

Assessing the *In Vitro* Antioxidant and Anti-inflammatory Activity of *Moringa oleifera* Crude Extract

Shanmugapriya Ramamurthy¹, Kavitha Thiagarajan², Sheeja Varghese³, Rajeshkumar⁴, Prem Karthick B⁵, Saranya Varadarajan⁶, Thodur Madapusi Balaji⁷

ABSTRACT

Background: Chronic inflammation and oxidative stress play a vital role in the pathogenesis of various diseases of the oral cavity including periodontal disease. Phytochemicals present in plants with antioxidant (AO) and anti-inflammatory properties could aid as a therapeutic adjunct in the management of these diseases.

Aim: To assess the antioxidant and anti-inflammatory effects of aqueous and ethanolic extracts of *Moringa oleifera* Lam. (MOL) in an *in vitro* environment.

Materials and methods: Aqueous and ethanolic extracts of *M. oleifera* Lam. were prepared by maceration. Antioxidant activity was assessed by FRAP, hydroxyl radical scavenging activity, and DPPH radical scavenging assay. Anti-inflammatory activity was assessed by Albumin Denaturation Assay. Experiments were repeated thrice, and mean and standard deviation were calculated.

Results: Both the test substances exhibited significant antioxidant and anti-inflammatory activity, and aqueous extracts exhibited higher activity than ethanolic extract.

Summary and conclusion: The anti-inflammatory and antioxidant activity of *M. oleifera* Lam. could be further explored for the management of periodontal disease as a local drug delivery system with the extract could be developed.

Keywords: Anti-inflammatory, Antioxidant, *Moringa oleifera*, Periodontal disease.

The Journal of Contemporary Dental Practice (2022): 10.5005/jp-journals-10024-3323

INTRODUCTION

Moringa oleifera is a plant commonly termed as horseradish tree, the ben oil tree, or the drumstick tree. This plant of Indian origin with several medicinal properties has been used for several hundred years by various countries.¹ The leaves of this wonder tree are rich in phytochemicals and nutrients such as beta carotene, essential minerals, and proteins, and hence the tree is often known as a "miracle tree" and "mother's best friend".² The leaves of this miracle tree can be consumed in several methods that will aid in the long-term maintenance and enhancement of nutritional status. Some countries of the African subcontinent have emphasized the development of these leaves as therapeutic nutritional supplements.³ In traditional medicine, these leaves have been explored for their use in the management of obesity, some cancers, hysteria, diabetes mellitus, and vitamin C deficiency.^{4,5}

The vital phytochemical content of this plant includes alkaloids,⁶ saccharides,⁷ tannins,⁸ saponins,⁹ glucosinolates,¹⁰ nitrile glycosides,¹¹ along with the well-known polyphenols¹² and flavonoids,¹³ which are responsible for the medicinal property of the tree. Evidence has shown that the leaves of this plant exhibit antioxidant,¹⁴ anticancer,¹⁵ antidiabetic,¹⁶ antimicrobial,¹⁷ anti-inflammatory,¹³ anti-hypertensive,¹⁸ and hepato-protective¹⁹ properties. Interestingly, the seeds and roots of this plant also have several medicinal properties like antioxidant (AO), anti-inflammatory, antitumor, and hypoglycemic activities.²⁰ Thus, recently *M. oleifera* has gained importance in the field of pharmacognosy, and several research studies are being conducted to assess its use for the management of chronic illness.

It is noteworthy that periodontal disease is an oral inflammatory condition of polymicrobial etiology, where inflammation, microbial

¹Department of Periodontics, Sri Venkateswara Dental College and Hospital; Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

²Department of Dental Surgery, Government Stanley Medical College and Hospital, Chennai, Tamil Nadu, India

³Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

⁴Department of Pharmacology, Nanobiomedicine Laboratory, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India

⁵Department of Oral Pathology and Microbiology, Priyadarshini Dental College and Hospital, Pandur, Tamil Nadu, India

⁶Department of Oral Pathology and Microbiology, Sri Venkateswara Dental College and Hospital, Chennai, Tamil Nadu, India

⁷Tagore Dental College and Hospital, Chennai, Tamil Nadu, India

Corresponding Author: Kavitha Thiagarajan, Department of Dental Surgery, Government Stanley Medical College and Hospital, Chennai, India; Phone: +91 9159344959, e-mail: drkavi81@gmail.com

How to cite this article: Ramamurthy S, Thiagarajan K, Varghese S, et al. Assessing the *In Vitro* Antioxidant and Anti-inflammatory Activity of *Moringa oleifera* Crude Extract. *J Contemp Dent Pract* 2022;23(4): 437–442.

