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ESSENTIAL TRACE ELEMENTS OF *MORINGA OLEIFERA* AND THEIR APPLICATION IN ORAL HEALTH

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ABSTRACT

Continuous change in socio-economic status has made people concerned about their oral health. Utilization of natural plant product has gained popularity since last few years. *M.oleifera* is a plant with abundant trace elements present in all its parts in good concentration. These trace elements need extraction in their good concentrated form for future application in oral healthcare for management of various diseases caused due to deficiency of micronutrients. Various methods for extraction have been adopted by different researchers. This review focuses on the benefits of essential trace elements from *Moringa* in oral health and directs to a comparative study of commonly used methodologies on basis of their advantages, limitations and economic convenience which help to estimate suitability and economic viability of the methods to obtain the trace elements in their most concentrated form The trace elements from *M. oleifera* can be used as future alternative in oral health care

and on the basis of accuracy, simplicity in operation and cost- effective nature fame atomic absorption spectrometry would be effective one for analysis of the elements.

Key words: Trace elements, Oral health, Fame atomic absorption spectrometry, *M. oleifera,* Micronutrient profile.

INTRODUCTION

Our body needs nutrients for the growth, development, and regulation of the metabolic activity of cells. The essential nutrients are divided into two groups- macronutrients and micronutrients. Macronutrients are needed in larger amounts by our body like carbohydrates, proteins, and fats. Micronutrients are needed in little or trace amounts like vitamins and minerals. Trace elements play a vital role in regeneration, oxidative stress, and immunity against pathogens. Although present in tiny amount has a great influence on all body functions. They take part in biochemical reactions by acting as catalysts or co-factor for enzymatic reactions, their presence in surplus amount or deficiency causes disturbance in metabolic pathways which lead to diseases **[1]**.

General health is linked to oral health directly because, oral cavity is the passage to the rest of the body. Oral health problems effects the quality of life. Deterioration in general health is the common cause for occurrence of oral diseases **[2]**. Nutritional guidance is the significant component of preventive dentistry these days **[3]**. Therefore, regular intake of vitamins and minerals are of huge importance for maintenance of oral health. For example, when there is inflammation, fast immune response is required, which effectively occurs when there is a sufficient supply of micronutrients **[4]**. Herbal medicines are preferred mostly because they have low toxicity, have high curative value, and are cost-effective. Numerous plants have been identified as having medicinal properties. *Moringa oleifera* or "the drumstick tree" is the storehouse of minerals, loaded with various bioactive compounds and essential trace elements in good concentration. As there had been limited knowledge regarding the application of Moringa derived essential trace elements in oral health care, the current review focuses on the benefits of essential trace elements from Moringa in oral health and their preferable extraction method to get the trace elements in their most concentrated form.

Classification:

Different scientists have their own respective system of classification. Till date, around 90 elements have be identified that occurs naturally, out of which 26 have been found essential for us. Among the 26; 11 are named as major elements - C, H, O,

N, S, Ca, P, K, Na, Cl, Mg and 15 are categorised as trace elements- Fe, Zn, Cu, Mn, Ni, Co, Mo, Se, Cr, I, F, Sn, Si, V, As **[5]**.

Micronutrient profile of *Moringa oleifera* and its application in oral health:

Medicinal plants have been the natural sources of various compounds having pharmacological and nutritional values that help in management and treatment of various diseases **[6]**. Several such plants has been studied till date and Moringa is one among them. *Moringa oleifera* or the drumstick tree belongs to the *Moringaceae* family is a flowering tree, perennial in nature and is native to Indian sub-continent. It is cultivated in tropical regions and is resistant to adverse environmental conditions **[7]**. *M. oleifera* has stood out amongst the most helpful medicinal plants that can be used therapeutically for control of several diseases. Presence of secondary metabolites, macronutrients and micro nutrients in this plant increases the efficiency of being used as alternative medicinal therapies **[8]**.

The miracle tree- as the name suggests is the store house of nutrients. This tree, alone, has the potential of eradicating human micronutrient deficiencies **[9]**. All the parts of the plant has tremendous health benefits. The leaves are commonly cooked and consumed. It also has uses in traditional medicine like for treatment of jaundice. The leaves contain more Ca than milk, more K than bananas, more Vit C than oranges, more Fe than spinach and more Vitamin A than carrots **[10, 11]**. Roots of this tree can be used for treatment of treatment of diabetes, cough, fever, gout, cholesterol and goitre. Stems are used as fodder for animal, to treat stomach aches, coughs and fever. Pods are also cooked and consumed **[10]**.

