**

From the MIC dilutions tubes, 5 tubes were plated (which was sensitive in MIC) and incubated for 24 - 48 hrs then next day the colony count was taken. MBC was done to see whether there was bacteriostatic or bactericidal effect of the extract (Drug) against the organism.

If there is no growth, it is considered as bactericidal effect.

If there is growth, it is considered as bacteriostatic effect.

4.OBSERVATION AND RESULTS

P.gingivalis, P.intermedia and F.nucleatum were most sensitive to **leaf extract** at dilution of 50 µl/ml (fig 2A)followed by *T.forsythia* which was most sensitive to **leaf extract** at dilution of 100 µl//ml. (table no.1)

Both *P.intermedia and F.nucleatum* were sensitive to **root extract** at 50 µl//ml dilution (fig 2D) *Pgingivalis* and *T.forsythia* were sensitive to **root extract** at 100 µl//ml dilution.(fig 2B)

Leaf extract at a concentration of 50 µl/ml was found to be **bactericidal** to *P.gingivalis* (fig 3A) followed by *F.nucleatum, T.forsythia*(fig3C*)* at a dilution of 100 µl//ml . Leaf extract at a dilution of 100 µl/ml was **bacteriostatic** to *P.intermedia*.(table no.2)

Root extract at 100 µl/ml dilution was **bactericidal** to *T.forsythia* and *F.nucleatum*. (fig 3D) *P. gingivalis* and *P.intermedia* were most **bacteriostatic** to root extract at dilution of 100 µl/ml.(table no.3)(fig3B)

5.DISCUSSION-

Oral health is an inseparable part of general health since it has an impact on a person's speech, selection of food, quality of life, and well-being. Periodontitis can cause tooth loss, discomfort, and compromises the esthetics and function[17]. Also, studies suggest an association between periodontitis and systemic health problems such as preterm low birth weight, cardiovascular diseases, diabetes mellitus, and chronic obstructive pulmonary disease[15]

Antimicrobial therapy is given as adjunct to conventional periodontal therapy for the treatment of periodontal diseases. However, these allopathic medications have

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various ill effects and limitations. The increasing misuse and developing resistance against these drugs necessitate the search of alternate therapies to combat infections. One of the main advantages of using medical herbs over chemotherapeutic medicines is that adverse reactions, like hypersensitivity and the development of bacterial resistance to standard drugs, are less likely. Chlorhexidine, a substance frequently applied topically as an antibacterial, has been reported to cause negative effects such as allergic contact dermatitis and anaphylaxis.[18,19]

Natural herbs used either exclusively or in combination are proven to be safe and effective in the management of various oral health problems and have the advantage of minimal side effects since they are alcohol and/or sugar-free, which are the two most common ingredients found in other over-the-counter products[17].The phytochemicals present in medicinal plants offer an effective alternate pathway to antibiotics and represent a promising approach in the prevention and therapeutic strategies for oral infections [14]. Many side effects associated with modern medicine have been averted by using herbal medicines as they provide higher degree of safety. These plants contain chemical compounds such as flavonoids, tannins, saponins and various other phenolic contents which are known to have antimicrobial effects[14,20]. In developing countries, due to the expensive cost of antibiotics, the use of medicinal plants for treatment of infection because of its reasonable cost will be a boon to the lower socioeconomic class, if the antimicrobial property of this plant is proven.

Moringa oleifera or the drumstick tree is a well-known food plant across the world and can grow well in varied climate at temperatures of 25-35 degree C. They grow to a height of 30-40 feet an are deciduous perennial trees. *Moringa* can grow in tropical and subtropical areas on all types of soil and is resistant to dry seasons with drought tolerance of up to 6 months[13]. They are known to contain more than 90 types of nutrients in the form of essential vitamins, minerals, amino acids, anti-aging, antibacterial, and anti-inflammatory.[20] This herb is also reported to have varied pharmacological effects which include anti-tumor, antipyretic, antispasmodic, diuretic, antiulcer, hypotensive, hypolipidemic, hepatoprotective effects[22].

