UV- Visible Spectroscopic Analysis and High Performance Liquid Chromatographic Analysis of Moringa Oleifera Bark extract in non-polar solvents

Jyoti Rathore[1], Chinmoyee Rani Das[2]

[1]Research Scholar, Atal Bihari Vajpayee Vishwavidyalaya, Bilaspur 492001(C.G) India

[2]Department of Chemistry, Dr. J.P Mishra Govt. Science College Mungeli, Bilaspur 492001(C.G) India

Abstract

The well known plant named Moringa oleifera (Drum Stick) has enormous pharmacological activity. From ancient time its been a model for its therapeutic value. In various systems of medicine like Ayurvedic , Allopathy the crude part of plant has been transformed into formulation part and more advance nano formulations has also emerged. The chemical constituents present in Moringa oleifera are moringinine, 4-hydroxymellein, β-sitosterol, octacosanoic acid and moringine in bark part , kaempferitrin, rhamnetin in flower part, benzylglucosinolate, 4-(α-l-rhamnopyranosyloxy)-benzylglucosinolate in seed part. This research article focused on the introduction of Moringa plant with its part, cultivation , collection region , extraction method employed and analytical method involvement UV Spectrophotometer and High Performance Liquid Chromatography. The result showed significant outcomes which can be helpful for further validation and Formulation development of Moringa Bark.

Keywords – Validation, High Performance Liquid Chromatography, Pharmacological, Ayurvedic

DOI Number: 10.48047/nq.2022.20.22.NQ10004 NeuroQuantology2022; 20(22): 92-103

1.Introduction

Indian native plant recognized as moringa (Moringa oleifera Lam.) is used for therapeutic purposes. The family Moringaceae includes the vegetable Moringaoleifera, which is a member of the Brassica genus. Along with Anoma and Hyperanthera, the genus Moringa is one of the genera that make up the Moringaceae family. The family is frequently referred to as "drumstick" or "horseradish." There are 13 species of the Moringa genus, which are found in southwest Asia, southwest Africa, northeast Africa, and Madagascar. M. oleifera, an Indian native, is the subject of extensive research. Because of this, the species has been raised in several parts of the world, including Asia, Latin America, Florida, the Caribbean, and the Pacific Islands.(1-5) The Moringa genus has long been utilised extensively to enhance health. Moringa was utilised by kings and queens to

increase alertness and maintain good skin. M. oleifera leaves were fed to Indian warriors to increase their vitality and help them cope with the agony and stress of battle . (7-8)The genus has also been used traditionally to treat sore throats, fever, diarrhoea, wounds, anxiety, and skin infections. The species is very well known for having numerous applications. All plant components can be utilised medicinally, including the seeds for water purification, the leaves for dietary supplements, the oil for biofuel, the trunks for gum, the flowers for honey, and the oil from the flowers. Anti-inflammatory, antispasmodic, antihypertensive, anticancer, antipyretic, antioxidant, antiepileptic, antiulcer, antidiabetic, and anticholestrol properties are present in Moringa oleifera. (10-15)Originally from Northern India, M. oleifera is now found worldwide in the Americas, Africa, Europe, Oceania, and Asia. This tree's leaves, blossoms, pods, and seeds are regarded as food sources with great nutritional value. Leaves can be eaten raw or cooked, and dried powder can be kept out of the refrigerator for several months without losing any nutritional value. Without a doubt, M. oleifera provides significant health advantages to nations where hunger is a concern.(16-18)

2. Cultivation

The Moringa plant grows in a broad range of climates. It is an evergreen tree that grows quickly in conditions of moderate temperature and high humidity, but it can also appear to be a deciduous tree under stress conditions, such as low temperatures of less than 5 C or high temperatures of more than 50 C during periods of drought. (19-21)This tree is excellent for semi-arid tropical and semi-tropical environments because it also requires 200–2000 mm of annual rainfall and temperatures between 25 and 40 C for growth. The strength and nutrient content of the plant are significantly influenced by the soil. Different nutrient compositions were produced on plant sections depending on whether the fertilisers were used alone or in combination with others. To evaluate the impact on the nutritional content, NPK fertiliser, chicken manure, and organic base fertiliser were provided. It was discovered that poultry manure produced the best results when compared to phosphorous, potassium, sodium, and manganese(22-26).