The presence of innumerable pharmaceutical properties makes this plant a potent herbal drug that is used for treatment of many diseases and also having its application in dentistry. Use of Moringa in dentistry is also widely found. Moringa can be used as ingredient in toothpaste and mouthwash. It has uses in root canal irrigation and healing of wound after extraction. It can be used as medicine for gingivitis and canker sores and can be used to prevent dental caries **[10]**. The micronutrient contents of this plant and its uses are being studied for management and treatment of diseases **(Table 1) (Figure 1) (Figure 2)**.

Table 1: Trace elements present in different parts of *M. oleifera* and their benefits in oral health care

Parts of plant	Trace elements	Benefits in oral health	References
Leaves	Ca, Mg, S, Na, K, P, Fe, Zn, Cu, Mo, Ni, Se, Mn, Cl, Se, I	Antimicrobial activiy against pathogens like *Staphylococcus aureus* and Pseudomonas aerogenosa , Ca improvres health of our teeth, antimicrobial activity against S. mutans which inhibits formation of carcinogenic biofilm.	12,13,14,15,16,17,18,19,7,20,11,21
Pods	Ca, Mg, S, Na, K, P, Fe, Cu, Zn, Mn, Se, S, K	Ca improvres health of our teeth	12,6,18,7,11,21
Seeds	Ca, Mg, Fe, Cu	Antibacterial effect against Staphylococcus aureus, Vibrio cholerea, and Escherichia coli and pseudomonas aerogenosa, Ca improvres health of our teeth, oil inhibit the formation of dental plaque, caries, and strengthen the gums	12,14,6,18,7,11
Stem bark	Ca, Co, Fe, K, Mg, Mn, Ni, Zn	Ca improvres health of our teeth, paste helps relieving burning sensation, inflammatory conditions, and abscesses and wound healing, antibacterial property against *Staphylococcus* and *Streptococcus*	22,11
Flowers	Ca, Cu, Fe, Mg, Zn, Se, P, K	Ca improvres health of our teeth, boiled flower is inhaled for oral health	15,11
Roots	Mg, Ca, Na, K, Cu	Dental Caries/Toothache, Ca improvres health of our teeth, inhibits growth of *Staphylococcus* and *Streptococcus*	16,24,11,23

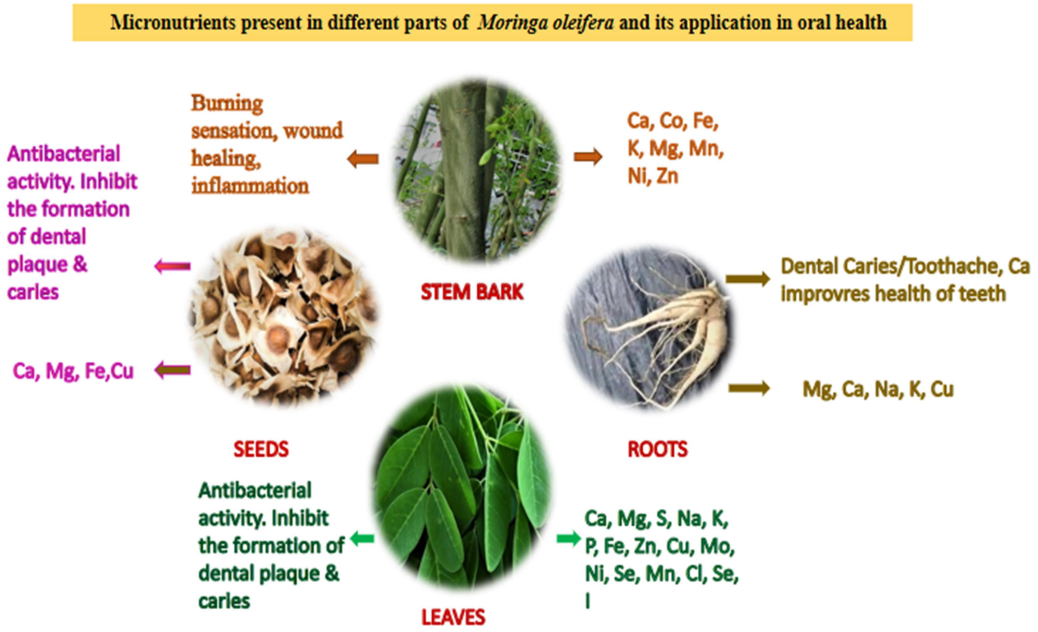

Figure 1: Micronutrients present in different parts of *M. oleifera* and its application in Oral health

