

ARTICLE

Comparative Study of the Phytochemical Profile, Proximate Content and Antioxidant Properties of Leaves, Seeds and Pods of *Moringa Oleifera*

Anita K. Asekunowo¹, Abosede M. Ebabhi² and Akintayo L. Ogundajo³

¹Department of Chemistry, Faculty of Science, University of Lagos, Nigeria.

²Biology Unit, Distance Learning Institute, University of Lagos, Nigeria.

³Organic and Natural Products Research Unit, Department of Chemistry, Lagos State University, PMB 001 LASU Post office, Ojo, Lagos, Nigeria

Received 26thth August 2022, Accepted 24th November, 2022

DOI: 10.2478/ast-2022-0010

*Corresponding author

Anita K. Asekunowo E-mail: aasekunowo@unilag.edu.ng, Tel: +2349091626826

Abstract

Moringa oleifera (Moringa) is a highly valued plant and various parts of this plant are employed for the therapeutic purposes in the indigenous system of medicine. This study was undertaken to compare the phytochemical, mineral, proximate and antioxidant constituents of the aqueous, ethanol and coconut oil extracts of *M. oleifera* leaf, seed and pod. Pulverized samples of the leaves, seeds and pods were extracted separately with distilled water, ethanol and coconut oil. The proximate analysis was carried out using standard AOAC protocols while the mineral contents were analyzed through atomic absorption spectrometry. The phytochemicals and antioxidants constituents of the extracts were analysed using standard protocols. The phytochemical screening of *M. oleifera* revealed the presence of alkaloids in each extract of the leaf, seed and pod in varying concentrations with the absence of phlobatannins in all sampled parts. Proximate composition of *M. oleifera* revealed higher percentage of crude protein (26.05 ± 0.01 %) and total fatty acid (5.42 ± 0.01 %) in the seed, while the leaf had higher percentage of fibre (8.12 ± 0.02 %) and ash (7.82 ± 0.02 %). The pod showed higher percentage of carbohydrate (72.05 ± 0.2 %) and the seed revealed lowest moisture content (3.12 ± 0.01 %) compared to other sampled parts. Sodium was revealed as the highest composition of mineral in each part of the plant. The result for antioxidant activity revealed that the leaf, seed and pod extracts have stronger scavenging effect on nitric oxide radical compared to the standards (ascorbic acid $8467.15 \mu\text{g/ml}$) and the least scavenging effect on FRAP. The results obtained from this study validate the pharmacological and nutritional potentials of *M. oleifera* and its use in treating different ailments as used in traditional medicine.

Key words: *Moringa oleifera*, ethanol, proximate, mineral, phytochemical, antioxidant.



1.0 Introduction

Medicinal plants are known to be rich sources of bio-active phytochemicals or bio-nutrients (Nandi et al., 2006). They are described as enriched resources that can be used in drug development and as vital deposit of nutrients and therefore, recommended for their therapeutic values (Khan, 2018).

Phytochemicals are defined as substances produced by plants, which exhibit biological activity; they include alkaloids, terpenes, steroids, stilbenes, saponins, xanthenes, carotenoids, flavonoids, glycosides, phenolic acids. The study of these chemicals includes the extraction, the biosynthesis of the chemicals, and the functions of the chemicals in plants, animals and humans (Mendoza and Silva, 2018).

Moringa oleifera Lam. (drum stick tree/ miracle tree/ ben oil tree/ horseradish tree) is a useful plant which has benefited humanities for centuries because of its medicinal properties and health benefits. Its antifungal, antiviral, antidepressant and anti-inflammatory properties have been reported in literatures such as Abdull Razis et al. (2014), Biswas et al. (2020) and Ahmadua et al. (2021). According to Palafox et al. (2012), oil extracted from Moringa seeds (Ben oil) contains 70% oleic acid and showed stronger antifungal activity against a zoophilic dermatophyte which causes marked inflammatory reactions in humans. The leaves of *M. oleifera* could serve as a great benefit to people who cannot obtain proteins from meat. Many companies across the world manufacture various products from Moringa leaf such as tea, tablets, capsules, leaf powder, soaps and facewash (Mishra et al., 2012). Dried ground powder made from young root of Moringa plant gives a flavour similar to that of horseradish and can be used as a hot seasoning base (Builders et al., 2014). This study was designed to compare the phytochemical components, mineral content and antioxidant properties of the leaves, seed and pod of *Moringa oleifera* using different media of extraction.

