Investigation on the Phenolic Content in *Moringa oleifera* and Its Antimicrobial Activity

Sourav Kumar Das, Bharanii Dharan J., Pavitra P.V., Sriya Das, Smruti Prangya Behera, P. Veilumuthu, J. Godwin Christopher

**ABSTRACT**

*Background:* One of the most commonly cultivated plant is *Moringa oleifera* because it has high medicinal in South India and nutritional values. The phytochemicals in it are widely used for the therapeutic purposes. It has a high economic value because of its usefulness traditionally and has a vast number of bioactive compounds. Phenol is one of the most common antimicrobial compounds found in the seeds of *M. oleifera*.

*Methods:* In this study we aim to analyse Total Phenolic Compound (TPC) and to observe its antimicrobial activity. Seed extracts were prepared using ethanol in Soxhlet apparatus for 36 hrs. The quantification of phenolic compound was done by spectrophotometric method using gallic acid as standard. The crude extract was characterized by HPLC and FTIR, showed that it contained phenol compounds confirmed by specific peak in some areas. FTIR and showed that it contained phenol compounds confirmed by specific peak in some areas. FTIR revealed the following bonds: 3280.92 cm\(^{-1}\) (O–H groups of phenols), 2922.16 cm\(^{-1}\) (C–H groups of phenols), 1544.98 cm\(^{-1}\) (C=O carbonyl group) and 1230.58 (C–O group of phenol) and 1743.65 cm\(^{-1}\) (C=O group of aromatic compounds). Whereas HPLC analysis of and library search confirmed peaks matches with Gallic acid standard. Moringa seeds were characterized by FTIR before extracted. Therefore the highest Total Phenolic Compound found to be 11.85 mg GAE/g (Gallic Acid Equivalent) was reached at 1: 5 ethanol solvent ratio and 3 days extraction time.

*Conclusion:* This *M. oleifera* extract showed antimicrobial activity against *Pseudomonas aeruginosa* (MTCC 1688), *Bacillus subtilis* (MTCC 8561), *Staphylococcus aureus* (MTCC 3160) and *Proteus mirabilis* (MTCC 3310). The anti-microbial activity of the *M. oleifera* seed is due to the presence of various phytochemicals like phenols. Also it exhibits, a broad-spectrum antimicrobial activity that proves further research is needed to elucidate other bioactive compounds over other pathogens.

*Key words:* Anti-microbial activity, FTIR, Gallic acid, HPLC, *Moringa oleifera*, Phytochemicals.

**INTRODUCTION**

*Moringa oleifera* commonly known as drumstick in India belongs to family Moringaceae is widely well-known for its excellent nutritional and medicinal properties (Kumar *et al.*, 2014). *M. oleifera* is known to possess anti-microbial, hypoglycemic, antioxidant immunomodulatory, antitumor activities and this is due to the presence of various secondary metabolites. It has also been used to treat gastro intestinal problems like gastritis, colitis and constipation. Some of the secondary plant metabolites present in *M. oleifera* are carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics that attribute to the biological activity of *M. oleifera* (Patel, 2017; Selmi *et al.*, 2019). Moringa is used as a vegetable and is also used for water purification (Delelegn *et al.*, 2018). It is a fast-growing tree commonly cultivated in tropical and sub-tropical areas. It is cultivated for its leaves, pods and seeds which are used for variety of purposes. All of its parts contain nutritional value including the oil pressed from seed. The seeds contain all vitamins mostly Vitamin B and C and minerals. The seeds can be used as fertilizers, water purifying agents and also as biofuel.

