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Original Research

Assessing the cytotoxic effect and antimicrobial activity of *Moringa oleifera* aqueous and ethanolic extract against oral pathogens extracted from periodontal and orthodontic patients – an in vitro study

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Abstract:

Background: Periodontitis is the result of inflammation caused due to the activity of microorganisms. The prevalence of anaerobic organisms is more when it comes to periodontal pockets and orthodontic patients. Plants with phytochemicals that could exert antimicrobial effects could aid in host modulation for management of periodontitis caused by these bacteria in periodontal and orthodontic patients.

Aim: To assess the antimicrobial effect of aqueous extract of Moringa oleifera Lam (MOL) and cytotoxic effect of aqueous and ethanol extracts of MOL.

Materials and methods: Moringa oleifera Lam. extracts were prepared by maceration. Subgingival plaque samples were collected, and microorganisms were cultured in anaerobic environment. The microorganisms were treated with the extracts and minimum inhibitory concentration and minimum bactericidal concentration was assessed. The cytotoxic effects were assessed by brine shrimp assay.

Results: Aqueous extract showed antimicrobial effect in dose and time dependent manner and both extracts exhibited cytotoxic effects in a dose and time dependent manner.

Summary and Conclusion: The antimicrobial effect of MOL could be utilized to develop a nature derived local drug delivery system for treating plaque induced periodontitis in different clinical situations.

Key words: anaerobic organism, anti-microbial, cytotoxicity, dental plaque, Moringa oleifera Lam, periodontitis.

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Introduction:

WHO has reported that in developing nations approximately eighty percent of the population prefers herbal and traditional medicine for disease management.[1] This could be attributed to the fact that herbs are easy to procure, cost effective and less likely to cause side effects as most of them can be included in staple diet. Hence the research interest for exploring the medicinal properties of herbs has increased recently.

Moringa oleifera Lam. also known as drumstick tree from India is rich in phytochemicals with diverse therapeutic effects.[2] The leaves of *Moringa oleifera* Lam. is rich in proteins, minerals and beta carotene, and is called as "mother's best friend" or "miracle tree" . [3] Particularly it is rich in saccharides [4] glucosinolates, [5] alkaloids, [6] saponins, [7] tannins, [8] nitrile glycosides, [9]flavonoids, [10] and polyphenols [11]which confer medicinal property to the tree. Studies have already reported the anti-cancer,[12] antimicrobial,[13] antidiabetic,[14] antioxidant,[15] anti-hypertensive,[16] hepato-protective,[17] and anti-inflammatory[10] properties of extracts of drumstick leaves. Also, the anti-inflammatory, antidiabetic, anticancer and antioxidant properties of root and seed of drumstick have also been reported.[18]

This awareness of the medicinal benefits of drumstick plant had brought about increased consumption of these leaves by many people as a part of their diet in different ways to maintain their nutritional status. In countries like Africa and India, research emphasizing on developing both therapeutic and nutritional supplement from Moringa leaves is in progress.[19] In Republic of Philippines and Niger, Moringa oleifera is included as therapeutic supplement in national nutrition rehabilitation program targeted for malnourished children.[20] Thus recently *M. oleifera* is being explored for the treating various chronic illness and systemic diseases. The leaves have been used for the treatment diabetes mellitus, cancer, obesity, scurvy, hysteria.[21, 22.] Among the various inflammatory condition affecting the oral cavity, periodontitis is the most common chronic inflammatory disease with a microbial etiology, wherein combined effect of oxidative stress, microbial activity and inflammation leads tissue destruction. [23] The chances of this type of tissue destruction is more when there are plaque retentive factors like multiple restorations of decayed teeth, restorations replacing missing teeth and in patients wearing orthodontic appliances. In these situations, the routine mechanical plaque control is challenging. Addition of Chemical plaque control agents in routine oral care in patients with these predisposing factors will aid in prevention of plaque induced diseases. And management of periodontal disease is by a combination of surgical and nonsurgical strategies along with local drug delivery. [24] Hence a local drug delivery agent with antimicrobial properties against could be a better choice in improving the prognosis of the condition. Hence various herbs and their active constituents are being explored to aid as a therapeutic adjunct for periodontal disease management. The pharmacological benefits of Moringa *oleifera* Lam (MOL) in the treatment of systemic diseases in humans and cattle are well established.[25 26] But literature of its actions in the management of oral diseases is scarce Few laboratory studies have explored its anti-microbial and host modulating activities in the management of oral diseases.[27]

In the microbial etiology of periodontal disease several anaerobic bacterial organisms have been attributed as a major cause.[28] Taking into consideration of this fact the present study was to assess the antimicrobial properties and cytotoxicity effects of aqueous (A) and ethanolic (Et) extracts of MOL against

anaerobic pathogens pooled from subjects with periodontitis and patients undergoing orthodontic treatment to explore its use as a chemical anti-plaque agent and local drug delivery system in future.

Methods

This invitro study design for testing the antimicrobial activity and cytotoxicity was approved by the ethics committee of Saveetha Dental College (SDC/Ph.D18/32).