Source of support: Nil

Conflict of interest: None

infection, and a jeopardized antioxidant response play a major role in mediating tissue destruction.²¹ Several surgical and nonsurgical strategies are being developed as therapeutic measures to manage periodontal disease.²² In this connection, several herbs and their

active phytoconstituents have been tried for periodontal disease management in various forms. So far very few studies have assessed the role of *M. oleifera* Lam. (MOL) as a host modulatory agent in periodontal therapy, and its properties in different extraction methods have not been fully exploited, which in turn forms the basis of the present study. Although there are several publications related to the anti-inflammatory and antioxidant activities of this plant, very little research has been done to compare the therapeutic properties of these leaves obtained as soluble extracts using distilled water (aqueous) and ethanol (ethanolic) extracts of leaves of this plant from Tamil Nadu, India as there are several genetic variations within the same species based on the place of cultivation and hybridization techniques for crop improvement. Moreover, crop improvement methods have been developed in Tamil Nadu and the commonly available subtypes in Tamil Nadu include Jaffna type, Moolanur moringa, Chavakacheri moringa, Valayapatti moringa, Kattumurungai, Palamedu moringa, Chemmurungai, Palmurungai, Kodikkalmurungai, and Punamurungai.^{23,24} The current research was performed to assess the AO and anti-inflammatory outcomes of aqueous and ethanolic extracts of MOL to check which extract is more superior to make a periodontal application with the extract for future use.

MATERIALS AND METHODS

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

Preparation of Crude Extracts of *Moringa* Leaves

Fresh MOL was obtained from the local market in Chennai. Leaves were thoroughly washed, kept for 10 days under the room temperature for drying. After weighing it was powdered.

Preparation of Aqueous Extract

To 100 gm of the powdered leaves, 1 liter of distilled water was added and macerated for 72 hours. Following filtration, the procedure was repeated with the same solvent for 48 hours, followed by 24 hours. Further concentration of the obtained pooled extracts was done with rotary flash and further concentrated with a vacuum desiccator and kept at 4°C for future use.

Ethanolic Extraction of MOL

To 100 gm of the powdered leaves, 1 liter of 100% pure ethanol was added and macerated for 72 hours. Following filtration, the procedure was repeated with the same solvent for 48 hours, followed by 24 hours. Further concentration of the obtained pooled extracts was done with rotary flash and further concentrated with a vacuum desiccator and preserved at 4°C.

Preparing Stock Solution and Working Solution from the Obtained Aqueous and Ethanol Extracts

To 1 mL of dimethyl sulfoxide and 9 mL of water, 10 mg of the extract (both aqueous and ethanol) was dissolved to obtain the stock solution. From the stock solution, serial dilutions were made to obtain a final concentration of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL for the experiment.

Hydroxyl Radical Scavenging Assay

For conducting this assay, the methodology described by Halliwell et al.²⁵ with a few minor changes was followed. Addition of 100 µL of 28 mM of 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4),

500 µL solution of various concentrations of the extracts (10–80 µg) or positive control vitamin E, 200 µL FeCl₃ (200 µM), 1.04 mM EDTA (1:1 v/v), 100 µL H₂O₂ (1.0 mM), and 100 µL ascorbic acid (1.0 mM) to 1.0 mL of the reaction mixture was done and incubated at 37°C for 60 minutes. TBA reaction was used to determine degradation of deoxyribose degradation. Measurement of absorbance was done at 532 nm.

FRAP Assay

Reagents for FRAP Assay (Obtained from Merck Company)

(a) Acetate buffer 300 mM pH 3.6: To 3.1 gm sodium acetate trihydrate, addition of 16 mL of glacial acetic acid was done, and volume was made up to 1000 mL with distilled water. (b) TPTZ (2,4,6-tripyridyl-s-triazine): (M.W. 312.34), 10 mM in 40 HCl (M.W. 36.46). (c) FeCl₃·6H₂O: (M.W. 270.30), 20 mM. Solutions a, b, and c at 10:1:1 ratio were mixed just before commencement of the experiment to obtain working solution of FRAP. Standard was FeSO₄·7H₂O: 0.1–1.5 mM in methanol.²⁶

Procedure

To 3.6 mL of FRAP solution, 0.4 mL of distilled water was added. Following 5 minutes of incubation at 37°C for 5 minutes, the addition of 80 mL of various concentrations of the extract or positive control was done and incubated for 10 minutes. Measurement of absorbance at 593 nm was done. Construction of the calibration curve was done with FeSO₄·7H₂O at concentrations 0.1, 0.4, 0.8, 1, 1.12, and 1.5 mM, and absorbance values were obtained for sample solutions.