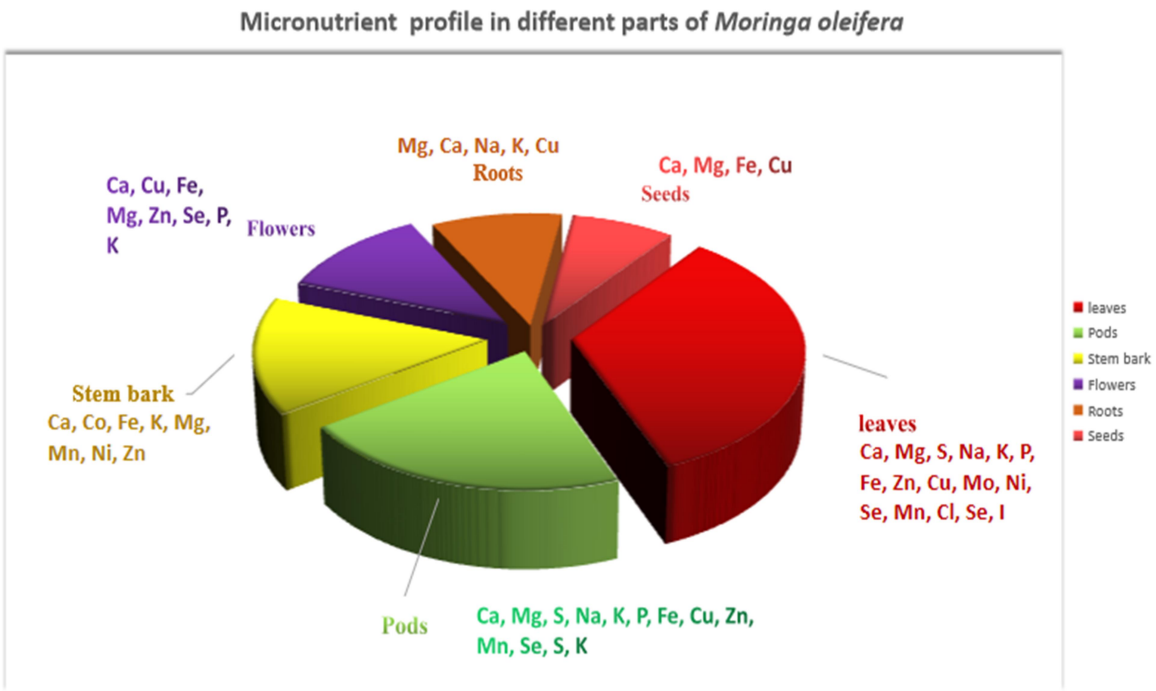

Figure 2: Micro-nutrients concentration in different parts of *M. oleifera*

Trace elements for a well-functioning of immune system

Role of micronutrients in optimising health and management or treatment of diseases is gaining interest in present day researches **[25]**. Micronutrients are dietary components having significant contribution to the immune system. Our health is completely dependent on the functioning of immune system **[26]**. Micronutrients has main role in maintenance of tissue function, formation of teeth and bones, act as co-factor and co-enzyme in enzyme system, aids co-ordination in various biochemical and physiological function in the body **[27]**. Sufficient vitamins and trace elements are needed in our body for the well-functioning of the immune system. Deficiency of micronutrients alters the innate T-cell mediated and adaptive antibody response, creating disturbance in host-pathogen interaction there by supressing the immune functions **[28]**.

Zn acts as catalyst in more than 300 enzymatic reactions. Zn plays role in cell communication, differentiation, proliferation and cell survival. Deficiency of Zn alternates the innate and adaptive immune response, induces inflammation leading to chronicity **[29]**. Deficiency of copper causes disturbances in haematopoiesis leading to anaemia, incomplete development of nervous system in prenatal stage, defective keratinization and loss of pigmentation of skin and hair, may also cause bradycardia **[30]**. Likewise

deficiency of trace elements also effects the oral health. In a study deficiency of Zn caused oral and periodontal tissues (Hyperkeratinisation) in rats **[31]**. Effects of Zn deficiency was studied on oral tissues of rat and the findings indicated increased mean pocket depth and apthous ulcer. Zn deficiency also leads to decrease in taste lingual trigeminal nerve sensitivity and reduction in salivary flow **[32]**. Patients suffering from iron deficiency anaemia had oral symptoms like burning sensation of oral mucosa, loss of taste, lingual varicosity, dry mouth, aphthous ulceration and oral lichen planus **[33]**. **(Table 2)**.