2.0 Experimental

Sample Collection

The leaves, pods and seeds of *Moringa oleifera* were obtained from trees in various locations in the eastern part of Nigeria. They were authenticated and deposited at the Lagos University Herbarium (University of Lagos, Nigeria) with voucher identification number LUH 8922 for the seeds and pods and LUH 8978 for the leaves.

Sample Treatment and Preparation

The fresh leaves and pods were separated manually, air dried for seven days and pulverized into fine particles. The seeds were de-shelled, air dried for two weeks and pulverized using an electric grinder. An amount of 25 g of each pulverized sample was macerated separately with ethanol for 72 h, coconut oil for 168 h and decocted with distilled water for ½ h. The ethanol extract was concentrated to dryness using rotator evaporator at 40°C, while the aqueous extract was concentrated to dryness using water bath. The dried powdered sample was used for the proximate analysis and mineral content determination. The extracts of each of the sampled parts of *M. oleifera* was used for the phytochemical screening and determination of the antioxidant activity.

Preliminary Phytochemical Screening

The preliminary phytochemical components of the leaves, seeds and pods extracts were analyzed using standard protocols (Ukoha et al., 2011; Dhani, 2012; Hossain et al., 2013; Auwal et al., 2014).

Mineral Analysis

Digestion method was according to the protocols of Hernández et al. (2010) and Paul et al. (2014). Elemental analysis was carried out using Atomic Absorption Spectrometry (AAS). Magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), and sodium (Na) were determined.

Proximate Analysis

The protocols described by the Association of Official Analytical Chemists (AOAC, 2012) was used for moisture, ash, crude fiber and crude protein content. The carbohydrate content was calculated by subtracting the sum of the percentage ash, crude lipid, moisture, crude protein and crude fibre from 100 % according to the methods described by Thiex (2009) and Maisarah et al. (2014)

Determination of Antioxidant Activities

Ferric Reducing Power Assay

The method of Rajurkar and Hande, (2011) was employed in determining the reducing power capacity of the extracts/fraction. The protocol involves a mixture containing 1 ml of sample (25-100 µg/ml), 2.5 ml of 0.1 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1%, w/v potassium ferrocyanate [K₃Fe(CN)₆] and incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 ml, 10%, w/v), was added to the mixture and centrifuged at 5000 rpm for 10 min. Equal volume of Fresh FeCl₃ (5ml 0.1%, w/v) was mixed, the upper layer (5 ml) of the centrifuged mixture and the absorbance was taken at 700 nm against a control (blank).

Free Radical Scavenging using DPPH Assay

The method of Kordali et al. (2005) was followed in accessing the free radical scavenging potential of the extracts/fraction. Precisely, 1 ml of 0.1 mM of DPPH in ethanol solution was added to 1 ml of extracts/fraction in water at different concentrations (25-100 µg/ml). The mixture was briskly agitated and allowed to stand at 25 °C for 30 min and the absorbance taken using a UV-Visible Spectrophotometer at 517 nm. The percent DPPH scavenging effect was calculated using the following equation:

$$DPPH\ Scavenging\ effect\ (\%) = \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

Where A₀ was the absorbance of the control and A₁ was the absorbance in the presence of the standard sample or extract. The IC₅₀ value represented the concentration of the compounds that caused 50 % inhibition of DPPH radical formation.

Nitric Oxide Assay

The antioxidant capacity of the extract using Nitric oxide assay was investigated using the methods of Lee et al. (2011) and Alam et al. (2013). In a beaker was added 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate-buffered saline (pH 7.4), and 0.5 ml of extracts/

fraction was added at varying concentrations. The mixture was incubated for 150 min at room temperature. In a test tube, 0.5 ml of the incubated mixture was added into 1.0 ml sulphanilamide solution (0.3 % in 20 % glacial acetic acid) and the mixture re-incubated for 5 mins at 25 °C. Lastly, 1.0 ml naphthylethylenediamine dihydrochloride (0.1 % w/v) was added and the resultant mixture incubated and the room temperature maintained for 30 min. The absorbance was taken at 546 nm for the sample and the standard. The percentage inhibition was calculated according to the following equation.

$$\% \text{ Inhibition} = \left(1 - \frac{A_i}{A_0}\right) \times 100$$

Where A_i = Absorbance of the extract; A_0 = Absorbance of the control/blank.