Antioxidant Activity

DPPH Method

The reagents were prepared by adding to 1 mL DPPH (0.1 mM conc.), Tris HCL buffer of pH 7.4. Various concentrations of the extracts were taken in microwells (1 mL). To this, the reagent was added and incubated for 30 minutes. Measurement of absorbance was done at 517 nm with butylated hydroxytoluene as the control.²⁷

$$\text{Inhibition \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Addition of different concentrations of the crude extract were added to 1 mL of 0.1 mM DPPH in methanol and 450 µL of 50 Tris-HCl buffer (pH 7.4). Incubation was done for half an hour. The absorbance was recorded at 517 nm. BHT was the control. Calculation of the percentage of inhibition was done as follows:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Anti-inflammatory Activity

Albumin Denaturation Assay

It was done according to Mizushima and Kobayashi²⁸ with minor changes.²⁹ To 0.05 mL of different concentrations of the extract, standard addition of 0.45 mL bovine serum albumin (1% aqueous solution) was done and pH was adjusted to 6.3 with 1 N hydrochloric acid. It was incubated for 20 minutes, followed by heating at 55°C in a water bath for half an hour. The samples were brought to the room temperature to determine the optical density at 660 nm with diclofenac sodium as the standard and DMSO as the control.

$$\text{Inhibition \%} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Following twenty-minute incubation at room temperature for 20 minutes, the mixture was heated at 55°C in a water bath for half an hour. Following cooling of the samples, measurement of absorbance was done at 660 nm. Standard was diclofenac sodium, and control was DMSO.

Percentage of protein denaturation was determined utilizing the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

All the experiments were repeated thrice, and calculation of mean and standard deviation was done.

RESULTS

Crude aqueous and ethanolic extracts of MOL demonstrated antioxidant effect in DPPH assay, hydroxyl radical scavenging activity assay, and FRAP assay in a dose-dependent manner with the greatest activity at 50 µg/mL and least at 10 µg/mL. The results were comparable to the standard or positive control. The results of the DPPH assay showed that the aqueous extract demonstrated antioxidant activity of 67.52% at 10 µL, 68.95% at 20 µL, 75.21% at 30 µL, 78.32% at 40 µL and 88.52% at 50 µL, whereas the ethanolic extract exhibited 69.32%, 72.52%, 74.85%, 79.52%, 84.52% at concentrations of 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL, respectively. The hydroxyl radical scavenging activity assay revealed that the aqueous extract exhibited 72.65%, 75.65%, 81.52%, 85.65%, and 90.21%, whereas the ethanolic extract exhibited 61.25%, 66.52%, 71.54%, 74.58%, and 82.21% at concentrations of 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL, respectively. In the FRAP assay, the aqueous extract showed an activity of 71.52%, 73.52%, 79.58%, 83.52%, and 89.25%, whereas the ethanolic extract exhibited an activity of 75.65%, 78.98%, 81.52%, 83.85%, and 89.65% at concentrations of 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL, respectively.

In comparison to the antioxidant activity of the aqueous and ethanolic extract, the aqueous extract exhibited significantly higher activity in the FRAP assay at all concentrations (Fig. 1). Similar results

were observed with the hydroxyl radical scavenging activity assay (Fig. 2). Interestingly in the DPPH assay at the highest concentration used in the study (50 µL), the aqueous extract exhibited greater activity, whereas at the lowest concentration, the alcoholic extract exhibited greater activity (Fig. 3).