Table 2: Effects due to deficiency of trace elements in oral health

Trace elements	Deficiency in oral health	References
Fe	**Lack of iron causes alternation In epithelium and iron-dependent enzymatic system and hampers the cell mediated immunity. Oral candidiasis, Atrophy of the lingual papillae, lowers the internal diversity in the oral flora, epithelium gets thinner, mucosal inflammatory, and changes in atrophy**	**34,35,36,37,38**
Zn	**Deficiency causes taste disorders, parakeratosis of oral mucosa, oral leukopakia, oral submucous fibrosis, oral squamous cell carcinoma, oral ulceration, Perioral dermatitis, flattened filiform papillae, impaired wound healing, and sensations of xerostomia, hypogeusia, and burning or sore mouth, mucosal membrane disintegrate is associated with the pathogenesis of lichen planus, gingival inflammation**	**1,35,39,38**
Cu	**Excess Cu results in oral leucoplakia, oral submucous fibrosis and oral squamous cell carcinoma.**	**1**
Co	**Pernicious anaemia characterized by glossitis, burning sensation, hunter's or moeller's glossites, shallow ulcer, adverse effect on peripheral neuropathy. Co from metal alloys damages basal epithelial keratinocytes.**	**1**
Se	**Deficiency causes oxidative stress leading to OSMF and oral cancer, reduction of immunoglobulin titer and cellular immunity leading to OLP**	**1,38**
Cr	**Deficiency leads to delayed wound healing, suppurative periodontitis, various oral fungal infections, premature periodontal diseases and hypo salivation. Cr from metal alloys has the potential of malignant formation.**	**1**
I	**Excess or deficiency disturbs the oral immune defence mechanism**	**1**
F	**Excess causes deformities of hard tissues, dental fluorosis.**	**34**
Ca	**Increase risk of periodontal diseases and tooth loss, oral ulcers such as aphthous stomatitis, indirectly affects periodontal disease**	**35,39,38**

Extraction of trace elements:

M.oleifera has versatile uses in medicine, nutraceutical, food and water purification. The use of Phytoactive compounds in health care had become common while the use of its micronutrients in management of different diseases has gained importance since past few years. Studies says all parts of the tree has storage of essential trace elements in good concentration. In order to use these trace elements effectively in health care there is a need for extraction and analysis in the best possible method. Various methods have been adopted by different researchers all over the world according to their requirement. Similarly, In a study carried out in India , the trace elements obtained from the stem bark of *M. oleifera* were: As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Ni, Mn, Pb and Zn after digestion with HNO_3 and $HClO_4$ (4:1) and determined by atomic absorption spectroscopy (AAS). The concentration of essential trace elements i.e, Fe, Zn, K, Mg, Mn, Ca, Co, Ni were found to be within limit whereas, the heavy trace elements i.e, Cu, Cd and Pb were found to be within limit and Cr was high.[22] In Ghana, South Africa a study aimed at measuring the macro and micro elements from dried *Moringa* leaves by the help of energy dispersive X-ray fluorescence spectroscopic (EDXRF) and its toxicity was tested in rats. The major elements detected were: Na, Mg, Al, Si, P, S, Cl K, Ca, Mn and Fe **[40]**. The nutrient content of *M. oleifera* leaves were analysed with the help of inductively coupled argon plasma atomic emission spectroscopy (ICP-AES) after digesting the leaves with HNO_3 and $HClO_4$ (4:1). The micronutrients detected were: Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Se, Zn. The concentration of Ca and K.[41] The leaves of *M. oleifera* were digested with HNO_3 and the trace elements were determined such as Ca, Cu, Fe, I, Mg, Se, Zn were determined by inductively coupled plasma- mass spectrometry(ICP-MS) **[15]**. Chemical composition and nutritional values were tested from dried *M. oleifera* leaves (obtained from market). The mineral elements were found to be- Ca, K, Mg. Analysis was done through inductively coupled plasma- mass spectrometry (ICP-MS) by dry ashing method.[42] Essential macro elements and essential trace elements were determined in dried leaves and seeds of *M. oleifera*. The samples were air dried, ground and digested with Nitric acid in in microwave, further analysis was done by inductively coupled plasma optical emission spectroscopy (ICP-OES). After analysis the presence of K, Fe, Mg, Mn were found in highest concentration in the seeds. Zn an Al was found highest in leaves.[43] The nutrient content of leaves were examined among 12 different species of Moringa, including *M. oleifera*. The

leaves were dried and digested with nitric acid and hydrogen peroxide. Then the samples were analysed for protein, macro and micro nutrient using inductively coupled plasma optical emission spectroscopy (ICP-OES). The result showed presence of Ca, Cu, Fe, K, Mg, Mo, Na, P, S, Zn and protein **[14]**. **(Table 3) (Table 4) (Figure 3)**.