EXTRACT PREPARATION

The drumstick plant leaves grown in Southern part of India were procured from the local market. The leaves were washed in running water and air dried in shade for two weeks. After weighing it was dry grounded and stored in airtight containers. For the preparation of alcoholic and ethanolic extracts to 100 gm of the powdered leaves 1 L of water and ethanol were added respectively. It was macerated for three days. Later the filtrate obtained with Whatman filter paper #1 was reduced further to obtain a solid residue. To prepare 5% aqueous and ethanolic of the extract 0.5 gm of the solid residue was dissolved in 10 ml of water or ethanol respectively.[29]

Antimicrobial Activity

Collection of plaque sample

A pooled plaque sample was collected from two periodontitis and two orthodontic patients visiting the dental college. Sampling was done only in patients with moderate periodontitis patient, with sites exhibiting 5-6 mm probing pocket depth and clinical attachment loss of 3-4mm sample according Armitage criteria 1999 was collected. And subjects who had undergone periodontal therapy or undertaken antibiotics in the past immediate six months were excluded. In orthodontic patients only subjects who is under orthodontic therapy for a minimum of 6 months was chosen for sample collection. After removal of supragingival plaque, the subgingival plaque samples were collected with sterile absorbable paper points. Samples contaminated with blood was discarded and immediately transferred to 2 ml Robertson's cooked meat medium that was preheated.

Anaerobic culture

Trypticase agar plate was used to obtain subculture from Robertson cooked meat media The Gaspak system had a transparent jar, air-tight lid, screened catalyst chamber with palladium-sized aluminium pellets. Addition of water was done to an aluminium foil packet with pellets of tartaric acid, sodium bicarbonate and sodium borohydride and placed inside the jar immediately. Carbon dioxide was produced as a result of chemical reaction to which the mounted agar plate was placed, and lid was tightly clamped. Incubation of the jar for 48 hours at 37°C was done in an incubator. Microbial growth was checked and CFU for each plate was done with a colony counter, CFUs for each plate were counted. [30]

Minimal inhibitory concentration:

To assess the minimum inhibitory concentration Brain Heart Infusion broth was prepared. And six ml of the prepared broth were subsequently taken in test tubes. To each test tube isolated bacterial colonies at a

density of 5×10^{5} CFU/mL. was added. The bacterial samples were treated with various concretions (25µL, 50 µL,100 µL) of 5% aqueous extract of *Moringa oleifera* Lam. An untreated bacterial suspension was considered as positive control and sample devoid of organisms were negative control. The test and control samples were incubated under anaerobic conditions for 1 hour, 2-hour, 3-hour, 4-hour and 5-hour. The percentage of dead cells was calculated at 600 nm at all the above time intervals. [31]

Minimum bactericidal concentration by Agar dilution method.

The prepared Brain Heart Infusion agar was poured in sterile petri plates. To the broth the anaerobic suspension and different concentrations of 5% aqueous extract of *Moringa oleifera* Lam. Extract were added to prepare the test samples. Finally, the test samples, positive and negative control were incubated in an anaerobic chamber for 24h following which colonies were counted.[32]

Brine Shrimp Lethality Assay:

Saltwater preparation:

2g of iodine free salt was weighed and dissolved in 200ml of distilled water to prepare the saline water for growth of Nauplii. Then 10-12 ml of this saline water was added to each of the six well plates. Followed by transfer of 10 nauplii slowly to each well. Then the wells were treated with various concentrations of 5%aqueous and ethanol extracts of (5µL,10 µL,20 µL,40 µL,80 µL) *Moringa oleifera* L. The untreated samples were treated as control. The samples were incubated for 24 hours and 48 hours. Following incubation period, the number of live nauplii's present were calculated. [33]

Results

Minimal inhibitory concentration:

The microbial culture samples when treated with 25µL, 50 µL,100 µL of aqueous extract of Moringa demonstrated antimicrobial activity in a dose dependent and time dependent manner with increase in activity with higher concentration and increased antimicrobial activity with incubation time ranging between one hour and 5 hours. The results were comparable with control or standard. On the contrary, negative control showed increased microbial growth with an increase in incubation time. The results are depicted in Figure 1.