Table no. 1 Phytochemicals Reported in Moringa Oleifera(28-31)

S.No.	Secondary Metabolites	Chemical Consitutents
1	Flavonoids	Rutin, quercetin, rhamnetin, kaempferol, apigenin,

		and myricetin
2	Glucosinolate	4-O-(αL-rhamnopyranosyloxy)-benzyl glucosinolate
3	Phenolic Acid	Gallic acid, gentisic acid (, syringic acid , ρ-coumaric acid , and sinapic acid
4	Terpenes	Lutein, d α-amyrin
5	Alkaloids	Marumoside A and marumoside B
6	Sterols	β-sitosterol-3-O-β-Dgalactopyranoside

2.1 Collection of Plant parts & solvents

Bark of Moringa oleifera was collected from the local areas of Korba region of state Chhattisgarh. Solvents used for the experiment were of AR grade named Chloroform, Ethyl acetate, Benzene and Hexane. All solvents were of molychem company and purchased from Ideal Chemicals Raipur,Chhattisgarh.

2.2 Method of Extraction

The extraction method involved was Soxhlet extraction. The bark after collection was washed and sun dried for 10days to be converted into coarse powder with the help of mortar and pestle. After that 30gms of bark powder was weighed accurately and treated with the solvent in soxhlet apparatus for 24hrs to get extracted liquid. This process was carried out for solvent name Chloroform, Ethyl acetate, Benzene and Hexane respectively to get the extracted liquid(31).

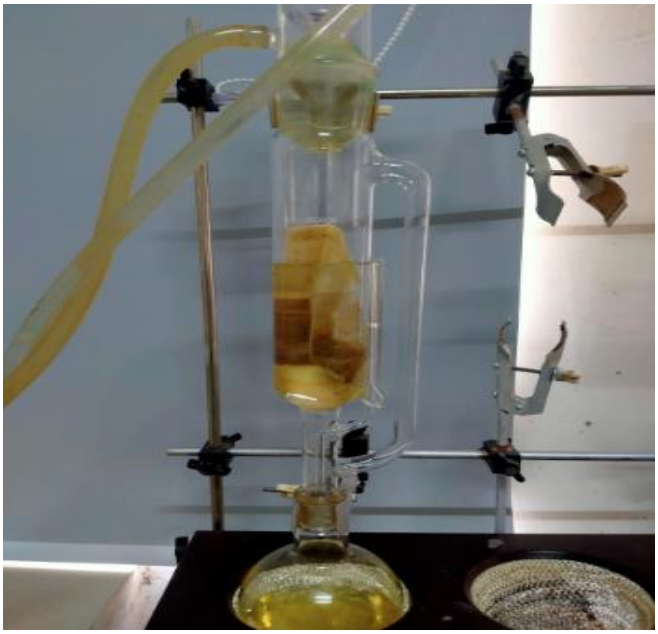


Figure 1 Glimpses of Soxhlet Extraction Process of Moringa Oleifera

2.4 UV-Vis Spectrophotometry Analysis

Sample Preparation

Chloroform bark extract , Benzene bark extract , Ethyl acetate bark extract and Hexane bark extract were prepared for Spectroscopic analysis. Each extract was prepared of 100µg/ml conc. in 100ml volumetric flask to get the absorbance. Then the each sample was scanned from 200nm-400nm.For the study Double beam UV-Vis Spectrophotometer was used of Labtronics Model LT 2700(32).