Considering anti-inflammatory activity, the aqueous extract exhibited 62%, 68%, 75%, 81%, and 84%, whereas the ethanolic extract exhibited 51%, 59%, 65%, 71%, and 79% at concentrations of 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL, respectively. Thus both the test substances showed anti-inflammatory activity in a concentration-dependent manner, with the maximum activity at 50 µg/mL and minimum activity at 10 µg/mL. The aqueous extract exhibited greater anti-inflammatory activity in comparison with the standard at all concentrations except 50 µL, where both exhibited the same activity. The anti-inflammatory activity of the alcoholic was slightly lesser than the standard and aqueous extract (Fig. 4).

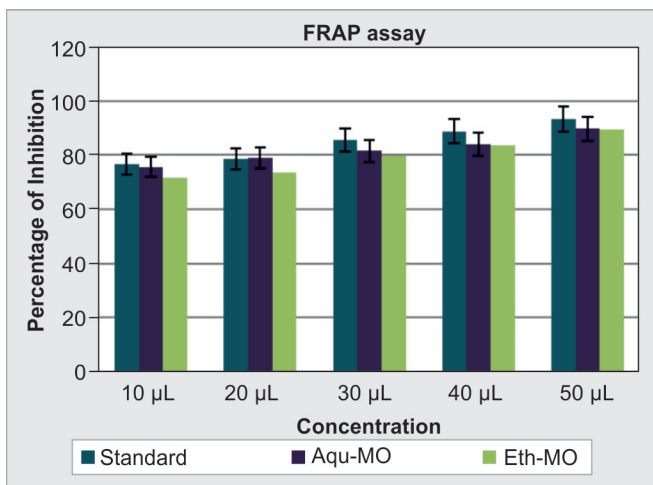


Fig. 1: Bar diagram representing FRAP scavenging activity of standard, aqueous extract of *Moringa oleifera* Lam. (Aqu-MO), and aqueous extract of *Moringa oleifera* Lam. (Eth-MO). All the extracts demonstrated antioxidant effect in a concentration-dependent manner

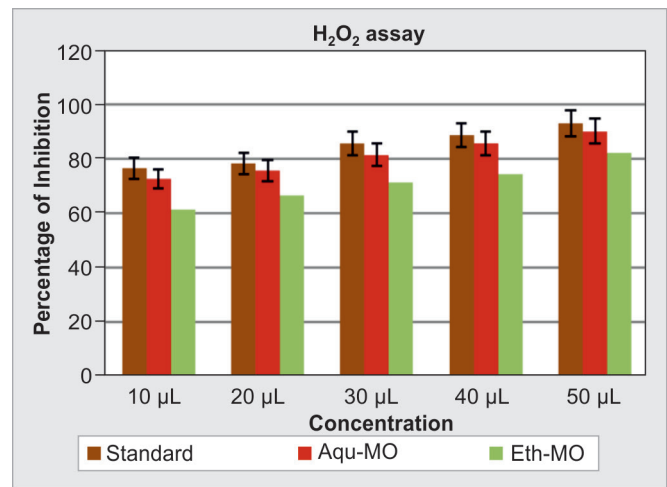


Fig. 2: Bar diagram representing hydroxyl scavenging activity of standard, aqueous extract of *Moringa oleifera* Lam. (Aqu-MO), and aqueous extract of *Moringa oleifera* Lam. (Eth-MO). All the extracts demonstrated antioxidant effect in a concentration-dependent manner

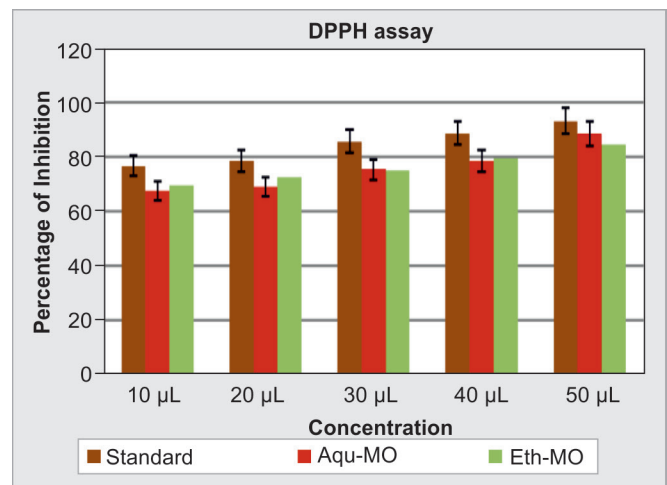


Fig. 3: Bar diagram representing DPPH scavenging activity of standard, aqueous extract of *Moringa oleifera* Lam. (Aqu-MO), and aqueous extract of *Moringa oleifera* Lam. (Eth-MO). All the extracts demonstrated antioxidant effect in a concentration-dependent manner