Lipid Peroxidation

The thiobarbituric acid assay protocol used by Ganhão, et al. (2011) was employed in determining the lipid peroxidation activity of the extracts/fraction. 10 µL of samples at different concentrations (25, 50, 75 and 100 µg/mL)/ standard solution (1,1,3,3-tetramethoxypropane, TEP) and 40 µL of 20 mM phosphate buffer (pH 7.0) were added to a test tube on ice bath. In each tube, 50 µL of 3% sodium dodecyl sulfate (SDS), 200 µL of 0.1 N HCl, 30 µL of 10% Phosphotungstic acid, and 100 µL of 0.7% of 2-thiobarbituric acid (TBA) were added. The tubes were firmly closed and boiled at 100 °C for 30 min in water bath. The reaction mixture was mixed with 400 µL of n-butanol and then centrifuged at 3000 rpm for 10 min. Supernatants were collected and pass through a UV/VIS spectrophotometer at a wavelength of 515 nm/555 nm.

$$\text{Lipid Peroxidation (\%)} = \frac{(A_{\text{control}} - A_{\text{test}}) \times 100}{A_{\text{control}}}$$

where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples of extracts or standards.

Statistical Analysis

All experiments were carried out in triplicates. Data were presented as mean and standard deviation. Statistical Package for Social Science (SPSS) version 22 was used for the data analysis.

3.0 Results and Discussion

Phytochemical Screening

The phytochemical assay revealed that *Moringa oleifera* seed, leaf and pod contain various components such as alkaloids, anthraquinone, flavonoids, phenols, reducing sugars, tannins, saponins, steroids, in varying concentrations with the absence of phlobatannins as shown in Table 1. This is similar to the results reported by Nepolean et al., (2009); Fowoyo and Oladoja, (2015), and Nkot et al., (2018) on *M. oleifera*. The coconut oil extract contains higher concentration of saponins, steroids and reducing sugars while aqueous extract contains high concentration of saponins. Ethanolic extract contains all tested phytochemicals except phlobatannins with high concentration of steroids and reducing sugars. Ethanol was observed to be the best solvent of extraction for the seed. The aqueous extract of the pod showed higher concentration of the phytochemicals presents than other extracts. Alkaloids were found to be the only phytochemical present

in all extracts of the leaf, seed and pod. Literatures have it that medicinal plants containing alkaloids, flavonoids and phenols as bioactive metabolites have good antibacterial properties and the presence of these metabolites in *Moringa oleifera* could make it a potent antibacterial agent (Akintelu et al., 2021). Flavonoids are responsible for the medicinal qualities accorded in the leaf. Foye et al. (2008) reported that they also induce mechanisms that may kill cancer cells and inhibit tumor invasion. Saponins were observed in the aqueous and ethanolic extract of the leaf, seed and pod samples as well as in the coconut oil extract of the seed and pod of *Moringa oleifera*. Due to its ability to form froth, soap can be produced locally for bathing from any plant containing Saponins (Oman, 2013). Saponins are also used as an adjuvant in the production of vaccines and also possess antioxidant, anti-inflammatory, anti-apoptosis and immune-stimulant as reported by Woods et al. (2017). Alkaloids are nitrogen-containing naturally occurring compounds, commonly found to have antimicrobial properties and are used as antimalarial, pesticides, tranquilisers and stimulants (Galeotti et al., 2008).

