International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Physicochemical Constituents and Photochemical Screening by GC-MS of Moringa Oleifera Seed grown in Blue Nile State, Sudan

Elhassein Ahmed Adam[1], Elmugdad Ahmed Ali[2]

Department of Chemistry, Sudan University of Science and Technology, Khartoum, Sudan

Abstract: ***The study of different quality attributes of Moringa Oleifera seed from Blue Nile State, Sudan was carried out. The study was examined the physicochemical characteristics, Phytochemical studying and concentrations of elements for Moringa oleifera seed. The physicochemical characteristics were determined using American Oil Chemists' Society (AOAS) methods. The n-hexane extracted oil content of Moringa seeds 40.39%. Protein, moisture, fiber and ash contents were 37.06%, 2.24%, 8.24% and 3.68%, respectively. The elements of the Moringa Oleifera seed in mg/kg dry matter was analyzed by (ICP-OES), the seed contained minor elements (Fe, Zn, Na, Mn, Al, Cu, Ba, Ni, Cd and Pb). This was followed by highest concentration elements P, K, Mg and Ca (8561, 5903, 4203 and 1240 mg/kg) respectively. The photochemical analyses by (GC-MS) showed presence of Alkaloids (2,6-Dimethyl pyrazine), (1-methyl-pyrrolidinone), (2-Methylpyrrolidine). Flavonoids (2(3H)-Furanone, dihydro-5-methyl-), (2(3H)-Furanone, 5-ethyldihydro-), (piperidone, 1-(2-ethoxy-5-tetrazol-1-). Phenolic complexes (Phenol, 2-methoxy-4-(2-propeny)-). Antioxidants (Phenol, 2,4-bis(1,1-dimethylethyl)-), (Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-). These results reveal that the seed contain an appreciable amount of oil which can be uses as food supplements and biofuel. The residual cake of seed oil extraction can be used for water and wastewater treatment or organic fertilization. Photochemical compounds consist antioxidant which can be developed for pharmaceutical uses.***

Keywords: Antioxidant, Element, Moringa Oleifera, photochemical, physicochemical.

1. Introduction

Moringa Oleifera is a tropical plant belonging to the family Moringaceae a single family of shrubs with 14 known species (Bergamasco et al., 2012). It was originally an ornamental tree in Sudan, planted during the British rule (Schwarz, 2001). It is believed to have originated from India but now largely cultivated in Sudan and many other countries (Marimax, 2011) (Ijarotimi et al., 2013). M. oleifera is known as a 'Miracle tree' or the 'horse radish tree' as almost every part of it is useful for humans (Fayazuddin et al., 2013). In the Nile valley, the name of the tree is 'Shagarat al Rauwaq', which means 'tree for purifying'. M. oleifera grows rapidly on favorable sites, with height increments of 1 to 2 m per year during the first 3 to 4 years (Rebecca et al., 2006) (Arora et al., 2013). Fruit production begins as early as 6 to 8 months after planting. Trees generally produce good seed crops for about 12 years (Ghebremichael, 2004). In locales with more constant seasonal temperatures and rainfall regimes, flowering typically occurs two times each year, the mature, dehiscent capsules, mature about 3 months after flowering (Mangale Sapana et al., 2012). Each tree can produce 15'000 – 25'000 seeds per year. (Roloff et al., 2009). The average of weight of non-shelled seed is 0.3 gm (300 mg) (Kumar et al., 2012). The plant has numerous medicinal applications, It has been established that the plants which naturally synthesize some secondary metabolites, like alkaloids, glycosides, tannins, volatile oils and containing minerals and vitamins, used as a traditional medicine for the treatment of various illnesses such as skin diseases, respiratory distress, ear and dental infections, hypertension, diabetes, anemia, and cancer (Al-Asmari et al., 2015) (Martin, 2015). Moringa seeds contain about 35% oil, is a sweet non-sticking, non-drying oil that resists rancidity (Fahey, 2005). The oil extracted from Moringa is known as Ben oil and reportedly contains 70% of oleic acid, an 18-carbon long mono unsaturated fatty acid (MUFA). It has found use in the food industry, as it allows for longer storage and high-temperature frying processing (Palafox et al., 2012). It can be used in salads, soap making, and burns without smoke (Marimax, 2011). The study also advocates that M. Oleifera seed oil could be used as a suitable feedstock for biodiesel production (Rashid et al., 2011). Currently, there is a nascent biodiesel industry in the Yucatan Peninsula, Mexico (Palafox et al., 2012). The leaves, seeds, flowers, bark, roots, and seed kernels of Moringa oleifera have been used for dietary purposes and cosmetic (Badejo et al., 2014). The seed powder of Moringa oleifera works as a natural coagulant which clarifies very turbid water (Lar et al., 2011), especially in the Sudan (Yarahmadi et al., 2009), and wastewater treatment (Sánchez-Martín et al., 2010) (Pallavi and Mahesh, 2013), without affecting the water pH, conductivity and alkalinity (Aho & Lagasi, 2012. Thereby making the M. Oleifera protein more attractive than aluminum salts in water treatment (Okoli, 2012). Furthermore, the study showed that M. oleifera had a degree of antibacterial properties (Walter et al., 2011). The shelled M. Oleifera seeds have been found effective for the removal of heavy metals such as cadmium by adsorption (Abaliwano et al., 2008) (Mataka et al., 2010).