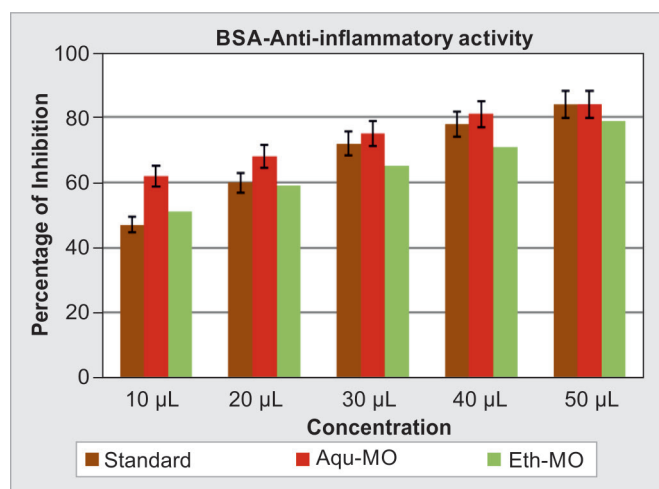


Fig. 4: Bar diagram representing the anti-inflammatory activity of standard, aqueous extract of *Moringa oleifera* Lam. (Aqu-MO), and aqueous extract of *Moringa oleifera* Lam. (Eth-MO). All the test substances showed anti-inflammatory activity in a concentration-dependent manner

DISCUSSION

Oxidative stress and inflammation play a vital role in the pathogenesis of various diseases including periodontal disease. Although several antioxidant mechanisms of the human body attempt to mitigate the free radical-induced damage, often overzealous production of free radicals and the role of external factors contribute to inflammation that results in a disease state of the periodontium. Dietary sources rich in phytochemicals confer therapeutic properties and can be explored for their antioxidant and anti-inflammatory properties for the management of chronic diseases such as periodontitis. Since these compounds are diet-derived, they are less likely to be associated with adverse effects. Hence our study aimed to assess the *in vitro* anti-inflammatory activity and antioxidant of aqueous and ethanolic extract of MOL. The present plant was chosen as it is indigenous to India, hence easily available and has several phytotherapeutic properties, and has been a part of the diet in India for several years. In the current study, the AO activity was assessed with three different assays, namely hydroxyl radical scavenging activity, DPPH assay, and FRAP assay, to determine the mechanism of action attributed to the various phytochemicals present in crude extracts.³⁰ Both the extracts demonstrated antioxidant effects in a dose-dependent manner. All three experiments and the results were comparable to the standard compound assessed. Considering AO activity of ethanolic extracts of MOL leaves in the DPPH and FRAP assay, the results are concurrent with the findings of Xu et al. who reported that the ethanolic extract of the leaves of *M. oleifera* exhibited the highest antioxidant activity in these two assays in comparison with that of roots and seeds.²⁰ Similarly, Atawodi et al. assessed the AO activity of methanolic extract of root bark, stem, and leaves of MOL, they found that all the plant parts exhibited antioxidant activity via xanthine oxidase model system and 2-deoxyguanosine assay model system with an IC₅₀ values of 40 µL for leaves, 58 µL for stem, and 72 µL for root bark.³¹ Siddhuraju and Becker³² compared the antioxidant activity of water, ethanol, and hydroalcoholic extracts of MOL from different sources such as India, Nicaragua, and Niger via reducing power, superoxide radical scavenging