*M. oleifera* is used to treat malnutrition and iron deficiency (Ravani *et al.*, 2017; Saini *et al.*, 2016). *M. oleifera* is rich in phenolic acids and flavonoids, it's extract exhibits significant antioxidant activity both *in vitro* and *in vivo*, especially seeds has the highest abundance of identified phenolic compound and antioxidant activity (Adebayo *et al.*, 2018). At present, the *M. oleifera* extract has been widely used in fields of medicine, functional food and cosmetics (Yadav and Ghimire, 2019; Zhao *et al.*, 2019). Moringa, native to parts of Africa and Asia, is the sole genus in the flowering plant family Moringaceae. The name is derived from *murungai*. The species of *M. oleifera* is a little tree with wide, spreading branches and fragrant, cream-shaded or white blossoms that sprout on long, hanging panicles. Depending on the species and
Investigation on the Phenolic Content in *Moringa oleifera* and Its Antimicrobial Activity

atmosphere. *Moringa* trees might be evergreen or semi-deciduous. Also, there are 13 known types of *Moringa*, few are developed outside their local natural surroundings. *Moringa arborea*, *M. borziana*, *M. longituba*, *M. pygmaea*, *M. rivea*, *M. ruspoliana*, *M. drouhardii* and *M. hildebrandtii* are the species.

*M. oleifera* seed flour was found to be a store house of phenolic compound produced from plant source act as free radical terminator (Shahidi et al., 1992). Most of the workers have reported the use of leaf and or bark extracts elsewhere, while the scientific and effective use of *M. oleifera* dried seed powder for total phenolic content (TPC) and antibacterial potential against gram positive and negative around the location of Vellore at Taminadu is not reported so far. In view of the above, the present study, we have described the FTIR, HPLC and antibacterial characteristics of crude seed extract *Moringa oleifera*, the seed extract was further explored the analysis of Total phenolic contents.

Therefore, the present study was designed to investigate TPC and characterization crude seed extract specifically FTIR and HPLC and the role of seed extracts against bacterial growth namely *Pseudomonas aeruginosa* (MTCC 1688), *Bacillus subtilis* (MTCC 8561), *Staphylococcus aureus* (MTCC 3160) and *Proteus mirabilis* (MTCC 3310).

**MATERIALS AND METHODS**

**Preparation of *M. oleifera* seed extract**

The experiment was conducted during 2020 at the School of BioSciences and Technology, VIT University. Mature seeds of *M. oleifera* were chosen from dry cracked fruits. The plucked fruits were cracked to obtain the seeds which were air-dried for 2 days. The shells surrounding the seed kernels were removed using a knife and the kernels were powdered using a laboratory mortar and pestle and sieved using a strainer with a pore size of 2.5 mm to obtain a fine powder. The powder was stored in a sterile bottle at room temperature for 4 weeks. The powdered sample was successively extracted with ethanol in increasing polarity. In this procedure, 50 g of *M. oleifera* powdered seeds were soaked in 250 mL of ethanol solvent (1:5). Solvent mixture was left shaking on a horizontal shaker for 3 days. Then, the extracts were filtered separately through sterile Whatman no.1 filter paper. The filtrates were then centrifuged at 5000 rpm for 15 min. The supernatant of each extract was evaporated by using Rota vapor (Laboratory 4000-efficient, Heidolph, Germany). The crude extracts were stored at 4°C.

**Estimation of Total Phenolic Content (TPC)**

Colorimetric method using Folin-Ciocalteau reagent was carried out for the estimation of total phenolic content as described by Siddiqui et al., 2017. A blank test tube was prepared using distilled water and gallic acid solution was used as standard. The working standard was prepared to contain a concentration of 0.1 mg/mL and a standard curve was obtained. A calibration curve of absorbance values against varying concentrations of gallic acid standard was plotted. A regression equation of the curve, absorbance value = 0.0248 (gallic acid concentration) + 0.0003 with *R* value, 0.9865 was obtained. Total phenolic contents of the samples in mg gallic acid equivalence (GAE)/g of sample were calculated using the equation.

**FTIR analysis**

FTIR relies on the fact that molecules absorb light in the infra-red region of the electromagnetic spectrum. The absorption corresponds specifically to the bands present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000 – 400 cm⁻¹. FTIR was done using Shimadzu IRAffinity-1.

**HPLC Analysis**

High Performance Liquid Chromatography, help us to get an accurate concentration of phenolic component in compare to the standard which is Gallic acid solution. The mobile phase was prepared using acetonitrile and 20 mM phosphoric acid in the ratio 9:1. It was degassed and filtered before using it for analytical purpose. The flow rate was set to 50 µl/ minute and the injection volume was 20 µl. The run time was 25 minutes. Gallic acid was used as standard with the concentration of 0.1 mg/mL. The wavelength was set to 280 nm and the chromatogram peaks were compared with the standard peaks and the results were interpreted (Gaafar et al., 2016).