Proximate Analysis

The results obtained from the proximate analysis of *M. oleifera* revealed high content of carbohydrates (72.05±0.2%) in the pod and protein (26.05±0.01%) in the seed compared to other compositions as confirmed with the result obtained by Sodamade et al. (2013). Carbohydrates was also found to be in high concentration (68.23±0.3%) in the leaf. Carbohydrate is suitable for optimal functioning of the brain, heart, nervous, digestive and immune system while depletion of body tissue is the consequence of its deficiency (Offor et al., 2014). Proteins are known to be body building blocks; thus, protein molecules carry out all the major structural and functional aspects of the body. According to Vasudevan et al. (2019) an abnormality in protein structure could lead to molecular diseases with severe alterations in metabolic functions. This supports the report that *M. oleifera* plant could serve as food supplement to increase the nutritional values of foods that lack protein, carbohydrate and lipid as asserted by Fowoyo and Oladoja, (2015). This would be a great boom to people who do not get protein from meat and who need more energy for metabolism. The seed showed low moisture content (3.12±0.01%) than other parts of the plant, as a result, slows down the growth of microorganisms hence increasing the storage life capacity. The result on the proximate content is as shown in Table 2. The pod showed the lowest amount of total fatty acid (1.15±0.01%).

Mineral Content

The mineral content of *Moringa oleifera* plant samples revealed the presence of varying amounts of minerals with sodium having the highest percentage as displayed in Table 3. Cobalt was not detected in any of the sample. The mineral content of the seed indicated that sodium (5.550±0.01 mg/L), potassium (1.511±0.00 mg/L), magnesium (0.872±0.00 mg/L) and zinc (0.361±0.00 mg/L) are the most abundant as justified by Kawo et al., (2009) and Sodamade et al., (2013) with little difference in their values. Sodium is present in higher concentration in the leaf and pod with a large significant difference. Sodium is both an electrolyte and mineral; it helps regulate blood fluids, balance the body and prevents low blood pressure, it is necessary for the health of the heart, liver and kidneys. Potassium plays a role in regulating the movement of nutrients into

Table 1: Presence of Phytochemicals Assayed in Sampled Parts of *Moringa oleifera*

PHYTOCHEMICALS	Seed			Leaf			Pod		
	AE	EE	CE	AE	EE	CE	AE	EE	CE
Alkaloids	++	+	+	+	++	+	+	+	+
Flavonoids	—	+	+	++	++	+	++	++	—
Tannins	+	+	—	+	+	+	++	+	—
Phenols	—	+	—	+	+	+	++	+	—
Saponins	++	+	++	+	+	—	+	+	+
Steroids	++	++	++	++	—	++	+	+	+
Phlobatannins	—	—	—	—	—	—	—	—	—
Reducing sugar	—	++	++	—	+	++	++	++	++
Anthraquinone	+	+	+	+	+	—	—	—	—

Keys: high (++); moderate (+); absent (-); AE-aqueous extract; EE-ethanolic extract; CE-coconut oil extract.

Table 2: Proximate Analysis of *Moringa oleifera* sampled parts

Proximate	Results (%)		
	Pod	Leaf	Seed
Ash content	6.25±0.01	7.82±0.02	4.64±0.01
Total fatty acid	1.15±0.01	1.96±0.00	5.42±0.01
Moisture content	4.20±0.02	5.42±0.03	3.12±0.01
Crude fibre	6.53±0.01	8.12±0.02	4.31±0.02
Carbohydrates	72.05±0.2	68.23±0.3	56.45±0.7
Crude protein	8.43±0.02	8.44±0.01	26.05±0.01

Results are presented as Mean±SD

cells and waste products out of cells; it also helps nerves to function and muscles to contract (Stone et al., 2016). Magnesium helps in promoting skin cells from damage. Majekodunmi (2015) reported that zinc is useful for protein synthesis, normal body development and recovery from illness.

Antioxidant Activity

The antioxidant potential of *Moringa oleifera* extracts revealed increase in reductive potential with increasing concentration of the extracts while the aqueous extract was observed to have the least scavenging activity as shown in Table 4. Antioxidants are inhibitors that the DPPH radical scavenging activities of *M. oleifera* leaf

increasing their antioxidant enzymes and inhibiting lipid peroxidation as observed by Robert et al. (2009). The ethanol extract of the pod had stronger antioxidant capacity on nitric oxide and ferric reducing antioxidant assay (37.76 µg/ml, 49923 µg/ml) and the aqueous extract exhibited more antioxidant activity (32.66 µg/ml) on DPPH assay. Coconut oil extract had more antioxidant capacity on lipid peroxidation assay (49.78 µg/ml). The result from this study revealed that *Moringa oleifera* pod exhibited an appreciable amount of antioxidant activity which tallies with the work carried out by Iram et al. (2016) and can therefore be safely ingested. The result obtained also supports the use of *M. oleifera* seed for natural antioxidant, anti-inflammatory, anti-diabetic, anti-hypertensive and anti-ulcer activities.