2.5 HPLC Analysis

For HPLC analysis of each bark extract sample was prepared of 10µg/ml conc. in volumetric flask. Shimadzu HPLC Binary system with column C18 and detector UV-Vis spectrophotometer was used(33-34).

3. Result and discussion

The M.Oleifera bark treated with solvents name Benzene, Chloroform, Ethyl Acetate and Hexane for soxhlet extraction .The extracted liquid also undergone for UV spectrophotometric Analysis and HPLC analysis. The spectrophotogram was between Absorbance (Abs) Vs Wavelength(nm).As the analytical study λmax was found to be 190nm for Benzene extract, 242nm for Chloroform extract, 213nm for Ethyl acetate and 214nm for Hexane extract. For High Performance Liquid Chromatography (HPLC) also all the extracted portion were analyzed to determine the Retention time ,Peak height, Peak width. HPLC column C18 of Shimadzu SPD VP10 model was used.N2000 Chromatostation software with UV Detector inbuilt. 20µl sample was injected for

analysis. Sample solution of 10µg/ml was prepared for analysis. Reported Retention time for Benzene Bark extract 5.015min with peak height 130738.789 and Peak Area 829309.875 at 190nm wavelength, Chloroform Bark extract Retention time 4.957minute, with peak height 247625.594 and Peak Area 2267337.500 at 242nm wavelength, For Ethyl Acetate bark extract 3 different Retention time was reported 4.432min, 4.907min and 10.86min with peak height 130465.898, 321477.500 and 161158.281 respectively , Peak Area 3114906.750, 7810399 and 5598475 respectively. In case of Hexane multiple retention time reported 0.357, 1.482, 2.873, 3.165, 4.557, 10.798 and 11.957 with Peak height 128079.305, 130023.453, 123246.977, 135021.578, 122575.219, 103950.820, 172260.750 and 115556.102 respectively, peak area 826434.375, 1053293.375, 919739.813, 720861.813, 891638, 869976.00, 1281326.375 and 823051 at 214.