The leaves of *Moringa oleifera* are known to contain certain secondary metabolites such as flavonoids, alkaloids and other phenolic compounds[21-22] and these active compounds from plant extract with antibacterial activity can be used to formulate various oral medications. The leaves are widely known to show antimicrobial activity against various other pathogens[22]. According to Anwar et al,[20] their roots contain powerful antibacterial agents like f 4-α-L-rhamnosyloxy benzyl isothiocyanate. Since there is paucity of studies regarding antibacterial effects of root extracts, the present study was carried out to compare the antimicrobial effects of both leaves and root extract against selected periodontal pathogens which are most commonly implicated in periodontal disease.

With this objective, the present in vitro experimental study evaluated and compared the antibacterial effects of ethanolic extract of leaves and roots of the plant against the 4 periodontal pathogens. Elgamily Hanaa et al[21] found that aqueous extracts of moringa oleifera plant generally exhibited little or no antimicrobial activities and ethanol as a solvent extracted maximum metabolites from plants as compared to aqueous extracts, so ethanol was used as a solvent in this study. The MIC results of our study showed that *Porphyromonas gingivalis, Prevotella intermedia ,Tannerella forsythia and Fusobacterium nucleatum* were **sensitive to both leaf and root extracts**. (Table 1). Similarly, Nagarajappa et al[23] in 2019 found that the Leaves and fruit extracts of *Moringa oleifera* were found to be effective with therapeutic potential against *Porphyromonas gingivalis and Prevotella intermedia*.

In the present study, Leaf extract showed **bactericidal** effect (No growth) against *P.gingivalis, F.nucleatum, T.forsythia* at a dilution of 100 µl//ml. Leaf extract at a dilution of 100 µl/ml was **bacteriostatic** to *P.intermedia*. However, **root extract** exhibited bactericidal effect at 100 µl/ml dilution only to T.forsythia and was **bacteriostatic** to *F.nucleatum*. *P.gingivalis and P.intermedia* at 100 µl/ml dilution. (Table 2and 3). Similarly in a study by Elgamily et al, the **leaves extract** of Moringa had the **highest mean inhibition zone** values followed by the roots, mix and at last seeds extracts against Staphylococcus aureus and Streptococcus mutans growth

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The observations of our study can be supported by the findings of Wang et al[24] who identified the phenol compounds in Moringa oleifera Lam leaf extract and stated that its anti-periodontitis activity is by regulating the p38α/MAPK14-OPG/RANKL pathway. They further stated that this extract not only alters the expression of inflammatory cytokines but also significantly reduces alveolar bone resorption in vivo and in vitro and therefore, can be used to clinically treat periodontitis.

The results of the present study can be supported by the findings of Sugiharto et al[25] who assessed the anti-inflammatory activities of the Moringa Leaf Extract in periodontitis cases through IL-6 cytokine analysis in Wistar rats. They found that *Moringa oleifera* leaf extract can reduce the production of the pro-inflammatory cytokine IL-6 induced by *P. gingivalis* bacteria in periodontitis.

The limitations of the present study were that experiments were not repeated multiple times for each strain and the colony count was taken into consideration for MBC.

Studies by Saquib et al[26] have shown that a combination of plant extracts and antibiotics possess a synergistic effect, which results in a significant decrease in levels of MIC for the antibiotics.

Hence future prospects could be studies involving combination of moringa plant extracts with commercially available antibiotics in order to develop antimicrobial agents against periodontopathic organisms thereby preventing the emergence of resistant bacteria and reducing the drug toxicity. Study of subgingival delivery of its extract after non-surgical periodontal therapy should be to performed to treat periodontal diseases, local-drug delivery of herbal gels.

6.CONCLUSION-

Findings from the current in vitro study confirmed that both leaves and root extracts of Moringa oleifera plant have antibacterial activity against periodontal pathobionts. Ethanolic extracts of leaves showed more bactericidal activity as compared to roots extracts. This plant can be therefore be used to formulate new dental products to inhibit microorganisms causing periodontitis owing to their antimicrobial potential. However, laboratory and clinical trials are required to ascertain effectiveness and safety of Moringa plant to render it as a reliable treatment modality for various periodontal ailments. This activity of this herbal extract can be enhanced by the synergism with known antibiotics. This could offer significant potential for the development of novel antimicrobial therapeutic agents and to reduce emerging bacterial resistance in the treatment of oral diseases.