activity, DPPH assay, liposome peroxidation induced by Fe³⁺/H₂O₂/ascorbic acid, and beta carotene-linoleic acid system. They reported that all the extracts exhibited antioxidant activity in all the assays, and predominantly alcoholic extract (methanol 80%) and ethanol (70%) from different sources had higher activity than aqueous extract. They also reported that the leaves of Indian origin exhibited the highest AO activity of 65.1% (methanol extract) and 66.8% (ethanol extract) in the β-carotene-linoleic acid system. The minor variations in the results of the current study with other studies could be attributed to the seasonal variations as we collected the plant material in the month of February. Huda et al. have assessed the seasonal variations of this plant and have reported that summer is the month of highest yield although spring, autumn, and monsoon are favorable.³³ In accordance with the results of our study, Tijani et al. reported the *in vitro* and *in vivo* hydroxyl radical scavenging activity of various fractions (ethyl acetate, chloroform, and N-hexane fractions) of aqueous extract of MOL.³⁴ They reported that ethyl acetate fraction had the highest activity, which could be due to the solubility and bioavailability of the phytochemicals with the particular solvent. The flavonoids and polyphenols in MOL could be responsible for the antioxidant effects.^{20,32} On comparing the antioxidant activity of aqueous and ethanol extracts, the aqueous extract exhibited higher activity in hydroxyl radical scavenging activity assay and FRAP, whereas, in DPPH, there was variation, wherein, at higher concentrations, the aqueous extract had higher activity, and at lower concentrations, the ethanol extract exhibited greater activity. These variations could be attributed to the solvent used for extraction and the number of days the leaves were left for drying, which alters the phytochemicals.³⁵ Although studies have shown that the total phenol content was higher in the ethanol extract, there may be other phytochemicals such as vitamin C and carotenoids that could confer the greater antioxidant property of the aqueous extract.

In the present study, the aqueous extract exhibited higher anti-inflammatory effects, whereas the alcoholic extracts had lesser activity in comparison with the standard. Xu et al. reported that the alcoholic extract leaves of *M. oleifera* exhibited increased activity than the seeds according to DPPH, FRAP, and ABTS assay with IC₅₀ values of 1.02 ± 0.13 mg/mL, 0.99 ± 0.06 mM Fe²⁺/gm, and 1.36 ± 0.02 mg/mL, respectively. In their study, both the extracts exhibited anti-inflammatory properties, shown by inhibition of nitric oxide. The results also correlated with the flavonoid content of the extracts, thereby showing the role of flavonoids in conferring antioxidant and anti-inflammatory activity.²⁰ Considering aqueous extract the findings of our study are concurrent with findings of Mittal et al. Similarly, Xiao et al., in their mini-review, have reported the anti-inflammatory properties of this wonder herb and its role in the management of chronic inflammatory condition, which is similar to the results of the present study. The anti-inflammatory properties could be attributed to the presence of quercetin which is known to inflammation NF-κB, thereby reducing inflammation.³⁶

From the data obtained, it appears that *M. oleifera* is an ideal herb for periodontal applications. It is to be reiterated at this point that inflammation and an overzealous production of reactive oxygen species dominate the scene of periodontal destruction, which is often described as a battle between the host immune system and periodontal pathogens.²¹ The antioxidant properties of *M. oleifera* extract as demonstrated by the hydroxyl radical scavenging activity assay, FRAP, and DPPH assays and the content of important phytoconstituents such as vitamin C, carotenoids,

and quercetin, make it a superior antioxidant. The other important property, as previously described, that makes this herb a superior periocutaneous agent is the NF- κ B inhibiting activity.³⁷ The major limitation of the study is that it has an *in vitro* design, and *in vivo* studies have to be done to determine the bioavailability and toxicity of the extract.

CONCLUSION

Thus, it can be concluded that the anti-inflammatory and antioxidant effect of *M. oleifera* could be explored further not only for systemic illness associated with inflammation but also inflammatory conditions of the oral cavity. A local drug delivery system with the extracts could be formulated and its use for the management of chronic inflammatory conditions such as periodontitis. It is noteworthy that several antimicrobial agents such as tetracycline, chlorhexidine, and doxycycline have been tried as local drug delivery formulations for periodontitis management.^{38,39} In this connection, *M. oleifera* extracts could also be tried as gels, ointments, and mouthwashes and in addition, they could be modified for intrapocket delivery in the form of microspheres, *in situ* gels, and nanoformulations. If fruitful clinical results are obtained, *M. oleifera* could be included in the list of nature-derived host modulatory agents for periodontitis management.