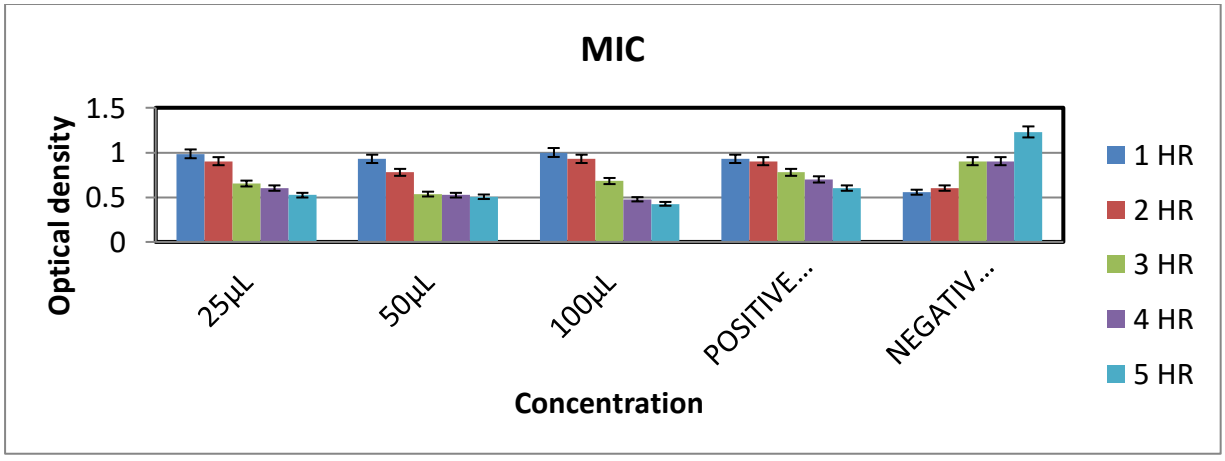

Figure 1- Minimum inhibitory concentration of aqueous extract of MOL at different time intervals.

Minimum bactericidal concentration

The plaque samples when treated with 25µL, 50 µL,100 µL aqueous extract of *M. oleifera* demonstrated significant antimicrobial effect in the dose dependent manner as depicted by decrease in colony count with increase in concentration 25µL (450), 50 µL (304),100 µL (206) which was comparable with positive control (57), whereas negative control had a colony count of (613). The results are shown in Figure 2.

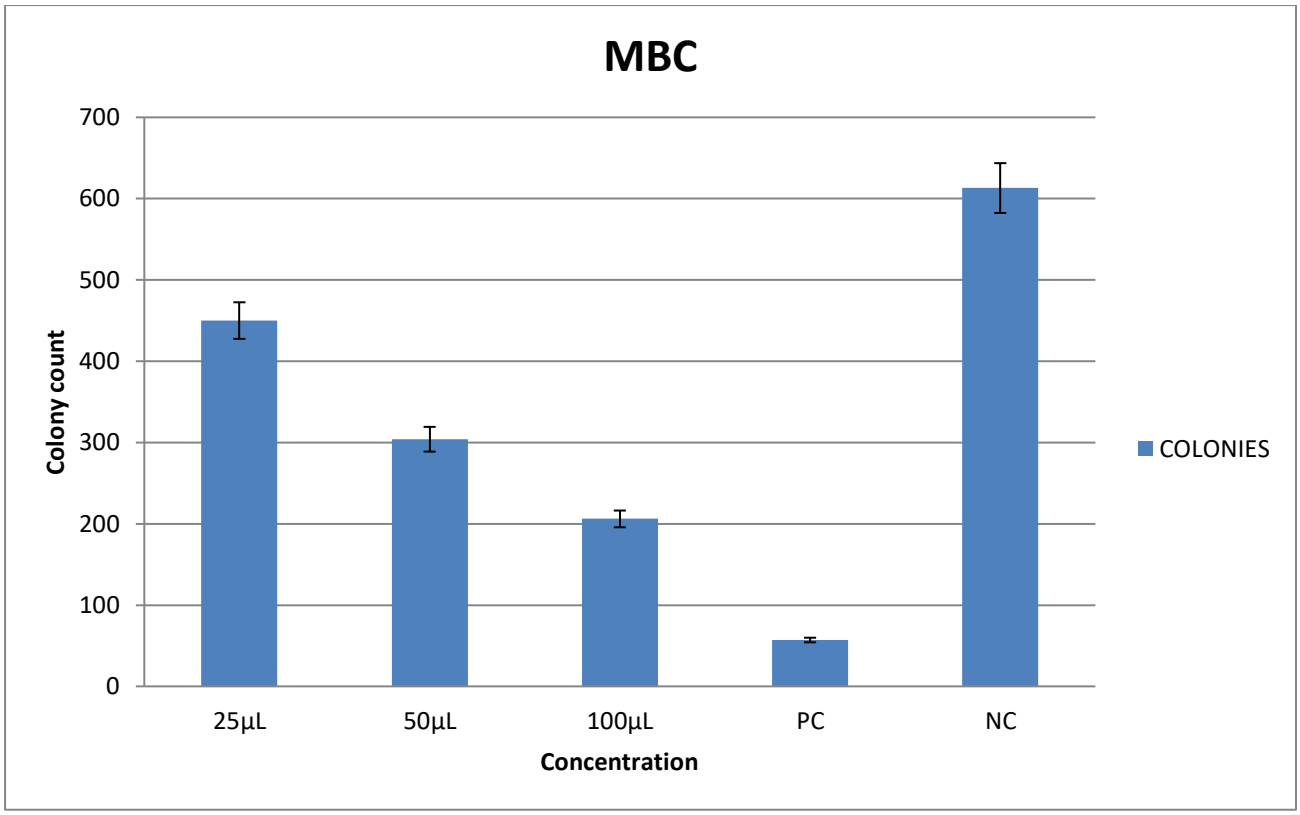

Figure 2- Minimum bactericidal concentration of aqueous extract of MOL.