2. Material and Methods

2.1 Collection of Moringa Oleifera seeds

The Seeds of Moringa Oleifera used in this study were collected from a farm in Ad-Damazin, Blue Nile State, Sudan.

Licensed Under Creative Commons Attribution CC BY

2.2 Preparation of M. Oleifera seed powder

The seeds collected from the farm were de-shelled and the endocarps were air dried at ambient temperatures (25°C) for a period of seven days before milling. Direct sunlight was avoided to prevent degradation of some of the plant photochemical constituents. The white kernel was crushed to a powder, using an electric grinder and sieved through a 500 µm stainless steel sieve. The fine powder obtained was stored in a sterile air-tight container in a dark place to prevent oxidation.

2.3 Physicochemical analysis of Moringa Oleifera seed

The oil, moisture, protein, fiber and ash contents were determined according to American Oil Chemists' Society (AOAS) method.

2.3.1 Determination of oil content

The soxhlet extraction method was used to extract the oil from Moringa Oleifera seeds. About 10 g of seeds powder were put into an extraction thimble. A 170 ml of n-hexane solvent was poured into around bottom flask. After set up the soxhlet apparatus, the solvent was heated for 45 min and the oil was extracted. The soluble extracts were filtered and evaporated in rotary evaporator (Buchi, Switzerland; temp: 50°C; pressure175 mbar). And the percentage of oil contain was determined.

2.3.2 Determination of moisture

The initial moisture content of the seed kernels was determined using the oven method. 5 g of seeds were taken in a tarred aluminum dish, then dry in an air oven at 100 ±20 ^{0}C for 6 hours, the dish was cool in a desiccator and weigh. The drying was replicated three time and the average value of moisture content was calculated and recorded.

2.3.3 Determination of total ash

About 5 gm of sample was weighed in a tarred silica / platinum dish and transferred ashed to a muffle furnace. The sample was ashed at a temperature of 550 ±10 °C for 1 hour until the ash is free of carbon, the dish was cooled in a desiccator and weighted it. The method was replicated until the difference between two successive weights is less than 1 mg, and the lowest weight was recorded.

2.3.4 Determination of crud fiber

The sample is boiled for 35 minutes in diluted sulphuric acid. After filtration and washing, the sample was boiled again for 35 minutes in diluted potassium hydroxide. After filtration, washing and drying the residue was ashes at 500ºC. The difference in weight before and after ash is called crude fiber.

2.3.5 Determination of crude protein

1 g of M. Oleifera seeds powdered was weighed into Kjeldahl flask. 0.5 g catalyst system made up of anhydrous sodium sulphate, copper (II) sulphate and selenium dioxide in the ratio 98:1:1 was added to the substrate in the flask. 12 ml of pure suphuriuc acid was added and the mixture boiled for 1 hour at 420°C. The solution was transferred into 100ml volumetric flask and diluted to 100ml. 10ml of the solution was placed in the Kjeldahl distillation apparatus (markehan still), and 20ml of 40% NaOH was added. The mixture was steam distilled and 20ml of 2% boric acid containing screened methyl orange indicator was added and titrated with standard HCl to end point, Then the percentage nitrogen was calculated.