**Antibacterial Assay**

The agar well diffusion method was used in assessing the antibacterial activity of the *M. oleifera* seed Extract. Nutrient agar medium was poured into sterile petri dishes and allowed to solidify. The test organisms were poured on into the solidified agar and was swab all over the surface of the agar. Three different organisms were tested namely *Pseudomonas aeruginosa* (MTCC 1688), *Bacillus subtilis* (MTCC 8561), *Staphylococcus aureus* (MTCC 3160) and *Proteus mirabilis* (MTCC 3310). After this the plate was kept for incubation at 37°C for 24 hrs. After 24 hrs the anti-bacterial activity was checked by measuring the diameter of the zone of inhibition. Bacterial broth culture was prepared to a density of 10⁶ cells ml of 0.5 McFarland standards. The aliquot was spread evenly onto Mueller–Hinton agar using a sterilized cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 min. On each plate, equidistant wells were prepared with a 9 mm diameter sterilized, cork borer, which were 5 mm from the edge of the plate. Fifty microliters of seed extract (50 mg/mL) was aseptically introduced into a respective agar well. Tetracycline (25 µg/mL) was used as standard (positive) control and sample free solutions as blank control. This was followed by allowing the agar plate on the bench for 40 min pre-diffusion followed by incubation at 37°C for 24-48 h. The formation of clear inhibition zone around the well was regarded as significant susceptibility of the organisms to the extract. The experiment was performed in triplicates (Delelegn et al., 2018).
**RESULT AND DISCUSSION**

**Sampling and Pre-treatment**

Pre-treatment makes the phenolic compound active, which was further undergone FIR, TPC and HPLC analysis. *M. oleifera* seeds contain phenolic compounds that are useful as natural antimicrobial agent. The ethanol extract for 50 gms of powdered *Moringa oleifera* seed yielded 0.6 gms of crude extract.

**Total phenolic content**

Phytochemical screening of the ethanolic extracts of *M. oleifera* seed was carried out by UV-Spectrometer. The graph (Fig 1) shows the presence of phenolic compounds. The total phenolic in terms of the gallic acid equivalent (GAE) in mg/g of the extract. The total phenolic content was calculated with the help of the graph shown in Fig 1 and the standard curve equation was $y = 0.0588x - 0.0262$, where $R^2 = 0.9599$. The average phenolic contents in the ethanolic extracts was calculated to be 11.85 GAE mg/g.

**FTIR**

The moringa seed extract was characterized by FTIR. The FTIR spectra (Fig 2 and Table 1) revealed that moringa seed contains phenolic compounds confirmed by specific peaks such as 3280.92 cm$^{-1}$ (O–H groups of phenols), 2922.16 cm$^{-1}$ (C–H groups of phenols), 1544.98 cm$^{-1}$ (C=O (Fig 2: FTIR analysis graph showing.)

![Fig 1: Graph showing UV-Visible Spectrophotometer (750nm) readings Total Phenolic compound.](image)

![Fig 2: FTIR analysis graph showing.](image)

**Table 1:** FTIR spectral data of the ethanolic extract of *Moringa oleifera* seeds with details of peaks values, stretching and its corresponding functional groups.

| Spectral analysis | Wavelength and peak Values (ν cm$^{-1}$) | Stretching | Interpretation |
|-------------------|-----------------------------------------|------------|----------------|
| In FTIR spectral region | (3280.92) | O–H | phenol group |
| (2922.16) | C–H stretching | phenol group |
| (2852.72) | O–H stretching | Alkyl group |
| (1743.65) | C=O stretching | Aromatic compounds group |
| (1643.35) | C=C stretching | Tertiary amide group |
| (1544.98) | C=O stretching | Carboxyl group |
| (1454.33) | C–H stretching | -CH$_2$- |
| (1230.58) | C–O stretching | Phenol group |
| (1157.29) | C–O stretching | Tertiary alcohol |
| (1097.50) | C–O stretching | Primary alcohol |
| (1053.13) | C–O stretching | Primary alcohol |
| (1028.06) | C–O stretching | C=O–C |
| (794.67) | C–O stretching | Distributed |
| (717.52) | C–H def | OH out-of-plane bend |
Investigation on the Phenolic Content in *Moringa oleifera* and Its Antimicrobial Activity