Table 3: Extraction and analysis methods used by various researchers

Sl. No.	Author	Year	Methods used for sample preparation	Analysis done with help of	Result obtained
1	Freiberger CE *et al*	1998	Dried leaves were ground and digested by concentrated HNO3 and 70% HClO4 (wet ashig)	inductively coupled argon plasma atomic emission spectroscopy (ICP-AES)	Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Se, Zn
2	Gyamfi ET *et al*	2011	Air, freeze and oven dried leaves were acid digested with NHCl and H2O2	fast sequential atomic absorption spectrometer(AAS)	Na, Mg, P, K, Ca, Mn, Cr, Fe, Cu, Cd, Zn
3	Gupta J *et al*	2014	Stem bark was dried and digested with HNO_3 and $HClO_4$ (4:1)	atomic absorption spectroscopy (AAS)	Fe, Zn, K, Mg, Mn, Ca, Co, Ni, Cu, Cd, Pb, Cr
4	Asiedu-Gyekye IJ *et al*	2014	Dried Moringa leaves were made to pellet and subjected for analysis	energy dispersive X-ray fluorescence spectroscopic (EDXRF)	Major elements detected were Na, Mg, Al, Si, P, S, Cl, K, Ca, Mn, and Fe
5	Yimer AM *et al*	2016	Dry Moringa leaves were digested by mixture of HNO3 (70%), HClO4 (70%) and H2O2 (30%) (2.5:1:1)	Atomic Absorption Spectrometry (AAS)	Fe, Cr, Pb and Cu
6	Kumssa DB *et al*	2017	Air and oven dried samples were digested with Nitric acid	ICP-MS	Ca, Cu, I, Fe, Mg, Se, and Zn
7	Goroya KG *et al*	2019	Dried roots were digested using HCl, HNO_3(3:1) and 30% H_2O_2	Flame Atomic Absorption Spectroscopy (FAAS)	Mg, Ca, Na, K, Cu
8	Ngigi AN et. al	2019	Dried leaves were digested with HNO_3 in a microwave	Inductively coupled plasma optical emission spectroscopy (ICP-OES)	K, Fe, Mg, Mn, Zn, Al
9	Aldakheel RK *et al*	2020	Dry Moringa leaves in pellet form digested with HNO3 of 69% and H2O2 of 30%	Inductively coupled plasma optical emission spectroscopy (ICP-OES).	Ca, K, S, Na, Mg, P, Fe, Mn, Zn, Cu, Cr, and Se
10	Adefa T *et al*	2020	Dried leaves were digested with HNO3 (69%), and HClO4 (70%)	Inductively coupled plasma optical emission spectroscopy (ICP-OES).	Cr,Cu, Zn.

Table 4: Comparative study of methods used for analysis of trace elements

Methods for analysis of trace elements	Advantages	Disadvantages	References
FAAS	1. Cost effective and low maintenance. 2. Simple to operate. 3. Capable of determining about 70 element. 4. Highly sensitive to many elements 5. Accuracy is higher 6. Portable in-run precision of 0.1-1%	1. Cannot determine non-metals. 2. Less sensitive to refractory elements. 3. Simultaneous elemental analysis is difficult.	48,49
ICP-OES	1.More than one element can be analysed sequentially. 2.Faster.	1.Costly in-run precision 0.3-2%	40

	3.Detection limit is higher but less than ICP-MS		
ICP-AES	**1. Wide variety of element can be determined. 2.Accurate 3.Faster rate of analysis than FAAS**	**1. Dilution factor of the sample caused by dissolution. 2. Spectral overlap due to presence of other elements in sample. 3. High temperature of plasma effects the calibration curve.**	**50**
ICP-MS	**1. Analyses multiple element and is faster. 2. Highly sensitive to elements. High detection limit**	**1. New and still growing. 2. High cost in-run precision is 1-3%**	**51,49**