2.4 Determination of Photochemical compounds

2.4.1 Extraction and isolation of M. Oleifera seeds for GS-MS analysis

The seeds were dehusked and air dried at room temperature. Then grinded into a fine powder (500µm) using a scientific electric blender. After oil was extracted, the 50 g of the deoiled seed powder were soaked by ethanol 80%. The obtained filter was concentrated using a rotary evaporator at 45ºC. The extract acidified with H2SO4 to remove water, after that the pH adjusted to 10 by ammonium hydroxide and extracted two times using chloroform followed by dichloromethane solvent. The obtained extract was kept for GC.MS analysis.

2.4.2 GC-MS analysis

GC-MS analysis of the extracts was carried out in a GC system equipped with split/splitles injector and auto-sampler attached to a polar 5-MS (5% phenyl polymethyl siloxane) capillary column (Rtx-5MS; 30 m×0.25 mm I. d. and 0.25-µm film thickness) and fitted to Mass Detector. The flow rate of the carrier gas, helium (He) was set to be at 50 ml.min−1 in splitless mode. The injector temperature was adjusted at 280°C, while the detector temperature was fixed to 280°C. The column temperature was kept at 70°C for 1 min followed by linear programming to raise the temperature from 200 °C to 250°C (at 8°C min−1 with 2.5 min hold time), and 200°C to 250°C (at 10°C min−1 with 2 min hold time). The transfer line was heated at 280°C. Total run time was 27.2 min. Mass spectra were acquired in scan mode (70 eV); in the range of 50 to 550 m/z. Twenty microliter each of the extracts (250 mg/ml stock) were further diluted in 2 ml of methanol. One microliter of this diluted sample was injected for GC-MS analyses.

2.4.3 Identification of compounds

Interpretation of mass spectra was conducted using the database of the National Institute of Standards and Technology (NIST, USA). The database caters for more than 62,000 patterns of known compounds. The spectrum of the extracts was matched with the spectrum of the known components stored in the NIST library.

2.5 Determination of elements

2.5.1 Seeds preparation

A mass of 0.1 g ground seed powder was placed in a microwave digestion vessel and concentrated nitric acid (8 mL) and hydrogen peroxide (2 mL) were added. Digestion was carried out for about 30 min in the microwave. After digestion, the samples were transferred to a 25mL volumetric flask and made up to volume with deionized water. The solutions were directly analyzed for metal content using ICP-MS and results were expressed as ppm (mg/kg) of dry weight.

Licensed Under Creative Commons Attribution CC BY

2.5.2 Seeds analyses (ICP-MS)

A prepared solution containing analyte element was aspirated into the plasma generated by inductive coupled plasma source. The atomized elements produced characteristic emission spectra lines, which are separated by a simultaneous optical spectrometer. The intensity of spectral line of an element is proportional to concentration.