Table 3: Mineral Analysis of *Moringa oleifera* Sampled Parts

Minerals	Concentration (mg/L)		
	Seed	Leaf	Pod
Lead	0.004±0.00	0.003±0.00	0.003±0.00
Iron	0.082±0.00	0.111±0.00	0.111±0.00
Calcium	0.083±0.00	0.293±0.00	0.261±0.00
Sodium	5.55±0.01	14.8±0.1	14.4±0.1
Zinc	0.361±0.00	0.193±0.00	0.193±0.00
Magnesium	0.872±0.00	1.219±0.00	0.823±0.00
Potassium	1.511±0.00	1.055±0.00	1.041±0.00
Copper	0.022±0.00	0.0125±0.00	0.021±0.0
Cobalt	ND	ND	ND
Manganese	0.024±0.00	0.057±0.00	0.019±0.00

Results are presented as Men±SD

ND-Not Detected

Table 4: IC₅₀ (µg/ml) of Different Antioxidant Activities of Samples Parts of *Moringa oleifera*

ASSAY	Standard		Leaf			Seed			Pod	
	AA (µg/ml)	EE (µg/ml)	AE (µg/ml)	CE (µg/ml)	EE (µg/ml)	AE (µg/ml)	CE (µg/ml)	EE (µg/ml)	AE (µg/ml)	CE (µg/ml)
NITRIC OXIDE	8467.15	95.33	53.19	58.18	58.94	42.09	59.73	37.76	61.42	50.57
DPPH	28.42	30.82	42.29	50.10	23.76	77.73	57.52	55.25	32.66	67.63
LIPID PEROXIDATION	33.46	48.49	59.13	70.76	58.94	42.09	59.73	69.29	71.00	49.78
FRAP	8467.17	15620.53	41589.33	14693.24	29381.47	29386.59	29390.41	49923.00	166576.00	49949.00

Keys: FRAP: Ferric reducing antioxidant power; DPPH: 2, 2-diphenyl-1-picryl hydrazine; AA: Ascorbic acid; EE: Ethanol extract; AE: Distilled water extract; CE: Coconut oil extract.

Conclusion

Moringa oleifera is one of the medicinal plants reported in folklore to have been explored for its beneficial purposes. The presences of various phytochemicals, nutritional and high antioxidant activities as asserted in this study lay credence to the fact the leaf, seed and pod of *M. oleifera* may be a good source of nutrient and therapeutic material to treat a number of diseases.

Conflict of interest

All authors have declared no conflict of interest

Individual author's contributions

AKA, AME and ALO designed the research.