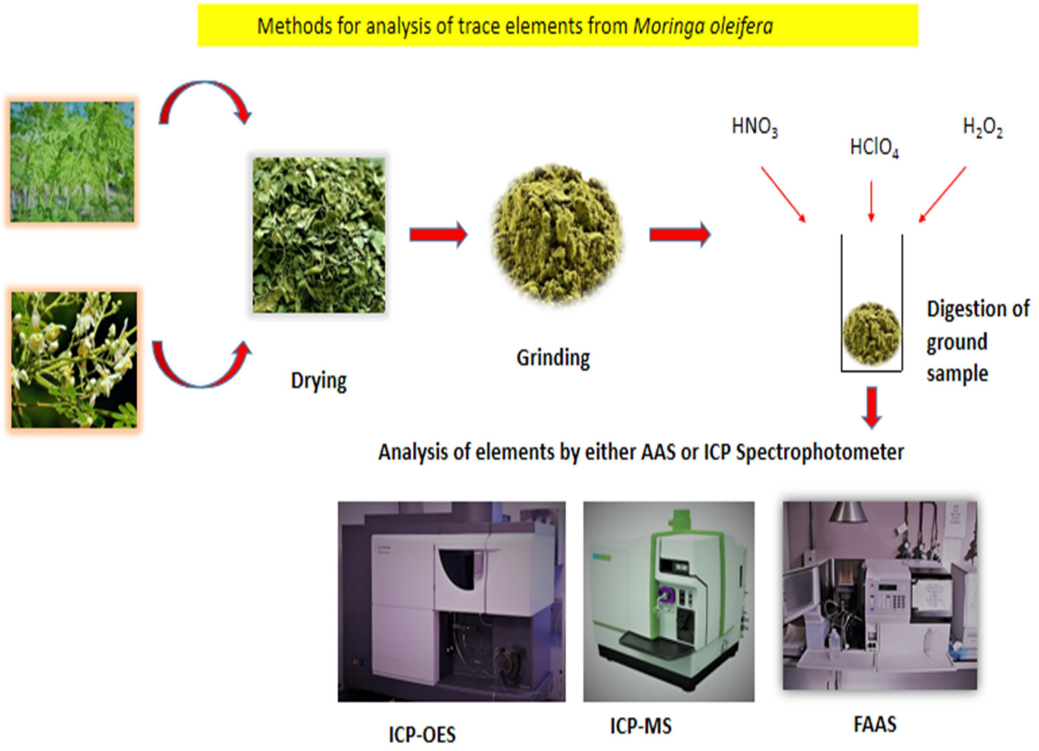

Figure 3: Methods for extraction and analysis of trace elements

CONCLUSION:

The changing socio-economic status has made the population more concerned about their oral health, particularly in the developing countries due to unhealthy eating habits and lack of good oral hygiene. Use of natural plant products has gained popularity since last few years due to their minimum side effects. Innumerable medicinal plants has been known for their nutraceutical properties and *Moringa oleifera* is one among them. Along with the Phytoactive compounds the trace elements present in various parts of the tree can be used as an alternative and effective measure without having any side effects. The extracts from various parts of the plant has already shown medicinal properties like antibacterial, anti-inflammatory and in prevention of tooth decay. Deficiency of

micronutrients like Zinc, Iron, Calcium, cobalt, selenium, chromium, iodine, calcium etc., causes various diseases in the oral cavity hence these micronutrients have been a choice of therapy by the Dental surgeons in management of various oral diseases. Therefore, trace elements from *Moringa* could be used in oral health care.

The trace elements should be in its best concentrated form so that the medicinal effect will be more. Hence, the method of extraction and analysis is a matter of concern. Based on the advantages and disadvantages of the instrument, the process of fame atomic absorption spectrometry would be one of the best process for analysis of the elements as it is simple to operate, accuracy is high, portable, determines about 70 elements and cost effective.

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REFERENCES

[1] Bhattacharya PT, Misra SR, Hussain M. Nutritional aspects of essential trace elements in oral health and disease: an extensive review. Scientifica. 2016 Jun 28; 2016.

[2] Haumschild MS, Haumschild RJ. The importance of oral health in long-term care.
J AM MED DIR ASSOC. 2009 Nov 1; 10(9): 667-71.

[3] Shaik PS, Pachava S. The role of vitamins and trace elements on oral health: a systematic review. IJMR. 2017 Mar 1; 4(1): 22-31.

[4] Willershausen B, Ross A, Försch M, Willershausen I, Mohaupt P, Callaway A. The influence of micronutrients on oral and general health. Eur J Med Res. 2011 Dec; 16(11): 514-8.

[5] Underwood EJ. Trace elements in human and animal nutrition. Ed. 3. New York, USA, Academic Press, Inc.; 1971.

[6] Brilhante RS, Sales JA, Pereira VS, Castelo DD, de Aguiar Cordeiro R *et al*. Research advances on the multiple uses of *Moringa oleifera*:

A sustainable alternative for socially neglected population. Asian Pac. j. trop. med. 2017 Jul 1; 10(7): 621-30.

[7] Meireles D, Gomes j,Lopes L, Hinzmann M,Machado J. A review of properties, nutritional and pharmaceutical applications of *Moringa oleifera*: integrative approach on conventional and traditional Asian medicine. 2020 Aug 17: 20: 495–515.

[8] SWATIą AK, Kumari CH, Ali AA, Garg PR *et al*. *Moringa oleifera* -A Never Die Tree: An Overview. Asian J Pharm Clin Res. 2018; 11(12): 57-65.

[9] Kumssa DB, Joy EJ, Young SD, Odee DW, Ander EL, Broadley MR. Variation in the mineral element concentration of *Moringa oleifera* Lam. and M. stenopetala (Bak. f.) Cuf.: Role in human nutrition. PloS one. 2017 Apr 7; 12(4): e0175503.

[10] Nurul M, Harun AM. Systematic Review of *Moringa oleifera* 's Potential as Antibacterial and Anti-Inflammatory in the Oral Cavity. Eur. J. Mol. Clin. Med.. 2020 Dec 8; 7(10): 144-61.