Cytotoxic effects of aqueous extract, ethanolic extract of *M. oleifera*

In the first 24 hour of incubation, there was no difference in number of live nauplii at various concentrations of 5µL,10 µL,20 µL,40 µL,80 µL in both aqueous and ethanol extract of *M. oleifera* . The number of live nauplii was 10 which was the same as the control. But at 48 hours there was a decrease in live Nauplii in both the test groups. And this decrease to 5 viable Nauplii, was more pronounced in wells with highest extract concentration of 80 µL. As the concentration of extract increased, there was a dose dependent decrease in live nauplii. When aqueous (10) and ethanol(8) groups were compared, the aqueous group showed a greater number of live cells at 5 µl. But as the concentration increased both the groups were comparable (5 viable Nauplii) but more cytotoxic than control group (10 Nauplii). The results are shown in Figure 3, 4 and Table 1.

Table 1: Percentage viable Nauplii after treatment with aqueous and ethanolic extracts of MOL at 24 and 48h

Conc in µl	Viable nauplii			
	Aqueous extract 24 h	**Ethanolic extract 24 h**	**Aqueous extract 48 h**	**Ethanolic extract 48 h**
5µL	10	10	10	8
10µL	10	10	8	8
20µL	10	10	6	7
40µL	10	10	6	6
80µL	10	10	5	5
CONTROL	10	10	10	10