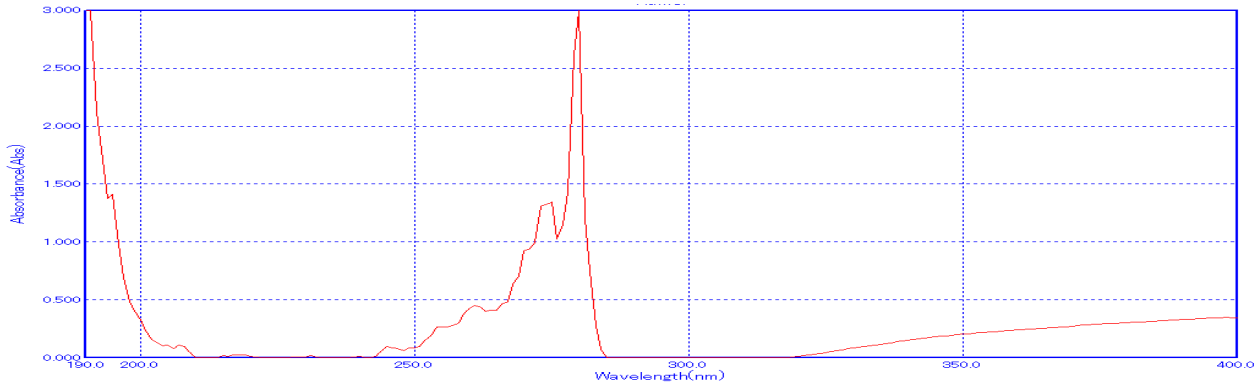

Figure 2: Benzene Extract Spectra of Moringa Oleifera

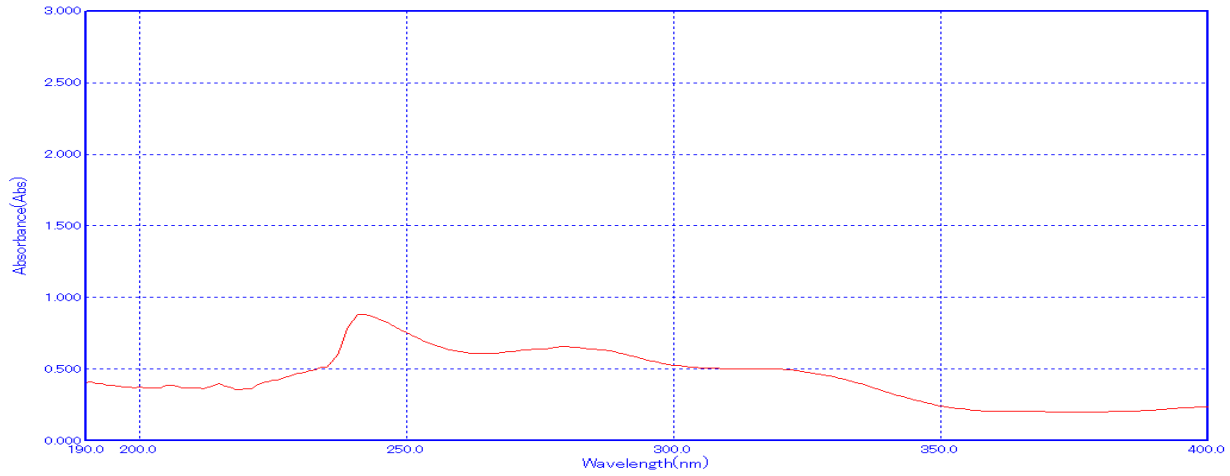

Figure 3 Chloroform Extract Spectra of Moringa Oleifera