carbonyl group of phenols) and 1230.58 (C-O group of phenols) and 1743.65 cm\(^{-1}\) (C=C group of aromatic compounds) as shown in Table 1.

**HPLC**

HPLC chromatogram of the phenolic compounds in crude extract in the seeds of *M. oleifera* and standard res are shown in Fig 3a and b respectively. Identified phenolic compounds are listed in Table 2b. The major constituents of the extract are phenolic acids such as, gallic acid, ellagic acid, ascorbic acid, acetyl salicylic acid. These phenolics were identified by comparing with the retention times of the standards. The major phenolic constituents are listed with their RT (Retention Time) and Peak area for each standard with peak area of seed sample in Table 2a and b.

**Antibacterial activity**

From the agar well diffusion result, it was found that *P. aeruginosa, B. subtilis, S. aureus* and *P. mirabilis* was significantly susceptible to ethanol extracts. The ethanol extract had the maximum (17mm) antibacterial activity against *S. aureus*, while minimum (7mm) antibacterial activity against *P. mirabilis*. The *M. oleifera* seed extract of ethanol was found to be effective against all the four pathogenic organisms showing a zone of inhibition ranging from 10- 17mm. It was found to be highly effective against *S. aureus* and least against *P. aeruginosa*.

*M. oleifera* is a versatile horticulture tree with important medicinal, nutritional and industrial applications, widely distributed and used in India (Pandey et al., 2019). *M. oleifera* is among the most common plants usually consumed by Indian medicine and almost all the parts of the plant, roots, leaves, flowers and seeds have been used in one way or other in the treatment of various ailments in the indigenous system (Ramachandran and Gopalakrishnan, 2010). In this study, antimicrobial activity of *M. oleifera* seed extract are due to the total phenolic contents of the seed (Abdulkadir et al., 2015). Total phenolic contents of *M. oleifera* seeds of different polar fractions yields are displayed in Fig 1. Ethanol extract (11.85 mg GAE/g sample) has showed the highest amount of phenolics. The same scenario reported (Singh et al., 2013) found that ethanolic extract had TPC range from 7.6 to 11.5 g/100 g defatted *M. oleifera* seed. The values of phenolic content in this current study slightly varied compared to those in the literatures. This may be due to the presence of different amounts of sugars, carotenoids or ascorbic acid, or the duration, geographical variation or methods of extraction, which may alter the amount of phenolics (Burri et al., 2017).

According to our study, FTIR range of (4000-500) cm\(^{-1}\) having many peaks recorded in the Table 1. For more confirmation, the FTIR was compared with the graph of other study, where *M. oleifera* seeds from Indonesia was used, which show similar peaks, like for –OH phenolic stretch at 3280.92 cm\(^{-1}\), C-O stretch of phenol at 1097.50 cm\(^{-1}\) and –

**Table 2a:** Showing details for the HPLC chromatogram its peak values, area and their retention time measured at 280nm for gallic acid standard [Fig 3a].

| Peak name | Interested Area | Area (%) | Retention Time | Peak Area |
|-----------|----------------|----------|---------------|-----------|
| G1        | Ascorbic acid  | 1414     | 2.123         | 8120      |
| G2        | Gallic acid    | 213223   | 3.164         | 1124273   |
| G3        | Ellagic acid   | 99408    | 4.150         | 626060    |
| G4        | Acetyl salicylic acid | 9343 | 5.998 | 80194 |
| G5        | Cinnamic acid  | 5433     | 5.275         | 42632     |

**Table 2b:** Showing details for the HPLC chromatogram its peak values, area and their retention time measured at 280nm for *M. oleifera* seed extract.