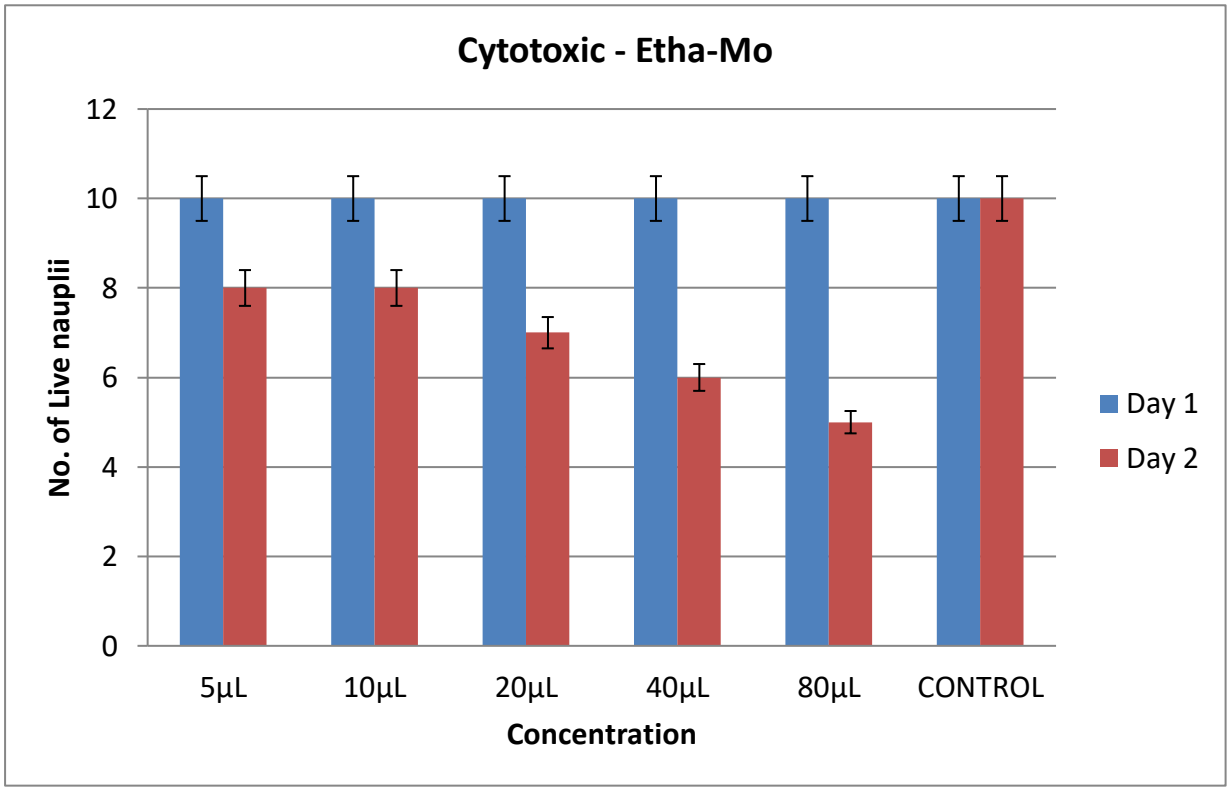

Figure 3- Cytotoxic effect of ethanolic extract of MOL against brine shrimp

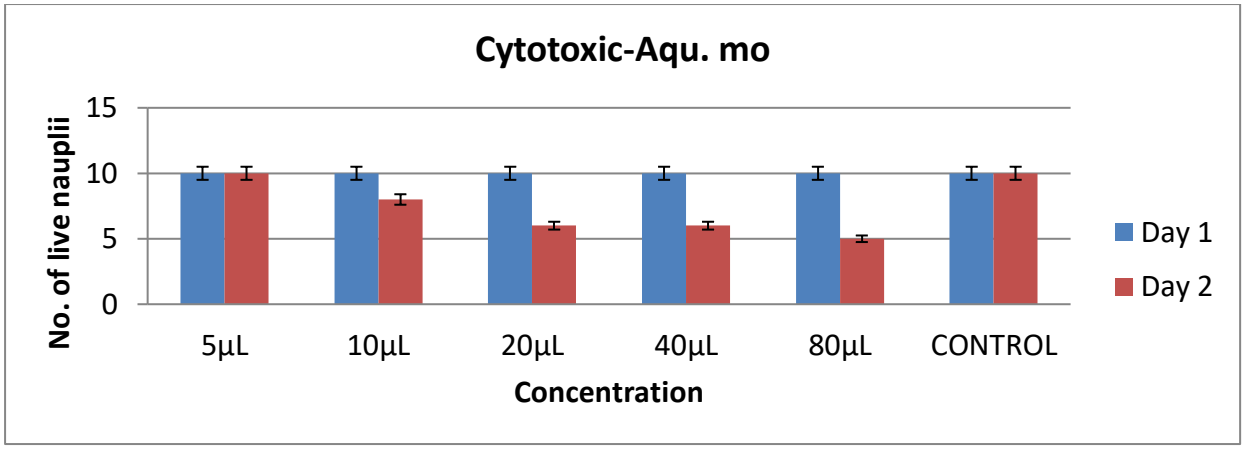

Figure 4- Cytotoxic effect of aqueous extract of MOL against brine shrimp

Discussion

Periodontitis an inflammatory condition of tooth supporting tissues with microbial etiology. This leads to tissue destruction as a result of inflammation associated with host-microbial interaction. This is of concern to all patients with poor plaque control and in patients with plaque retentive factors which makes them vulnerable to these damages. In periodontal patients even shallow periodontal pockets of 5 mm is inaccessible for routine periodontal care might precede to further progression.[34] Similarly in orthodontic patients daily oral hygiene practices are complex which allows for a microbial shift from aerobic to anaerobic microorganisms and thus nurturing an increased bacterial load. [35] The anaerobic organisms causes more destructive changes in the periodontal apparatus and jeopardizes both periodontal and orthodontic treatment outcome. Hence antimicrobial agents could aid in reduction of periodontal pathogens thereby preventing further tissue destruction. Although several antimicrobial agents have been used as an adjunct therapy, search for herbal alternative continue owing to diverse pharmacological effects such as antioxidant, antimicrobial and anti-inflammatory properties.

In this regard *Moringa oleifera* L. also known as drumstick is known for its diverse pharamcognostic effects. Although antimicrobial effects of drumstick leaves against aerobic bacteria have been reported so far, the antimicrobial effect of MOL against anaerobia bacteria from dental plaque samples have been less explored. Also prior to conduct of clinical trials, the determination of cytotoxic concentration is essential. Hence, we aimed to determine the antimicrobial and cytotoxic effects of MOL against anaerobic pathogens from subgingival dental plaque samples and cytotoxic effects.

The results of minimum inhibitory concentration assay revealed that MOL exhibited antimicrobial effects in a dose dependent manner 25µL, 50 µL, 100 µL, with decrease in activity with decrease in concentration and time dependent manner with increase in activity with increase in incubation period of one to five hours. Similarly, the results minimum bactericidal concentration revealed that MOL exhibited antimicrobial effects in a dose dependent manner with decrease in colony count with increase in concentration. The results are concurrent with the findings of Fouad et al 2019 who assessed the antimicrobial effect of aqueous and ethanol extract of leaves of drumstick against bacteria isolated from abscess of camels and reported that ethanol extract

showed better antimicrobial activity than aqueous extract.[36] Since in our study the cytotoxicity of aqueous was comparatively less than ethanol, the antimicrobial effect was assessed against 5 %aqueous preparation.

Similarly, Lucia et al assessed the antimicrobial effect of methanol extract of *M. oleifera* leaves against anaerobic bacterium *Enterococcus faecalis* . They reported MIC of 75 μg/ml bactericidal concentration *of* 75 μg/ml. Considering cytotoxic effects the IC 50 value was 70 μg/ml. [37] Kim et al reported the antimicrobial activity of methanol extract of drumstick leaves against dental plaque bacteria. They reported that the isolated compounds Niazinin A, ß-Stigmasterol, Quercetin-3-O-ß-DGlucopyranoside and Kaempferol-3-O-ß-D-Gluco pyranoside from Moringa exhibited antimicrobial activity against the tested bacteria. [38] Studies have reported that quercetin exerts antimicrobial effects against *Staphylococcus aureus* (at 10 × MIC). And E. coli (at 50 × MIC) by causing cell wall damage. [39] Hence the antimicrobial effect of MOL could also be via the same mechanism due to the presence of quercetin, however further molecular research warranted in this field.