AKA and AME executed the research

AKA, AME and ALO analysis of data and interpretation of the results

AKA, AME and ALO discussion and manuscript writing

References

- Abdull Razis, A.F., Din Ibrahim, M., and Kntayya, S.B. 2014, Health benefits of Moringa. *Asian Pacific Journal of Cancer Prevention*, 15 (20), 8571-8576. DOI: [10.7314/apjcp.2014.15.20.8571](https://doi.org/10.7314/apjcp.2014.15.20.8571)
- Ahmadua, T., Ahmada, k., Ismaila, S. I., Rasheda, O., Asiba, N., and Omara, D. 2021, Antifungal efficacy of *Moringa oleifera* leaf and seed extracts against *Botrytis cinerea* causing gray mold disease of tomato (*Solanum lycopersicum* L.). *Brazilian Journal of Biology*, 81(4), 1007-1022. DOI: [10.1590/1519-6984.233173](https://doi.org/10.1590/1519-6984.233173)
- Akintelu, S. A., Folorunso, A. S., and Oyebamiji, A. K. 2021, Phytochemical and antibacterial investigation of *Moringa oleifera* seed: experimental and computational approaches. *Eclética Química Journal*, 46(2), 17-25. DOI: [10.26850/1678-4618eqj.v46.2.2021.p17-25](https://doi.org/10.26850/1678-4618eqj.v46.2.2021.p17-25)
- Alam, M. N., Bristi, N. J., and Rafiquzzaman, M. 2013, Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21(2), 143-152. DOI: [10.1016/j.jsps.2012.05.002](https://doi.org/10.1016/j.jsps.2012.05.002)
- AOAC 2012, Methods of chemical analysis (19th Edition), Association of official analytical chemists, Washington D.C. USA.
- Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., and Ibrahim, A. 2014, Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum*, 5(2), 95-100. PMID: [25568701](https://pubmed.ncbi.nlm.nih.gov/25568701/)
- Biswas, D., Nandy, S., Mukherjee, A., Pandey, D.K., and Dey, A. 2020, *Moringa oleifera* Lam. and derived phytochemicals as promising antiviral agents: A review. *South African Journal of Botany*, 129, 272-282. <https://doi.org/10.1016/j.sajb.2019.07.049>
- Builders, P. F., Iwu, I. W., Mbah, C. C., Iwu, I. W., Builders, M. I., and Audu, M. M. 2014, *Moringa oleifera* ethosomes a potential hair growth activator: Effect on rats. *Journal of Pharmaceutical and Biomedical Science*, 4:611-618. Available online at www.jpbbms.info
- Dhani, R. 2012, Extraction and chemical investigation of leaves of *Pterospermum acerifolium*. *International Journal of Pharmaceutical Research and Bio-Science*, 1(6), 299-314. <http://www.ijprbs.com/issuedocs/2012/...>
- Fernandez-Hernandez, A., Mateos, R., Mesa, J. A. G., Beltran, G., and Escobar, R. F. 2010, Determination of mineral elements in fresh olive fruits by flame atomic spectrometry. *Spanish Journal of Agricultural Research*, (4), 1183-1190. Available online at www.inia.es/sjar
- Fowoyo, P., and Oladoja, E. 2015, Phytochemical screening, nutritional composition and antimicrobial activity of *Moringa oleifera* seed and leaf extract against selected gastrointestinal pathogens. *Journal of Pharmacy and Biological Sciences*, 10(6), 116-124. DOI: [10.9790/3008-1062116124](https://doi.org/10.9790/3008-1062116124)