REFERENCES

- Paliwal R, Sharma V, Pracheta. A review on horse radish tree (*Moringa oleifera*): a multipurpose tree with high economic and commercial importance. *Asian J Biotechnol* 2011;3(4):317–328. DOI:10.3923/ajbkr.2011.317.328.
- Leone A, Spada A, Battezzati A, et al. Cultivation, genetic, ethnopharmacology, phytochemistry, and pharmacology of *Moringa oleifera* leaves: an overview. *Int J Mol Sci* 2015;16(6):12791–12835. DOI:10.3390/ijms160612791.
- Rodríguez-Pérez C, Quirantes-Piné R, Fernández-Gutiérrez A, et al. Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves. *Ind Crops Prod* 2015;66:246–254. DOI:10.1016/j.indcrop.2015.01.002.
- Kasolo JN, Bimenya GS, Ojok L, et al. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *J Med Plants Res* 2010;4(9):753–757. DOI:10.5897/JMPR10.492.
- Gupta S, Jain R, Kachhwaha S, et al. Nutritional and medicinal applications of *Moringa oleifera* Lam.—review of current status and future possibilities. *J Herb Med* 2018;11:1–11. DOI:10.1016/j.hermed.2017.07.003.
- Panda S, Kar A, Sharma P, et al. 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;23(4):959–962. DOI:10.1016/j.bmcl.2012.12.060.
- Roy SK, Chandra K, Ghosh K, et al. Structural investigation of a heteropolysaccharide isolated from the pods (fruits) of *Moringa oleifera* (Sajina). *Carbohydr Res* 2007;342(16):2380–2389. DOI:10.1016/j.carres.2007.07.020.
- Bhatta R, Saravanan M, Baruah L, et al. Nutrient content, *in vitro* ruminal fermentation characteristics and methane reduction potential of tropical tannin-containing leaves. *J Sci Food Agric* 2012;92(15):2929–2935. DOI:10.1002/jsfa.5703.
- Mathur M, Yadav S, Katariya PK, et al. *In vitro* propagation and biosynthesis of steroidal sapogenins from various morphogenetic stages of *Moringa oleifera* Lam., and their antioxidant potential. *Acta Physiol Plant* 2014;36:1749–1762. DOI:10.1007/s11738-014-1549-1.
- Maldini M, Foddai M, Natella F, et al. Identification, and quantification of glucosinolates in different tissues of *Raphanus raphanistrum* by liquid chromatography tandem mass spectrometry. *J Food Compos Anal* 2017;61:20–27. DOI:10.1016/j.jfca.2016.06.002.
- Sahakitpichan P, Mahidol C, Disadee W, et al. Unusual glycosides of pyrrole alkaloid and 4'-hydroxyphenylethanamide from leaves of *Moringa oleifera*. *Phytochemistry* 2011;72(8):791–795. DOI:10.1016/j.phytochem.2011.02.021.
- Coz-Bolaños X, Campos-Vega R, Reynoso-Camacho R, et al. *Moringa* infusion (*Moringa oleifera*) rich in phenolic compounds and high antioxidant capacity attenuate nitric oxide pro-inflammatory mediator *in vitro*. *Ind Crops Prod* 2018;118:95–101. DOI:10.1016/j.indcrop.2018.03.028.
- Coppin JP, Xu Y, Chen H, et al. Determination of flavonoids by LC/MS and anti-inflammatory activity in *Moringa oleifera*. *J Funct Foods* 2013;5(4):1892–1899. DOI:10.1016/j.jff.2013.09.010.
- Verma AR, Vijayakumar M, Mathela CS, et al. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food Chem Toxicol* 2009;47(9):2196–2201. DOI:10.1016/j.fct.2009.06.005.
- Jung IL. Soluble extract from *Moringa oleifera* leaves with a new anticancer activity. *PLoS One* 2014;9(4):e95492. DOI:10.1371/journal.pone.0095492.
- Jaiswal D, Kumar Rai P, Kumar A, et al. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. *J Ethnopharmacol* 2009;123(3):392–396. DOI:10.1016/j.jep.2009.03.036.
- Peixoto JRO, Silva GC, Costa RA, et al. *In vitro* antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. *Asian Pac J Trop Med* 2011;4(3):201–204. DOI:10.1016/S1995-7645(11)60069-2.
- Dangi SY, Jolly CI, Narayanan S. Antihypertensive activity of the total alkaloids from the leaves of *Moringa oleifera*. *Pharm Biol* 2002;40(2):144–148. DOI:10.1076/phbi.40.2.144.5847.
- Atta A, Nasr S, Almaweri A, et al. Phytochemical, antioxidant and hepatoprotective effects of different fractions of *Moringa oleifera* leaves methanol extract against liver injury in an animal model. *Asian Pac J Trop Med* 2018;11(7):423–429. DOI:10.4103/1995-7645.237186.
- 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;8(8):296. DOI:10.3390/antiox8080296.
- Sczepanik FSC, Grossi ML, Casati M, et al. Periodontitis is an inflammatory disease of oxidative stress: we should treat it that way. *Periodontol* 2000;2020;84(1):45–68. DOI:10.1111/prd.12342.
- Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. *Int Dent J* 2020;71(6):462–476. DOI:10.1111/idj.12630.
- Bhatnagar AS, Gopalakrishna AG. Natural antioxidants of the Jaffna variety of *Moringa Oleifera* seed oil of Indian origin as compared to other vegetable oils. *Grasas y Aceites* 2013;64(5):537–545. DOI: 10.3989/gya.010613
- Rajalakshmi R, Rajalakshmi S, Parida A. Evaluation of the genetic diversity and population structure in drumstick (*Moringa oleifera* L.) using SSR markers. *Curr Sci* 2017;112(6):1250–1256. DOI: 10.18520/cs/v112/i06/1250-1256
- Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem* 1987;165(1):215–219. DOI:10.1016/0003-2697(87)90222-3.
- Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. *Anal Biochem* 1996;239(1):70–76. DOI:10.1006/abio.1996.0292.
- Yokozawa T, Chen CP, Dong E, et al. Study on the inhibitory effect of tannins and flavonoids against the 1,1-Diphenyl-2-picrylhydrazyl radical. *Biochem Pharmacol* 1998;56(2):213–222. DOI:10.1016/S0006-2952(98)00128-2.
- Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol* 2011;20:169–173. DOI:10.1111/j.2042-7158.1968.tb09718.x.