[11] Mohanty M, Mohanty S, Bhuyan SK, Bhuyan R. Phytoperspective of *Moringa oleifera* for oral health care: An innovative ethnomedicinal approach. Phytother. Res.. 2021 Mar; 35(3): 1345-57.

[12] Gowrishankar R, Kumar M, Menon V, Divi SM *et al*. Trace element studies on *Tinospora cordifolia* (Menispermaceae), *Ocimum sanctum* (Lamiaceae), *Moringa oleifera* (Moringaceae), and *Phyllanthus niruri* (Euphorbiaceae) using PIXE. Biol. Trace Elem. Res. 2010 Mar; 133(3): 357-63.

[13] Dhakar RC, Maurya SD, Pooniya BK, Bairwa N, Gupta M. Moringa: The herbal gold to combat malnutrition. Chron. Young Sci. 2011 Jul 1; 2(3).

[14] Olson ME, Sankaran RP, Fahey JW, Grusak MA, Odee D, Nouman W. Leaf protein and mineral concentrations across the "Miracle Tree" genus Moringa. PloS one. 2016 Jul 26; 11(7): e0159782.

[15] Kumssa DB, Joy EJ, Young SD, Odee DW, Ander EL, Broadley MR. Variation in the mineral element concentration of *Moringa oleifera* Lam. and M. stenopetala (Bak. f.) Cuf.: Role in human nutrition. PloS one. 2017 Apr 7; 12(4): e0175503.

[16] Sujatha BK, Patel P. *Moringa oleifera* –Nature's Gold. Imperial J Interdisciplinary Res. 2017; 3(5): 1175-9.

[17] Sahay S, Yadav U, Srinivasamurthy S. Potential of *Moringa oleifera* as a functional food ingredient: A review. Magnesium (g/kg). 2017; 8(9.06): 4-90.

[18] Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera* : A review on nutritive importance and its medicinal application. Food Sci Hum Well. 2016 Jun 1; 5(2): 49-56.

[19] Jwa SK. Efficacy of *Moringa oleifera* leaf extracts against cariogenic biofilm. Prev Nutr Food Sci. 2019 Sep; 24(3): 308.

[20] Arif M, Singh M, Onte S, Dey D, Kumar R. Comparative evaluation of fodder qualities in different parts of locally available moringa (*Moringa oleifera*) strains. INDIAN J ANIM SCI 2020 Jan 1; 90(1): 80-4.

[21] Singh AK, Rana HK, Tshabalala T, Kumar R *et al*. Phytochemical, nutraceutical and pharmacological attributes of a functional crop *Moringa oleifera* Lam: An overview. S AFR J BOT. 2020 Mar 1; 129: 209-20.

[22] Gupta J, Gupta A, Gupta AK. Determination of trace metals in the stem bark of *Moringa oleifera* Lam. Int. J. Chem. Stud.2014;2(4):39-42.

[23] Trigo C, Castelló ML, Ortolá MD, García-Mares FJ, Desamparados Soriano M. *Moringa oleifera* : An Unknown Crop in Developed Countries with Great Potential for Industry and Adapted to Climate Change. Foods. 2021 Jan;10(1):31.

[24] Goroya KG, Kaba TM. Determination of concentration of some essential and heavy metals in roots of Moringa stenopetala using flame atomic absorption spectroscopy. J. Med. Plant Res. 2019 Feb 25;13(4):89-95.

[25] Shenkin A. Micronutrients in health and disease. Postgrad. Med. J. 2006 Sep 1; 82(971): 559-67.

[26] Akhtar S, Das JK, Ismail T, Wahid M, Saeed W, Bhutta ZA. Nutritional perspectives for the prevention and mitigation of COVID-19. Nutr. Rev.. 2021 Mar; 79(3): 289-300.

[27] Godswill AG, Somtochukwu IV, Ikechukwu AO, Kate EC. Health benefits of micronutrients (vitamins and minerals) and their Associated Deficiency Diseases: a systematic review. INT J FOOD

SCI NUTR. 2020 Jan 7; 3(1): 1-32.

[28] Wintergerst ES, Maggini S, Hornig DH. Contribution of selected vitamins and trace elements to immune function. Ann. Nutr. Metab. 2007; 51(4): 301-23.

[29] Sanna A, Firinu D, Zavattari P, Valera P. Zinc status and autoimmunity: a systematic review and meta-analysis. Nutrients. 2018 Jan; 10(1): 68.

[30] Altarelli M, Ben-Hamouda N, Schneider A, Berger MM. Copper deficiency: causes, manifestations, and treatment. Nutr Clin Pract Nutr Clin Pract. 2019 Aug; 34(4): 504-13.