In a systematic review by Nurul et al, the antimicrobial effect of MOL against oral pathogens and anti-inflammatory properties have been reported. They have also complied the results of a few studies that have reported the antimicrobial properties of MOL against drug resistant Staphylococcus aureus and *Escheria coli*. [40-42] The antimicrobial property of MOL could also be attributed to the presence of lectins as reported by Khatun et al 2009 who demonstrated the antimicrobial effects of three lectins obtained from MOL against E coli, S dysenteriae and S aureus. [43]

Considering cytotoxicity, but there was a dose dependent increase in cytotoxic effects as depicted by decrease in number of live nauplii in both aqueous and ethanol extract of *M. oleifera* at 48 hours of incubation. the results of the present study are concurrent with the findings of Jafarain et al who reported dose dependent cytotoxic effects of MOL in HeLA cell line. They attributed the cytotoxic properties to the phenols present in the leaves.[38] Similarly Khatun also reported the cytotoxic effect of MOL derived lectins by the brine shrimp (Artemia salina L.) lethality bioassay. [44]

The limitation of the study is that it has an in-vitro design and further well controlled clinical trials are warranted to determine the exact therapeutic effect. The cytotoxicity assay sheds light on selection of non-toxic concentration for developing local drug delivery system and the antimicrobial properties of MOL could be further explored clinically as an adjunct to dental plaque control. This study opens up new avenues for further research especially in relation to intraoral bacteria which are capable of causing corrosion in the intra oral environment. The study by Odusote et al has mentioned about the possibility of the inhibitory effect of moringa leaf extract on the corrosion of stainless steel. [45] This is of significance in the wake of increasing use of stainless-steel appliances intraorally.

Conclusion:

The 5% aqueous extract of *Moringa oleifera* Lam. demonstrated a dose dependent antimicrobial activity against oral anaerobic organisms. This effect was pronounced as the exposure time of the treated sample increased. And the aqueous extract was marginally better in lesser concentration compared with ethanol extract in cytotoxicity assay which was revealed by a greater number of live Nauplii. These observations together with other published research support the pharmacological effects of *M. oleifera* for the management of disease and

malnutrition in communities. [46] This difference in higher concentration was not observed Thus, the antimicrobial property of *M. oleifera* against anaerobic pathogens could be explored further for management of periodontitis an inflammatory condition of the tooth supporting structures. Development of a local drug delivery such as thermo-reversible gel ointments, mouthwashes containing ideal non-toxic concentration of MOL could be formulated and a clinical trial could be conducted for its effect in treatment of periodontitis similar to the current antimicrobial agents such as tetracycline, chlorhexidine, and doxycycline [47,48] Thus *M. oleifera* could be used as a nature-derived host modulatory agent for treating dental biofilm induced diseases caused by amicrobial aggregations as found in periodontal and orthodontic patients.

Ethical approval: The study was approved by the Institutional Ethics Committee of Saveetha Dental College ((SDC/Ph.D18/32).)

Conflict of interest: No conflict of interest among authors in this study.

Sources of Funding: Nil

Authorship Contributions: Conception and design of the work was presented by Shanmugapriya & Sheeja, laboratory studies, data collection was executed by Dr. Uma Revathy & Dr. Mayma, data analysis and interpretation was carried out by Dr.Mahesh, Dr.Jeyaram drafted the article and finally the article was critically reviewed by Dr.Shanmugapriya.

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References

1. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in–vitro antioxidant and anti–inflammatory activity of flowers of Moringa oleifera. Asian Pac J Trop Biomed . 2013 Aug;3(8):623–7.

2. Paliwal R, Sharma V, . P. A Review on Horse Radish Tree (Moringa oleifera): A Multipurpose Tree with High Economic and Commercial Importance. Asian J Biotechnol . 2011 Jun 15;3(4):317–28.

3. Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of Moringa oleifera Leaves: An Overview. Int J Mol Sci . 2015 Jun 5;16(12):12791–835.

4. Roy SK, Chandra K, Ghosh K, Mondal S, Maiti D, Ojha AK, et al. Structural investigation of a heteropolysaccharide isolated from the pods (fruits) of Moringa oleifera (Sajina). Carbohydr Res . 2007 Nov;342(16):2380–9.

5. Bhatta R, Saravanan M, Baruah L, Sampath KT. Nutrient content, in vitro ruminal fermentation characteristics and methane reduction potential of tropical tannin-containing leaves. J Sci Food Agric . 2012 Dec;92(15):2929–35.

6. Panda S, Kar A, Sharma P, Sharma A. Cardioprotective potential of N,α-l-rhamnopyranosyl vincosamide, an indole alkaloid, isolated from the leaves of Moringa oleifera in isoproterenol induced cardiotoxic rats: In vivo and in vitro studies. Bioorg Med Chem Lett . 2013 Feb;23(4):959–62.

7. Mathur M, Yadav S, Katariya PK, Kamal R. In vitro propagation and biosynthesis of steroidal sapogenins from various morphogenetic stages of Moringa oleifera Lam., and their antioxidant potential. Acta Physiol Plant . 2014 Jul 15;36(7):1749–62.

8. Maldini M, Foddai M, Natella F, Petretto GL, Rourke JP, Chessa M, et al. Identification and quantification of glucosinolates in different tissues of Raphanus raphanistrum by liquid chromatography tandem-mass spectrometry. J Food Compos Anal . 2017 Aug;61:20–7.

9. Sahakitpichan P, Mahidol C, Disadee W, Ruchirawat S, Kanchanapoom T. Unusual glycosides of pyrrole alkaloid and 4′-hydroxyphenylethanamide from leaves of Moringa oleifera. Phytochemistry . 2011 Jun;72(8):791–5.