| Peak name | Interested Area | Area (%) | Retention Time | Peak Area |
|-----------|----------------|----------|---------------|-----------|
| M1        | Noise Peaks    | 339431   | 1.370         | 1353229   |
| M2        | Noise Peaks    | 74965    | 1.534         | 342840    |
| M3        | Noise Peaks    | 84632    | 1.650         | 538087    |
| M4        | Noise Peaks    | 410453   | 1.845         | 1887513   |
| M5        | Gallic acid    | 1783     | 3.631         | 14581     |
| M6        | Ellagic acid   | 5401     | 4.406         | 53168     |
| M7        | Benzoic acid   | 5639     | 6.606         | 90763     |
| M8        | *              | 5270     | 10.146        | 164404    |

![Fig-3.a : HPLC chromatogram of *M. oleifera* seed crude ethanolic extract showing peaks for the presence of phenolic compounds (measured at 280nm).](image-url)
Investigation on the Phenolic Content in *Moringa oleifera* and Its Antimicrobial Activity

OH bending of phenolics at 1057.65 cm⁻¹; these similarity in peak values confirm the presence of phenolic compounds (Izza *et al.*, 2018). This mild variation in peak values, due to the variation in amount and type of phenolic compounds due to geographic, climatic or environmental changes, which varies from place to place. Phenolic compounds are an important group of active compounds in herbs since they act disrupting the bacterium cell wall, interfering with the ATP pool and altering its membrane potential, resulting in bacterium’s death (Aliyu *et al.*, 2016).

The HPLC graph recorded for 280 nm (Fig 3.a and 3.b) was compared with a standard graph studies by (Mradu *et al.*, 2012), where they analysed eight well known phenolic compounds which were collected from different compounds. Their recorded HPLC retention time were Ellagic acid (11.86 min), Catechol (9.08 min), Gallic acid (3.50 min), Resorcinol (7.15 min), Vanillin (12.77 min), Acetyle Salicylic Acid (17.46 min), Benzoic acid (19.19 min) and Ascorbic acid (2.56 min). This study is in accordance with Mradu *et al.*, (2012) which showed a similar values for the seed extract. The similarity with their standard, represent the same phenolic compound in ethanol extract of *M. oleifera* seed powder. The slight variation in retention times, could be due to difference in calibration of HPLC, same solvent from different manufacturer and run time. Interestingly similar report was observed by Kadam *et al.* (2017) the retention time of ascorbic acid, galic acid and catechol were 2.56, 3.5 and 9.08 respectively.

The antibacterial activity of ethanolic extracts of dried *M. oleifera* seeds was determined using four bacterial species such as *Bacillus subtilis*, *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa*, *Proteus mirabilis* (Gram negative). The ethanolic extract had appreciable antimicrobial activity proved against all the 4 tested pathogens. Similar report were recorded by (Anwar and Rashid, 2007) and (Bello and Jamiu, 2017). which also had both bactericidal and bacteriostatic activity on Gram positive and negative organisms. This indicates that the seed extracts could also be used in the treatment of some gastrointestinal or wound infections caused by the above tested pathogens.
Gram negative and positive bacteria. Other studies have also shown that the antibacterial activity of *M. oleifera* seeds ethanolic extract is due to the presence of phenolic compound (Abdulkadir et al., 2015; Gebregiorgis Amabye and Mekonen Tadesse, 2016; Ruttaratnammongkol and Petrasch, 2015) Phenolic acid has a beneficial role of forming both ester and ether linkage on reacting with carboxylic and hydroxyl group respectively, this bifunctional nature resulted into efficient anti-microbial activity by cross link with cell wall macromolecules (Yu et al., 2001).