3. Results and Discussion

3.1 Physicochemical analysis

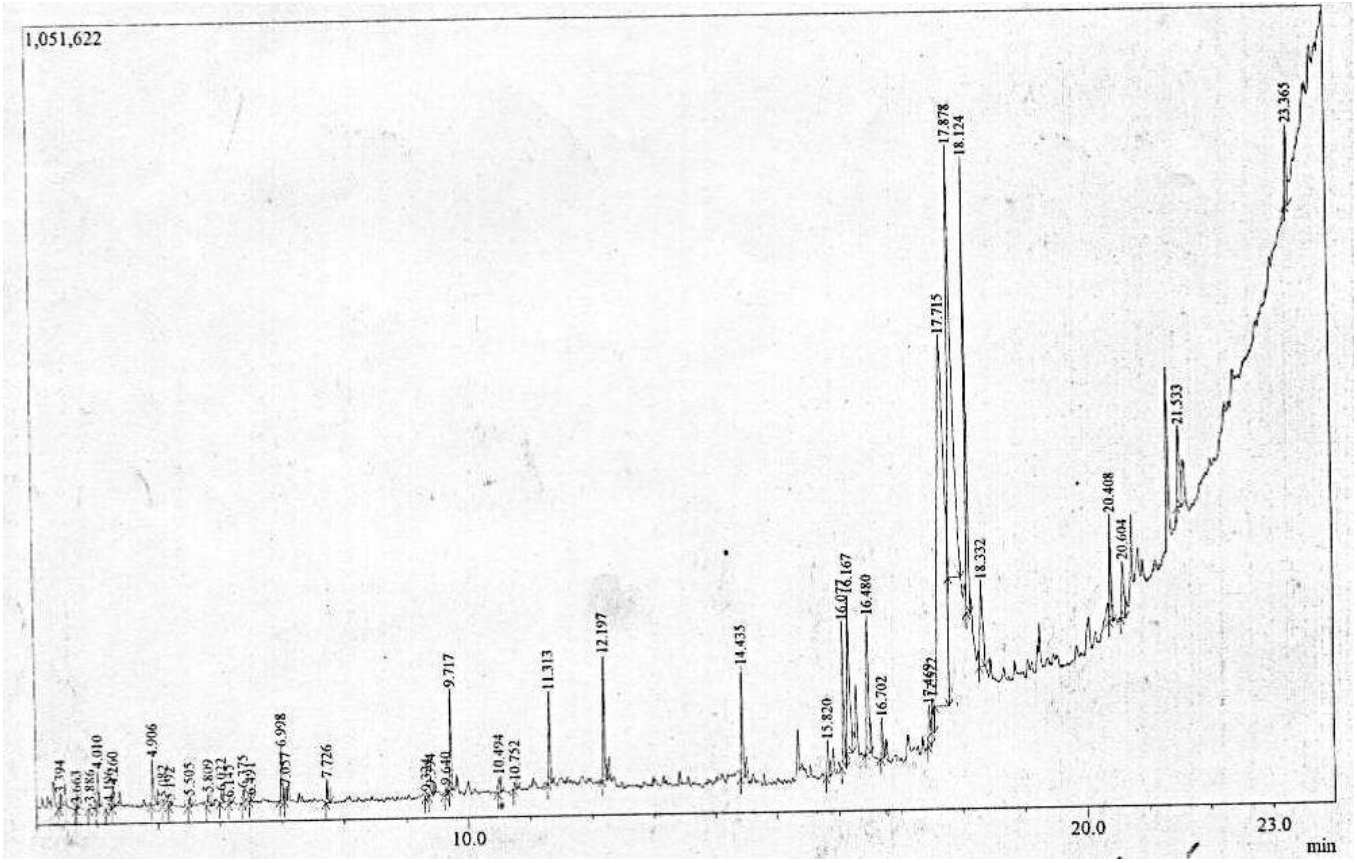
Table 1: Physicochemical analysis of M. Oleifera seed

Constituents	Moringa Oleifera
Oil contain (%)	40.39
Moisture contain (%)	2.24
Protein contain (%)	37.06
Fiber contain (%)	8.24
Ash contain (%)	3.68

From the table (1) the n-hexane extracted oil content of Moringa Oleifera seeds was found to be 40.39%, this highest oil yield of M. Oleifera seeds might be attributed to the natural habitats and geoclimatic constraints. The moisture content of seeds was found to be 2.24 %. The moisture usually depends on factors such as harvesting time as well as storage conditions. The protein and fiber was found to be 37.06% and 8.24% respectively. The oil seed residue left after the extraction of oil (protein) could also be explored as a potential source of natural coagulants for water treatment. The ash content of the Moringa oleifera seed was determined to be 3.68 %. This indicates a high mineral content in the seeds.

3.2 Photochemical analysis

Mass spectrum of moringa oleifera seeds



3.2.1 GC-MS chromatogram of moringa oleifera seed extraction

GC method was optimized by varying the oven temperature. In current gradient oven temperature programming, a good resolution of the extracts has been seen in a relatively short duration of time. The fragmented ions were separated by the analyzer, according to their mass to charge ratio. GC-MS analyses of M. Oleifera seeds show several complexes. Alkaloids (2,6-Dimethyl pyrazine), (1-methyl-pyrrolidinone), (2-Methylpyrrolidine) Flavonoids Dihydro-5-methyl-5-phenyl-2(3H)-furanone (2(3H)-Furanone, 5-ethyldihydro-), (piperidone, 1-(2-ethoxy-5-tetrazol-1-). Phenolic compound (Phenol, 2-methoxy-4-(2-propeny)-) and Antioxidant (Phenol, 2,4-bis(1,1-dimethylethyl)-), (Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-).