10. Coppin JP, Xu Y, Chen H, Pan M-H, Ho C-T, Juliani R, et al. Determination of flavonoids by LC/MS and anti-inflammatory activity in Moringa oleifera. J Funct Foods . 2013 Oct;5(4):1892–9.

11. Coz-Bolaños X, Campos-Vega R, Reynoso-Camacho R, Ramos-Gómez M, Loarca-Piña GF, Guzmán-Maldonado S. Moringa infusion (Moringa oleifera) rich in phenolic compounds and high antioxidant capacity attenuate nitric oxide pro-inflammatory mediator in vitro. Ind Crops Prod . 2018 Aug;118:95–101.

12. Jung IL. Soluble Extract from Moringa oleifera Leaves with a New Anticancer Activity. Pandey S, editor. PLoS One . 2014 Apr 18;9(4):e95492.

13. Peixoto JRO, Silva GC, Costa RA, de Sousa Fontenelle J res L, Vieira GHF, Filho AAF, et al. In vitro antibacterial effect of aqueous and ethanolic Moringa leaf extracts. Asian Pac J Trop Med . 2011 Mar;4(3):201–4.

14. Jaiswal D, Kumar Rai P, Kumar A, Mehta S, Watal G. Effect of Moringa oleifera Lam. leaves aqueous extract therapy on hyperglycemic rats. J Ethnopharmacol . 2009 Jun;123(3):392–6.

15. Verma AR, Vijayakumar M, Mathela CS, Rao C V. In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food Chem Toxicol. 2009 Sep;47(9):2196–201.

16. Dangi SY, Jolly CI, Narayanan S. Antihypertensive Activity of the Total Alkaloids from the Leaves of Moringa oleifera. Pharm Biol . 2002 Jan 29;40(2):144–8.

17. Atta A, Nasr S, Almaweri A, Sedky D, Mohamed A, Desouky H, et al. Phytochemical, antioxidant and hepatoprotective effects of different fractions of Moringa oleifera leaves methanol extract against liver injury in animal model. Asian Pac J Trop Med . 2018;11(7):423.

18. Xu Y-B, Chen G-L, Guo M-Q. Antioxidant and Anti-Inflammatory Activities of the Crude Extracts of Moringa oleifera from Kenya and Their Correlations with Flavonoids. Antioxidants . 2019 Aug 9;8(8):296.

19. Rodríguez-Pérez C, Quirantes-Piné R, Fernández-Gutiérrez A, Segura-Carretero A. Optimization of extraction method to obtain a phenolic compounds-rich extract from Moringa oleifera Lam leaves. Ind Crops Prod . 2015 Apr;66:246–54.

20. Manzo, Mahamane , Mahaman , Doudou, Maimouna, Daouda, Paluku, Salimata, Katia, Michèle, Philippe. Effect of Moringa supplementation in the management of moderate malnutrition in children under 5 receiving ready-to-use supplementary foods in Niger: A randomized clinical trial. Advanced Research and Reviews. 2021; 8. 071-086.

21. Kasolo, J.N, Bimenya, G.S. Ojok, L.Ochieng, J. Ogwal-Okeng JW. Phytochemicals and uses of Moringaoleifera leaves in Ugandan rural communities. J Med Plants Res . 2010 Jan 16;4:753–7.

22. Gupta S, Jain R, Kachhwaha S, Kothari SL. Nutritional and medicinal applications of Moringa oleifera Lam.—Review of current status and future possibilities. J Herb Med. 2018 Mar;11:1–11.

23. Sczepanik FSC, Grossi ML, Casati M, Goldberg M, Glogauer M, Fine N, et al. Periodontitis is an inflammatory disease of oxidative stress: We should treat it that way. Periodontol 2000 . 2020 Oct 25;84(1):45–6

24. Kwon T, Lamster IB, Levin L. Current Concepts in the Management of Periodontitis. Int Dent J . 2021 Dec;71(6):462–76.

25. Fouad EA, Abu Elnaga ASM, Kandil MM. Antibacterial efficacy of Moringa oleifera leaf extract against pyogenic bacteria isolated from a dromedary camel (Camelus dromedarius) abscess. Vet World. 2019 Jun;12(6):802-808.

26. Ramamurthy S, Thiagarajan K, Varghese S, Kumar R, Karthick BP, Varadarajan S, Balaji TM. Assessing the In Vitro Antioxidant and Anti-inflammatory Activity of Moringa oleifera Crude Extract. J Contemp Dent Pract. 2022 Apr 1;23(4):437-442.

27. The antimicrobial effects of MOL on aerobic caries causing organisms to have been researched Nurul M , Muhammad Harun. Systematic Review of Moringa oleifera's Potential as Antibacterial and Anti-Inflammatory in the Oral Cavity. European Journal of Molecular & Clinical Medicine, 2020; 7(10): 144-161.

28. Adriaens PA, De Boever JA, Loesche WJ. Bacterial Invasion in Root Cementum and Radicular Dentin of Periodontally Diseased Teeth in Humans. J Periodontol . 1988 Apr;59(4):222–30.

29. Mallikarjun S, Rao A, Rajesh G, Shenoy R, Pai M. Antimicrobial efficacy of Tulsi leaf (Ocimum sanctum) extract on periodontal pathogens: An in vitro study. J Indian Soc Periodontol. 2016 Mar-Apr;20(2):145-50.