### CONCLUSION

The anti-microbial activity of the *M. oleifera* seed is due to the presence of various phytochemicals like phenols. The total value phenolic content was estimated in the *M. oleifera* seed. By using these natural extracts, the biggest emerging problem like anti-microbial resistance can be overcome and it is a promising alternative. The total phenolic content estimated 11.85mg GAE/g of dried seed, by observing the phenolic content of –OH stretches and bindings along with C-O stretches by FTIR, further HPLC analysis proves the proved the presence of Ascorbic acid, Gallic acid and Catechol in 280 nm wavelength. This study could further analyse in-depth for the unknown peaks of HPLC, which could be some unique phenolic compounds. All these are quite beneficial in microbial susceptibility tested on species *Pseudomonas aeruginosa* (MTCC 1688), *Bacillus subtilis* (MTCC 8561), *Staphylococcus aureus* (MTCC 3160) and *Proteus mirabilis* (MTCC 3310), which may be a broad-spectrum activity from *M. oleifera* can be proved by further research which using active bioactive compounds over other pathogens.

### Conflict of Interest Statement

There is no conflict of interest.

### REFERENCES

Abdulkadir, I.S., Nasir, I.A., Sofowora, A., Yahaya, F., Ahmad, A., and Hassan, I.A. (2015). Phytochemical Screening and Antimicrobial Activities of Ethanolic Extracts of Moringa oleifera Lam on Isolates of Some Pathogens. J. App. Pharm. 7: 4. https://doi.org/10.14172/1920-4159.1000203

Adebayo, I.A., Arsad, H. and Samian, M.R. (2018). Total phenolics, total flavonoids, antioxidant capacities and volatile compounds gas chromatography-mass spectrometry profiling of Moringa oleifera ripe seed polar fractions. Pharmacognosy Magazine. 14(54): 191-194. https://doi.org/10.4103/pm.pm_212_17

Aliyu, A., Darlington Chukwuma, U., Omoregie, H., and Falolashade, K.O. (2016). Qualitative phytochemical analysis of the leaf of Moringa oleifera lam. from three climatic zones of Nigeria. Available Online Ww. Jocpr. Com Journal of Chemical and Pharmaceutical Research. 8(8): 93-101. www.jocpr.com

Anwar, F., and Rashid, U. (2007). Physico-chemical characteristics of moringa oleifera seeds and seed oil from a wild provenance of pakistan. In Pak. J. Bot (Vol. 39, Issue 5).

Bello, S.A., and Jamiu. (2017). Antibacterial Activity of *Moringa oleifera* Seed Extracts On Escherichia coli, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. In Nigerian Journal of Microbiology (Vol. 2017, Issue 1). www.nsmjournal.org

Bidve S. C., Kadam V.B., Shindikar M.R. and N.P. Malpathak. (2017). Antioxidant potential of bark and leaves extracts of mangrove plant aegiceras curriculatum l. World Journal of Pharmaceutical Research. 6(13): 495-505.

Burri, S.C.M., Ekholm, A., Häkansson, Å., Tornberg, E., and Petrasch, S.C.M. (2017). Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. Journal of Functional Foods. 38(Pt A). 119-127. https://doi.org/10.1016/j.jff.2017.09.003

Delelegn, A., Sahile, S., and Husen, A. (2018). Water purification and antibacterial efficac of Moringa oleifera Lam. Agriculture and Food Security. 7(1): 1-10. https://doi.org/10.1186/s40066-018-0177-1

Gaafar, A.A., Ibrahim, E.A., Asker, M.S., Moustafa, A.F., and Salama, Z.A. (2016). Characterization of Polyphenols, Polysaccharides by HPLC and Their Antioxidant, Antimicrobial and Antiinflammatory Activities of Defatted Moringa (*Moringa oleifera* L.) Meal Extract. In International Journal of Pharmaceutical and Clinical Research. (Vol. 8, Issue 6).

Gebregiorgis Amabye, T., and Mekonen Tadesse, F. (2016). Phytochemical and Antibacterial Activity of Moringa Oleifera Available in the Market of Mekelle. Journal of Analytical and Pharmaceutical Research. 2(1). https://doi.org/10.15406/japlr.2016.02.00011

Govardhan Singh, R.S., Negi, P.S., and Radha, C. (2013). Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of Moringa oleifera seed flour. Journal of Functional Foods. 5(4): 1883-1891. https://doi.org/10.1016/j.jff.2013.09.009