29. Das P, Ghosal K, Jana NK, et al. Green synthesis and characterization of silver nanoparticles using belladonna mother tincture and its efficacy as a potential antibacterial and anti-inflammatory agent. *Mater Chem Phys* 2019;228:310–317. DOI:10.1016/j.matchemphys.2019.02.064.
30. Zhu M, Liu T, Zhang C, et al. Flavonoids of lotus (*Nelumbo nucifera*) seed embryos and their antioxidant potential. *J Food Sci* 2017;82(8):1834–1841. DOI:10.1111/1750-3841.13784.
31. Atawodi SE, Atawodi JC, Idakwo GA, et al. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of *Moringa oleifera* Lam. *J Med Food* 2010;13(3):710–716. DOI:10.1089/jmf.2009.0057.
32. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agric Food Chem* 2003;51(8):2144–2155. DOI: 10.1021/jf020444+.
33. Huda NH, Haque KS, Roy BK, et al. Seasonal weather impacts on biomass production of *Moringa oleifera* at different fertilizer doses. *Int J Health Anim Sci Food Saf* 2017;4(1):12–23. DOI: 10.13130/2283-3927/8001
34. Tijani AA, Adekomi DA, Adewole SO, et al. In-vitro and in-vivo determination of hydroxyl radical scavenging activity (hrsa) of fractions of aqueous extract of moringa oleifera leaves (aemol). *Eurasian J Med Oncol* 2018;2(4):209–216. DOI:10.14744/ejmo.2018.46330.
35. Nobossé P, Fombang EN, Mbofung CMF. Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food Sci Nutr* 2018;6(8):2188–2198. DOI:10.1002/fsn3.783.
36. Das N, Sikder K, Ghosh S, et al. *Moringa oleifera* Lam. leaf extract prevents early liver injury and restores antioxidant status in mice fed with a high-fat diet. *Indian J Exp Biol* 2012;50(6):404–412. PMID: 22734251
37. Abdel-Daim MM, Khalil SR, Awad A, et al. Ethanolic extract of *Moringa oleifera* leaves influences NF-κB signaling pathway to restore kidney tissue from cobalt-mediated oxidative injury and inflammation in rats. *Nutrients* 2020;12(4):1031. DOI:10.3390/nu12041031.
38. Panwar M, Gupta, S. Local drug delivery with tetracycline fiber : an alternative to surgical periodontal therapy. *Med J Armed Forces India* 2009;65(3):244–246. DOI:10.1016/S0377-1237(09)80014-2.
39. Da Rocha HAJ, Silva CF, Santiago FL, et al. Local drug delivery systems in the treatment of periodontitis: a literature review. *J Int Acad Periodontol* 2015;17(3):82–90. PMID:26373225.