30. Shahin M, Jamal W, Verghese T, Rotimi VO. Comparative Evaluation of Anoxomat and Conventional Anaerobic GasPak Jar Systems for the Isolation of Anaerobic Bacteria. Med Princ Pract . 2003;12(2):81–6.

31. Navarini A, Martino MD V, Sasagawa SM, Massaia IFD, Mimica MJ. Accuracy of a vancomycin brain heart infusion screening plate for the screening of Staphylococcus aureus isolates with increased vancomycin minimum inhibitory concentrations. New Microbiol . 2015 Jul;38(3):423–6.

32. Rodrigues JZ de S, Passos MR, Silva de Macêdo Neres N, Almeida RS, Pita LS, Santos IA, et al. Antimicrobial activity of Lactobacillus fermentum TcUESC01 against Streptococcus mutans UA159. Microb Pathog . 2020 May;142:104063.

33. Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD. A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnol. 2002 Sep;2:17.

34. Matuliene G, Pjetursson BE, Salvi GE, Schmidlin K, Brägger U, Zwahlen M, Lang NP. Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. J Clin Periodontol. 2008;35:685–695.

35. Umarevathi et al, Prevalence of Anaerobic Microbiota in Orthodontic Patients – Scoping Review. Int J Orthod Rehabil 2022; 13(2):1-13.

36. Fouad EA, Abu Elnaga ASM, Kandil MM. Antibacterial efficacy of Moringa oleifera leaf extract against pyogenic bacteria isolated from a dromedary camel (Camelus dromedarius) abscess. Vet World . 2019 Jun 14;12(6):802–8.

37. Arévalo-Híjar L, Aguilar-Luis MÁ, Caballero-García S, Gonzáles-Soto N, Del Valle-Mendoza J. Antibacterial and Cytotoxic Effects of Moringa oleifera (Moringa) and Azadirachta indica (Neem) Methanolic Extracts against Strains of Enterococcus faecalis. Int J Dent . 2018 Sep 25;2018:1–5.

38. Elgamily H, Moussa A, Elboraey A, EL-Sayed H, Al-Moghazy M, Abdalla A. Microbiological Assessment of Moringa Oleifera Extracts and Its Incorporation in Novel Dental Remedies against Some Oral Pathogens. Open Access Maced J Med Sci . 2016 Nov 28;4(4):585–90.

39. Wang S, Yao J, Zhou B, Yang J, Chaudry MT, Wang M, et al. Bacteriostatic Effect of Quercetin as an Antibiotic Alternative In Vivo and Its Antibacterial Mechanism In Vitro. J Food Prot . 2018 Jan 1;81(1):68–78.

40. Nurul M, Harun AM. European Journal of Molecular & Clinical Medicine Systematic Review of Moringa oleifera's Potential as Antibacterial and Anti-Inflammatory in the Oral Cavity. European Journal of Molecular & Clinical Medicine, 2020; 7(10): 144-161.

41. Zaffer M, Ahmad S, Sharma R, Mahajan S, Gupta A, Agnihotri RK. Antibacterial activity of bark extracts of Moringa oleifera Lam. against some selected bacteria. Pak J Pharm Sci . 2014 Nov;27(6):1857–62.

42. B M, PJ M, V M. Antimicrobial activities of Moringa oleifera Lam leaf extracts. Afrcan J Biotechnol . 2012 Feb 7;11(11).

43. Khatun S, Khan M, Ashraduzzaman M, Pervin F, Bari L, Absar N. Antibacterial Activity and Cytotoxicity of Three Lectins Purified from Drumstick (Moringa oleifera Lam.) Leaves. J bio-sci. . 2011 Feb. 18 [cited 2022 Nov. 15];17:89-94.

44. Jafarain A, Asghari G, Ghassami E. Evaluation of cytotoxicity of Moringa oleifera Lam. callus and leaf extracts on Hela cells. Adv Biomed Res . 2014;3:194.

45. Odusote, Jamiu & Owalude, David & J. Olusegun, Sunday & Abolore, Yahya. Inhibition Efficiency of Moringa Oleifera Leaf Extract on the Corrosion of Reinforced Steel Bar in HCl Solution. The West Indian J. Eng. 2016. 38.

46. Ramamurthy S, Varghese S, Sudarsan S, Muruganandhan J, Mushtaq S, Patil PB, Raj AT, Zanza A, Testarelli L, Patil S. Moringa oleifera: Antioxidant, Anticancer, Anti-inflammatory, and Related Properties of Extracts in Cell Lines: A Review of Medicinal Effects, Phytochemistry, and Applications. J Contemp Dent Pract. 2021 Dec 1;22(12):1483-1492.

47. Panwar M, Gupta S. Local Drug Delivery with Tetracycline Fiber : An Alternative to Surgical Periodontal Therapy. Med J Armed Forces India . 2009 Jul;65(3):244–6.

48. Da Rocha HAJ, Silva CF, Santiago FL, Martins LG, Dias PC, De Magalhães D. Local Drug Delivery Systems in the Treatment of Periodontitis: A Literature Review. J Int Acad Periodontol . 2015 Jul;17(3):82–90.

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