Izza, N., Dewi, S.R., Setyandra, A., Sukoyo, A., Utoro, P., Riza, D.F. Al, and Wibisono, Y. (2018). Microwave-assisted extraction of phenolic compounds from Moringa oleifera seed as anti-biofouling agents in membrane processes. MATEC Web of Conferences. 204: 03003. https://doi.org/10.1051/ MATECCONF/201820403003

Kumar, A.R., Prabhu, M., Ponnuswami, V., Lakshmanan, V., and
Nithyadevi, A. (2014). Scientific seed production techniques in Moringa. Agricultural Reviews. 35(1): 69. https://doi.org/10.5958/j.0976-0741.35.1.009

Mradu, G., Saumyakanti, S., Sohini, M., and Anup, M. (2012). HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. International Journal of Pharmacognosy and Phytochemical Research. 4(3). Available online on www.ijppr.com

Pandey, V.N., Chauhan, V., Pandey, V.S., Upadhyaya, P.P. and Kopp, O.R. (2019). Moringa oleifera Lam.: A Biofunctional Edible Plant from India, Phytochemistry and Medicinal Properties. Journal of Plant Studies. 8(1): 10. https://doi.org/10.5539/jps.v8n1p10

Patel, P. (2017). Moringa Oleifera-Nature's Gold. Imperial Journal of Interdisciplinary Research (IJIR, 3.

Ramachandran, C., and Gopalakrishnan, K. (2010). Drumstick (Moringa oleifera): a multipurpose Indian vegetable. Economic Botany. 34(3): 276-283. https://doi.org/10.2307 /4254186

Ravani, A., Prasad, R.V., Gajera, R.R., and Joshi, D.C. (2017). Potentiality of Moringa oleifera for food and nutritional security - A review. Agricultural Reviews. 38(03): 228-232. https://doi.org/10.18805/ag.v38i03.8983

Ruttarattanamongkol, K., and Petrasch, A. (2015). (n.d.). Antimicrobial activities of Moringa oleifera seed and seed oil residue and oxidative stability of its cold pressed oil compared with extra virgin olive oil. In Songklanakarin J. Sci. Technol (Vol. 37, Issue 5). Retrieved May 27, 2020, from http://www.sjst.psu.ac.th

Saini, R.K., Sivanesan, I., and Keum, Y.S. (2016). Phytochemicals of Moringa oleifera: a review of their nutritional, therapeutic and industrial significance. In 3 Biotech (Vol. 6, Issue 2), Springer Verlag. https://doi.org/10.1007/s13205-016-0526-3.

Selmi, H., Bahi, A., Ferchichi, A., and Rouissi, H. (2019). Effect of supplementing Moringa oleifera essential oils on milk quality and fatty acid profile in dairy sheep. Indian Journal of Animal Research. 54(7): 879-882. https://doi.org/10.18805/ijar.b-1085.

Shahidi, F., Janitha, P.K., and Wanasundara, P.D. (1992). Phenolic Antioxidants. Critical Reviews in Food Science and Nutrition. 32(1): 67-103. https://doi.org/10.1080/10408399209527581

Siddiqui, Nazish, Latif, Abdul and Mahmood, Zeenat. (2017). Spectrophotometric determination of the total phenolic content and spectral fluorescence of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). Journal of Taibah University Medical Sciences. 12. 10.1016/j.jutmed.2016.11.006.

Yadav, B.P., and Ghimire, T.R. (2019). Moringa oleifera: A Plant Critical for Food Security, Nutraceutical Values and Climate Change Adaptation in the Hindu-Kush Himalayan Region: A Review. Asian Journal of Dairy and Food Research. 38(04): 322-328. https://doi.org/10.18805/ajdfr.dr-132

Yu, J., Vasanthan, T., and Temelli, F. (2001). Analysis of phenolic acids in barley by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry. 49(9): 4352-4358. https://doi.org/10.1021/jf0013407

Zhao, B., Deng, J., Li, H., He, Y., Lan, T., Wu, D., Gong, H., Zhang, Y., Chen, Z., and Khan, M. K. (2019). Optimization of Phenolic Compound Extraction from Chinese Moringa oleifera Leaves and Antioxidant Activities. https://doi.org/10.1155/2019/5346279