3.3 Elements content in M. Oleifera seeds:

Table 2: Concentration (mg/kg; dry weight) of minor and the major elements in Moringa Oleifera seeds

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Elements	Pb	Zn	P	Mg	Mn	Ni	Na	Fe	K	Cu	Ca	Cd	Ba	Al
Concentration (mg/kg)	<0.015	35.12	8561	4203	11.98	0.2797	12.46	37.50	5903	4.933	1240	0.1121	4.273	8.152

Licensed Under Creative Commons Attribution CC BY

Both minor elements: (Pb, Cd, Ni, Ba, Cu, Ba, Al, Mn, Na, Zn and Fe) and major elements (P, K, Mg and Ca) were present in seeds of M. Oleifera. Among the micro nutrients, lead (Pb) had the lowest concentration (<0.015 mg/kg). This was followed by Cd, Ni, Cu, Ba, Al, Mn, Na, Zn and Fe (0.1121, 2797, 4.273, 4.933, 8.152, 11.98, 12.46, 35.12 and 37.50 respectively). Phosphorus (8561 mg/kg) had the highest concentration followed by the K (5903mg/kg), Mg (4203 mg/kg) and Ca (1240 mg/kg). The concentration of P, K, Mg and Ca was highest compared to other elements among the macro nutrients. This might be due to the highest concentration in plants absorbed from soil.

4. Conclusion

This research work showed that Moringa Oleifera is rich of oil and protein. Also, contains some of minerals such as calcium, potassium, phosphorus, magnesium and iron hence it is a good source of food, and the oil can be use as biofuel. Photochemical analyzed showed Alkaloids, Flavonoids and Antioxidants complexes which can be developed for pharmaceutical uses.

References

[1] Abaliwano, J. K., Ghebremichael, K. A. & Amy, G. L. 2008. Application of the purified Moringa oleifera coagulant for surface water treatment. *WaterMill Working Paper Series,* 5**,** 1-19.

[2] Al-Asmari, A. K., Albalawi, S. M., Athar, M. T., Khan, A. Q., Al-Shahrani, H. & Islam, M. 2015. Moringa oleifera as an Anti-Cancer Agent against Breast and Colorectal Cancer Cell Lines. *PLoS One,* 10**,** 14.

[3] Arora, D. S., Onsare, J. G. & Kaur, H. 2013. Bioprospecting of Moringa (Moringaceae): microbiological perspective. *Journal of Pharmacognosy and Phytochemistry,* 1.

[4] Badejo, A. A., Damilare, A. & Ojuade, T. D. 2014. Processing Effects on the Antioxidant Activities of Beverage Blends Developed from Cyperus esculentus, Hibiscus sabdariffa, and Moringa oleifera Extracts. *Prev Nutr Food Sci,* 19**,** 227-33.

[5] Bergamasco, R., Vieira, A. M. S., Nishi, L., Araújo, Á. & Silva, G. 2012. Application of Hybrid Process of Coagulation/Flocculation and Membrane Filtration to Water Treatment. *Ecological Water Quality Water Treatment and Reuse,* 14**,** 2.88

[6] Fahey, J. W. 2005. Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. *Trees for life Journal,* 1**,** 1-15.

[7] Fayazuddin, M., Ahmad, F., Kumar, A. & Yunus, S. 2013. An Experimental Evaluation of Anti-inflammatory activity of Moringa Oleifera Seeds. *International Journal of Pharmacy and Pharmaceutical Sciences,* 5**,** 717-721.

[8] Ghebremichael, K. A. 2004. *MORINGA SEED AND PUMICE AS ALTERNATIVE NATURAL MATERIALS FOR DRINKING WATER TREATMENT.* Trita-LWR. PH.D, Royal Institute of Technology.