[31] Orbak R, Kara C, Özbek E, Tezel A, Demir T. Effects of zinc deficiency on oral and periodontal diseases in rats. J. Periodontal Res. 2007 Apr; 42(2): 138-43.

[32] Uwitonze AM, Ojeh N, Murererehe J, Atfi A, Razzaque MS. Zinc adequacy is essential for the maintenance of optimal oral health. Nutrients. 2020 Apr; 12(4): 949.

[33] Wu YC, Wang YP, Chang JY, Cheng SJ, Chen HM, Sun A. Oral manifestations and blood profile in patients with iron deficiency anemia. J. Formos. Med. Assoc. 2014 Feb 1; 113(2): 83-7.

[34] Fordyce FM. Fluorine: human health risks. Fordyce, Fiona M.. 2011 Fluorine : human health risks. In: Nriagu, J.O., (ed.) *Encyclopedia of environmental health Vol. 2.* Elsevier, 776-785.

[35] Tolkachjov SN, Bruce AJ. Oral manifestations of nutritional disorders. Clin. Dermatol. 2017 Sep 1; 35(5): 441-52.

[36] Aggarwal N, Bhateja S, Arora G, Yasmin T. Candidiasis-The most common fungal infection of oral cavity. BJSTR. 2018: 1-5.

[37] Xi R, Wang R, Wang Y, Xiang Z *et al*. Comparative analysis of the oral microbiota between iron-deficiency anaemia (IDA) patients and healthy individuals by high-throughput sequencing. BMC oral health. 2019 Dec; 19(1): 1-3.

[38] Gholizadeh N, Sheykhbahaei N. Micronutrients Profile in Oral Lichen Planus: a Review Literature. Biol. Trace Elem. Res. 2021 Mar; 199(3): 912-24.

[39] Kaur K, Sculley D, Wallace J, Turner A *et al*. Micronutrients and bioactive compounds in oral inflammatory diseases. J. Nutr.

Intermed. Metab. 2019 Dec 1; 18: 100105.

[40] Asiedu-Gyekye IJ, Frimpong-Manso SA, Awortwe C, Antwi DA, Nyarko AK. Micro-and macroelemental composition and safety evaluation of the nutraceutical *Moringa oleifera* leaves. J. Toxicol. 2014 Jul 22; 2014.

[41] Freiberger CE, Vanderjagt DJ, Pastuszyn A, Glew RS, Mounkaila G *et al*. Nutrient content of the edible leaves of seven wild plants from Niger. Plant Foods Hum Nutr 1998 Mar;5 3(1): 57-69.

[42] Amabye TG, Gebrehiwot K. Chemical compositions and nutritional value of *Moringa oleifera* available in the market of Mekelle. JFNS. 2015 Sep 8;3(5):187-90.

[43] Ngigi AN, Muraguri BM. ICP-OES determination of essential and non-essential elements in *Moringa oleifera* , Salvia hispanica and Linum usitatissimum. Sci African. 2019 Nov 1; 6: e00165.

[44] Gyamfi ET, Kwarteng IK, Ansah MO, Anim AK *et al*. Effects of processing on *Moringa oleifera* . Proceedings of the International Academy of Ecology and Environmental Sciences. 2011 Dec 1; 1(3-4): 179.

[45] Yimer AM, Khan MA. Atomic absorption spectrometric determination of some heavy metals from the leaves of *Moringa stenopetala* grown in Gamo Gofa Zone, Ethiopia. Curr. j. appl. sci. technol. 2016: 1-6.

[46] Aldakheel RK, Gondal MA, Nasr MM, Almessiere MA, Idris N. Spectral analysis of Miracle Moringa tree leaves using X-ray photoelectron, laser induced breakdown and inductively coupled plasma-optical emission spectroscopic techniques. Talanta. 2020 Sep 1; 217: 121062.

[47] Adefa T, Tefera M. Heavy metal accumulation and health risk assessment in *Moringa oleifera* from Awi zone, Ethiopia. Chemistry Africa. 2020 Dec; 3(4): 1073-9.

[48] Aruscavage PJ, Crock JG. Atomic absorption methods. US Geological Survey Bulletin. 1770.

[49] Tyler G, Jobin Yvon S. ICP-OES, ICP-MS and AAS Techniques Compared. ICP Optical Emission Spectroscopy Technical Note. 1995; 5.

[50] Lichte FE, Golightly DW, Lamothe PJ. Inductively coupled

plasma-atomic emission spectrometry. In Methods for Geochemical Analysis 1987 (Vol. 1770, pp. B1-B10). Washington, DC: USDI US Geological Survey.

[51] Beaty RD, Kerber JD. Concepts, instrumentation and techniques in atomic absorption spectrophotometry. USA: Perkin-Elmer; 1978.