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FIGURE LEGENDS

FIG 1- A) Leaf extract of *Moringa oleifera* plant

B) Root extract of *Moringa oleifera* plant

FIG 2 -A) MIC of Leaf extract against *P.gingivalis*.

B) MIC of root extract against *P.gingivalis*.

C) MIC of leaf extract against *F.nucleatum*.

D) MIC of root extract against *F.nucleatum*.

FIG 3-- A) MBC of leaf extract against *P.gingivalis*

B) MBC of root extract against *P.gingivalis*

C) MBC of leaf extract against *T.forsythia*

D) MBC of root extract against *T.forsythia*

TABLES

Table.1 MIC results of ethanolic extract of leaves and roots of *M.oleifera* against 4 periodontal pathogens.

Sr No.	Samples	100 µl/ml	50 µl/ml	25 µl/ml	12.5 µl/ml	6.25 µl/ml	3.12 µl/ml	1.6 µl/ml	0.8 µl/ml	0.4 µl/ml	0.2 µl/ml	B+C	B+0
	Pg												
01	**Leaf extract**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
02	**Root extract**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
	Pi												
01	**Leaf extract**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**

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02	**Root extract**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**
	Fn												
01	**Leaf extract**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**
02	**Root extract**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**
	Tf												
01	**Leaf extract**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**
02	**Root extract**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**

Pg:P.gingivalis, Pi :P.intermedia , Fn:F.nucleatum , Tf:T.forsythia ;

S-Sensitive R-Resistant

B+O: broth +organism(positive control) ; B+C: broth+control (negative control)

Table.2: MBC results of ethanolic extract of leaves and roots of *M.oleifera* against 4 periodontal pathogens

Sr No.	**Samples**	**100 µl/ml**	**50 µl/ml**	**25 µl/ml**	**12.5 µl/ml**	**6.25 µl/ml**	**3.12 µl/ml**	**1.6 µl/ml**	**0.8 µl/ml**	**0.4 µl/ml**	**0.2 µl/ml**	**B+C**	**B+O**
	Pg												
01	**Leaf extract**	**NG**	**NG**	**28**	**32**	**63**	**49**	**74**	**82**	**118**	**136**	**NG**	**430**
02	**Root extract**	**12**	**18**	**69**	**78**	**94**	**142**	**154**	**160**	**191**	**200**	**NG**	**328**
	Pi												
01	**Leaf extract**	**18**	**32**	**54**	**60**	**72**	**80**	**84**	**96**	**212**	**218**	**NG**	**361**
02	**Root extract**	**34**	**48**	**62**	**78**	**90**	**93**	**108**	**123**	**148**	**200**	**NG**	**312**
	Fn												
01	**Leaf extract**	**NG**	**20**	**32**	**48**	**54**	**69**	**72**	**84**	**102**	**112**	**NG**	**309**
02	**Root extract**	**NG**	**24**	**38**	**64**	**81**	**96**	**112**	**182**	**196**	**218**	**NG**	**380**
	Tf												

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01	**Leaf extract**	**NG**	**24**	**68**	**71**	**84**	**92**	**102**	**136**	**154**	**210**	**NG**	**336**
02	**Root extract**	**NG**	**32**	**54**	**76**	**98**	**134**	**186**	**199**	**218**	**318**	**NG**	**336**

NG:no growth

Table 3: MIC /MBC comparison of ethanolic extract of roots and leaves of *M.oleifera* plant against 4 periodontal pathogens.

MICRO-ORGANISMS	LEAF EXTRACT		ROOT EXTRACT	
	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)
Pg	50	50	100	-
Pi	50	-	50	-
Fn	50	100	50	100
Tf	100	100	100	100

-= No MBC found (Only Bacteriostatic Activity)