[9] Ijarotimi, O. S., Adeoti, O. A. & Ariyo, O. 2013. Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented Moringa oleifera seed flour. *Food Sci Nutr,* 1**,** 452-63.

[10] Kumar, K. V., Rubha, M., Manivasagan, M., Babu, N. & Balaji, P. 2012. Moringa oleifera-The Nature's Gift. *Universal Journal of Environmental Research & Technology,* 2**,** 203-209.

[11] Lar, P., Ojile, E., Dashe, E. & Oluoma, J. 2011. Antibacterial Activity on Moringa Oleifera Seed Extracts on Some Gram Negative Bacterial Isolates. *African Journal of Natural Sciences,* 14**,** 57 - 62.

[12] Mangale Sapana, M., Chonde Sonal, G. & Raut, P. 2012. Use of Moringa oleifera (drumstick) seed as natural absorbent and an antimicrobial agent for ground water treatment. *Research Journal of Recent Sciences,* 2277**,** 2502.

[13] Marimax, A. M. 2011. *Comparative studies on the effects of moringa oleifera in improving water quality for some communities in Sekyere South District.* Kwame Nkrumah University of Science and Technology.

[14] Martin, W. 2015. Do the benefits of Moringa oleifera trees extend to KELEA activation of water. *Advances in Plants & Agriculture Research,* 2.

[15] Mataka, L., Sajidu, S., Masamba, W. & Mwatseteza, J. 2010. Cadmium sorption by Moringa stemopetala and Moringa oleifera seed powders: Batch, time, temperature, pH and adsorption isotherm studies. *International Journal of Water Resources and Environmental Engineering,* 2**,** 50-59.

[16] Okoli, C. 2012. *Development of protein-functionalized magnetic iron oxide nanoparticles: potential application in water treatment.* Doctoral Doctoral, AlbaNova University Center.

[17] Palafox, J. O., Navarrete, A., Sacramento-Rivero, J. C., Rubio-Atoche, C., Escoffie, P. A. & Rocha-Uribe, J. A. 2012 .Extraction and characterization of oil from Moringa oleifera using supercritical CO 2 and traditional solvents. *American Journal of Analytical Chemistry,,* 3**,** 946-949.

[18] Pallavi, N. & Mahesh, S. 2013. Feasibility study of Moringa oleifera as a natural coagulant for the treatment of dairy wastewater. *International Journal of Engineering Research,* 2**,** 200-202.

[19] Rashid, U., Anwar, F., Ashraf, M., Saleem, M. & Yusup, S. 2011. Application of response surface methodology for optimizing transesterification of Moringa oleifera oil: Biodiesel production. *Energy Conversion and Management,* 52**,** 3034-3042.

[20] Rebecca, H., Sharon, M., Arbainsyah, A. & Lucienne, D. Moringa oleifera: medicinal and socio-economic uses. International Course on Economic Botany. National Herbarium Leiden, 2006 Netherlands. National Herbarium Leiden, 2-6.

[21] Roloff, A., Weisgerber, H., Lang, U. & Stimm, B. 2009. Moringa oleifera LAM., 1785. *Sea,* 10.

[22] Sánchez-Martín, J., Ghebremichael, K. & Beltrán-Heredia, J. 2010. Comparison of single-step and two-step purified coagulants from Moringa oleifera seed for turbidity and DOC removal. *Bioresource Technology,* 101**,** 6259–6261.

[23] Schwarz, D. 2001. Water clarification using Moringa olifera. *GATE-ESCHBORN-***,** 17-20.

[24] Walter, A., Samuel, W., Peter, A. & Joseph, O. 2011 .Antibacterial activity of Moringa oleifera and Moringa

Licensed Under Creative Commons Attribution CC BY

stenopetala methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. *African Journal of Microbiology Research*, 5**,** 153-157.

[25] Yarahmadi, M., Hossieni, M., Bina, B., Mahmoudian ,M., Naimabadie, A. & Shahsavani, A. 2009. Application of Moringa oleifera seed extract and poly aluminium chloride in water treatment. *World Appl Sci J*, 7**,** 962-967.

Licensed Under Creative Commons Attribution CC BY