- Foye, W.O., Lemke, T.L., and Williams, D.A. 2008, *Foye's Principles of Medicinal Chemistry*. 6th Ed Lippincott Williams and Wilkins. Philadelphia, 1377pp. ISBN: [9780781768795](https://doi.org/9780781768795), [0781768799](https://doi.org/0781768799)
- Galeotti, F., Barile, E., Curir, P., Dolci, M., and Lanzotti V. 2008, Flavonoids from carnation of *Dianthus caryophyllus* and antifungal activity, *Phytochemistry Letters*, 1(1), 44-48. <https://doi.org/10.1016/j.phytol.2007.10.001>
- Ganhão, R., Estévez, M., & Morcuende, D. (2011). Suitability of the TBA method for assessing lipid oxidation in a meat system with added phenolic-rich materials. *Food Chemistry*, 126(2), 772-778. <https://doi.org/10.1016/j.foodchem.2010.11.064>
- Hossain, M. A., AL-Raqmi, K. A. S., Al-Mijzy, Z. H., Weli, A. M., and Al-Riyami, Q. 2013, Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pacific Journal of Tropical Biomedicine*, 3(9), 705-710. DOI: [10.1016/S2221-1691\(13\)60142-2](https://doi.org/10.1016/S2221-1691(13)60142-2)
- Gull, I., Javed, A., Aslam, M.S., Mushtaq, R., and Muhammad, A.A. 2016, Use of *Moringa oleifera* flower pod extract as natural preservative and development of SCAR Marker for its DNA based identification. *BioMed Research International*. Article ID 7584318, 12 pages <http://dx.doi.org/10.1155/2016/7584318>
- Islam, Z., Islam, S.M.R., Hossen, F., Mahtab-Ul-Islam, K., Hasan, M.R., Karim, R. 2021, *Moringa oleifera* is a prominent source of nutrients with potential health benefits. *International Journal of Food Science*, 10, 2021:6627265. doi: [10.1155/2021/6627265](https://doi.org/10.1155/2021/6627265)
- Jahan, I. A., Hossain, M. H., Ahmed, K. S., Sultana, Z., Biswas, P. K., and Nada, K. 2018, Antioxidant activity of *Moringa oleifera* seed extracts. *Oriental Pharmacy and Experimental Medicine*, 18(4), 229-307. <https://doi.org/10.1007/s13596-018-0333-y>
- Kawo, A., Abdullahi, B., Halilu, A., Gaiya, Z., Dabai, M., and Dakare, M. 2009, Preliminary phytochemical screening, proximate and elemental composition of *Moringa oleifera* lam seed powder. *Bayero Journal of Pure and Applied Sciences*, 2(1), 96-100. Available online at <file:///C:/Users/pc/Downloads/58476-Article%20Text-104058-1-10-20100825.pdf>
- Khan, M. A. 2016, Introduction and importance of medicinal plants and herbs. *National Health Portal (NHP)*, India. https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs_mtl
- Khowala, S., Verma, D. and Bank, S. P. 2008, *Biomolecules: (Introduction, Structure and Function)*. Carbohydrate. Drug Development and Biotechnology, Kolkata. 93pp. <http://nsdl.niscair.res.in/xmlui/bitstream/handle/123456789/802/Carbohydrates.pdf?sequence=1&disAllowed=y>
- Kordali, S., Kahir, A., Mavi, A., Kilic, H., and Yildirim, A. 2005, Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia* species. *Journal of Agricultural and Food Chemistry*, 53(5), 1408-1416. DOI: [10.1021/jf048429n](https://doi.org/10.1021/jf048429n)
- Lee, N. J., Lee, J. W., Sung, J. H., Lee, Y. J., and Kang, J. K. 2011, In vitro antioxidant properties of a ginseng intestinal metabolite IH-901. *Laboratory Animal Research*, 27(3), 227-234. doi: [10.5625/lar.2011.27.3.227](https://doi.org/10.5625/lar.2011.27.3.227)

- Maisarah, A. M., Asmah, R., and Fauziah, O. 2014, Proximate analysis, antioxidant and antiproliferative activities of different parts of *Carica papaya*. *Journal of Tissue Science and Engineering*, 5(1), 1. DOI: [10.4172/2155-9600.1000267](https://doi.org/10.4172/2155-9600.1000267)
- Majekodunmi, S. O., 2015, Review of extraction of medicinal plants for pharmaceutical research. *Merit Research Journal of Medicine and Medical Sciences*, 11, 521-527. Available online at <http://www.meritresearchjournals.org/mms/index.htm>
- Mendoza, N., and Silva, E. M. E. 2018, Introduction to phytochemicals: secondary metabolites from plants with active principles for pharmacological importance. *Phytochemicals: Source of Antioxidants and Role in Disease Prevention*, 25. DOI: [10.5772/intechopen.78226](https://doi.org/10.5772/intechopen.78226)
- Nandi, D., Patra, R. C., and Swarup, D. 2006, Oxidative stress indices and plasma biochemical parameters during oral exposure to arsenic in rats. *Food and Chemical Toxicology*, 44(9), 1579-1584. DOI: [10.1016/j.fct.2006.04.013](https://doi.org/10.1016/j.fct.2006.04.013)
- Nepolean, P., Anitha, J., and Emilin, R. R. 2009, Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica*, 3(1), 33-37. Available online at www.currentbiotica.com
- Nkot, J. L., Ngono Bikobo, D. S., Abouem A Zintchem, A., Nyemeck, N. M., Moni Ndedi, E. D. F., Betote Diboué, P. H., and Koert, U. 2018, Antitubercular evaluation of root extract and isolated phytochemicals from *Lophira lanceolata* against two resistant strains of *Mycobacterium tuberculosis*. *Pharmaceutical Biology*, 56(1), 318-324. DOI: [10.1080/13880209.2018.1476559](https://doi.org/10.1080/13880209.2018.1476559)
- Oman, M., Škerget, M., and Knez, Z. 2013, Application of supercritical fluid extraction for separation of nutraceuticals and other phytochemicals from plant material. *Macedonian Journal of Chemistry and Chemical Engineering*, 32(2), 183-226. <https://doi.org/10.20450/mjcc.2013.443>
- Palafox, J. O., Navarrete, A., Sacramento-Rivero, J. C., Rubio-Atoche, C., Escoffie, P. A., and Rocha-Urbe, J. A. 2012, Extraction and characterization of oil from *Moringa oleifera* using supercritical CO₂ and traditional solvents. *American Journal of Analytical Chemistry*, 3(12A), 10.4236/ajac.2012.312A125
- Paul, B. N., Chanda, S., Das, S., Singh, P., Pandey, B. K., and Giri, S. S. 2014, Mineral assay in atomic absorption spectroscopy. *The Beats of Natural Sciences*, 1(4), 1-17. <https://www.researchgate.net/publication/279195165>
- Rajurkar, N. S., and Hande, S. M. 2011, Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences*, 73(2), 146. DOI: [10.4103/0250-474x.91574](https://doi.org/10.4103/0250-474x.91574)
- Roberts, R.A. Laskin D.L. Smith C.V. Robertson F.M. Allen E.M.G., and Doorn J.A. 2009, Nitrate and oxidative stress in toxicology and disease. *Toxicological Sciences*, 112:4-16. DOI: [10.1093/toxsci/kfp179](https://doi.org/10.1093/toxsci/kfp179)
- Sharma, S. Mahotra P., and Bhattacharyya, A.K. 2008, Effect of electroplating industrial waste on "available phosphorus" of soil in relation to other physico-chemical properties. *African Journal of Environmental Science and Technology*, 2(9): 257- 264. Available online at <http://www.academicjournals.org/AJest>
- Sodamade, A., Bolaji, O. S., and Adeboye, O. O. 2013, Proximate analysis, mineral contents and functional properties of *Moringa oleifera* leaf protein concentrate. *IOSR Journal of Applied Chemistry*, 4(6), 47-51. <https://www.researchgate.net/publication/284483349>
- Tejada-Jiménez, M., Galván, A., Fernández, E., and Llamas, Á. 2009, Homeostasis of the micronutrients Ni, Mo and Cl with specific biochemical functions. *Current Opinion in Plant Biology*, 12(3), 358-363. DOI: [10.1016/j.pbi.2009.04.012](https://doi.org/10.1016/j.pbi.2009.04.012)
- Thiex, N. 2009, Evaluation of analytical methods for the determination of moisture, crude protein, crude fat, and crude fiber in distillers dried grains with solubles. *Journal of AOAC International*, 92(1), 61-73. <https://doi.org/10.1093/jaoac/92.1.61>
- Ukoha, P. O., Cemaluk, E. A., Nnamdi, O. L., and Madus, E. P. 2011, Tannins and other phytochemical of the *Samanea saman* pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry*, 5(8), 237-244. Available online at <http://www.academicjournals.org/AJPAC>
- Vasudevan, D. M., Sreekumari, S., and Vaidyanathan, K. 2019, Textbook of Biochemistry for Medicinal Students. Jaypee brothers Medical publishers. 672pp. www.jaypeebrothers.com
- Woods, N., Niwasabutra, K., Acevedo, R., Igoli, J., Altwaijry, N.A., Tusiimire, J., Gray, A.I., Watson, D.G., and Ferro, V.A. 2017, Natural vaccine adjuvants and immunopotentiators derived from plants, fungi, marine organisms, and insects. *Immunopotentiators in Modern Vaccines*. 211-229. <https://doi.org/10.1016/B978-0-12-804019-5.00011-6>
- Yadav, A., Kumari, R., Yadav, A., Mishra, J. P., Scrivata S., and Prabha S. 2016, Antioxidants and its functions in human body-A Review. *Research in Environment and Life Sciences*, 9(11), 1328-1331. <https://www.researchgate.net/publication/311674771>