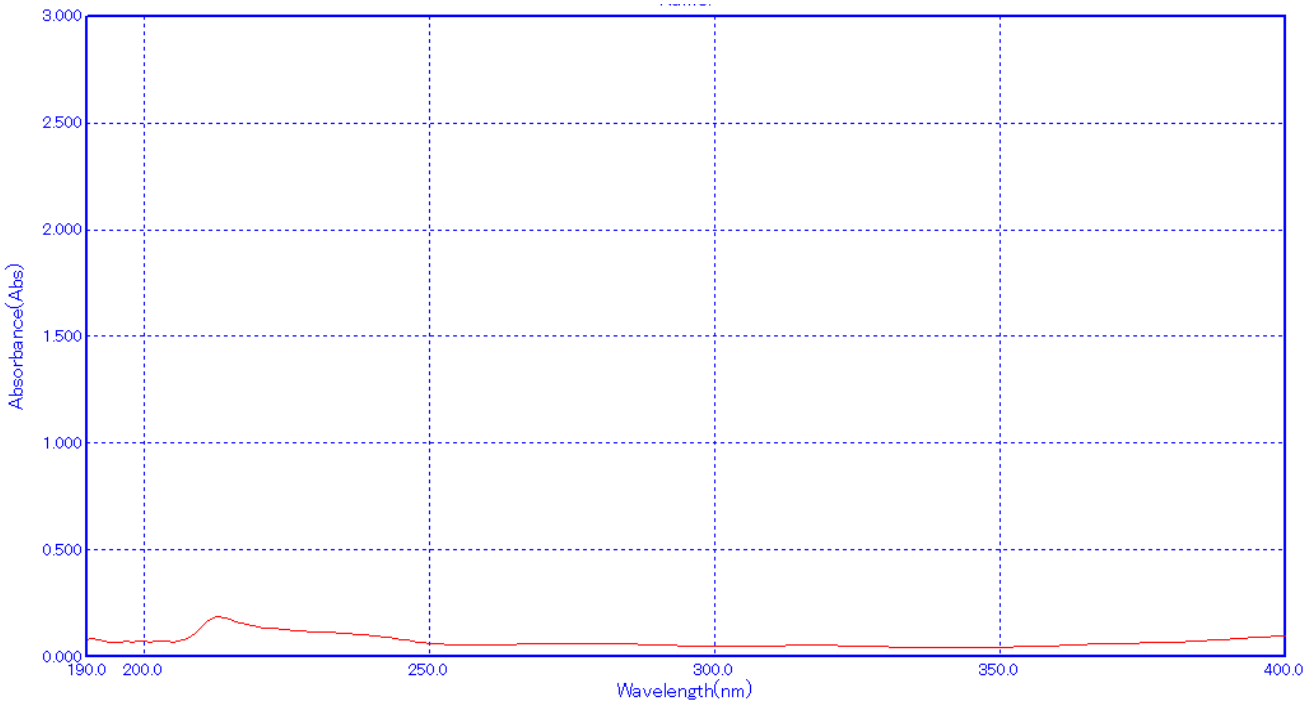

Figure 4 Ethyl acetate Spectra of Moringa Oleifera

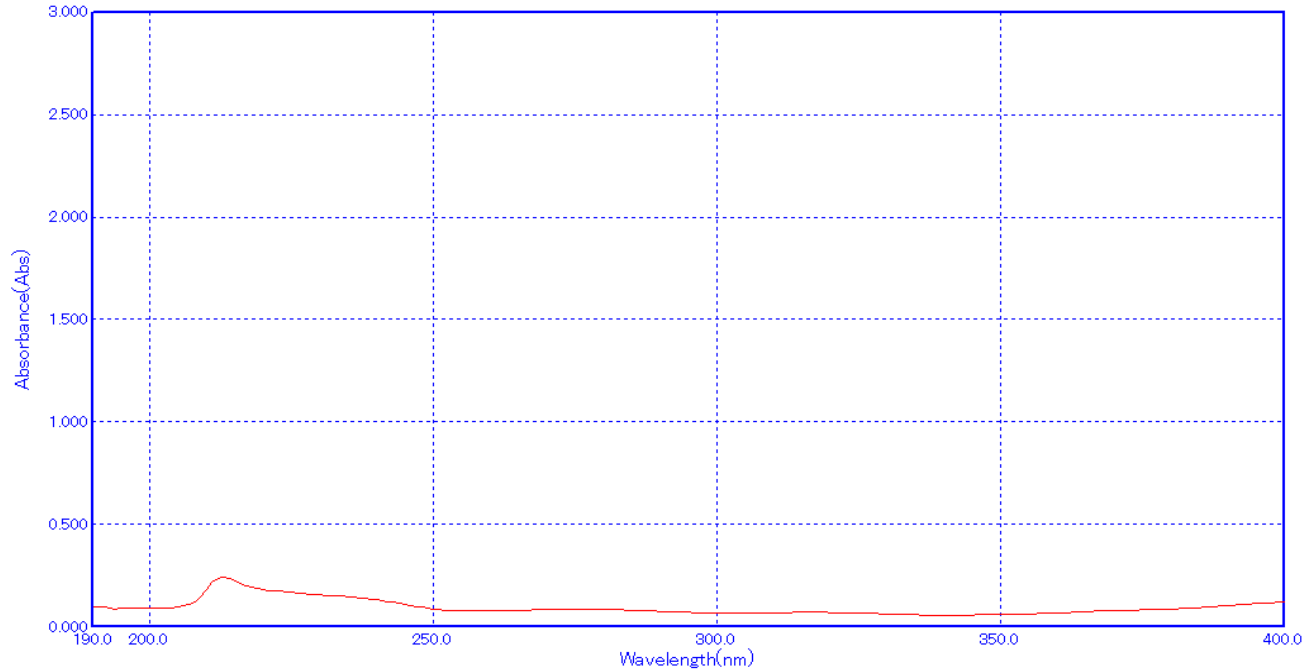

Figure 5 Hexane Extract Spectra of Moringa Oleifera

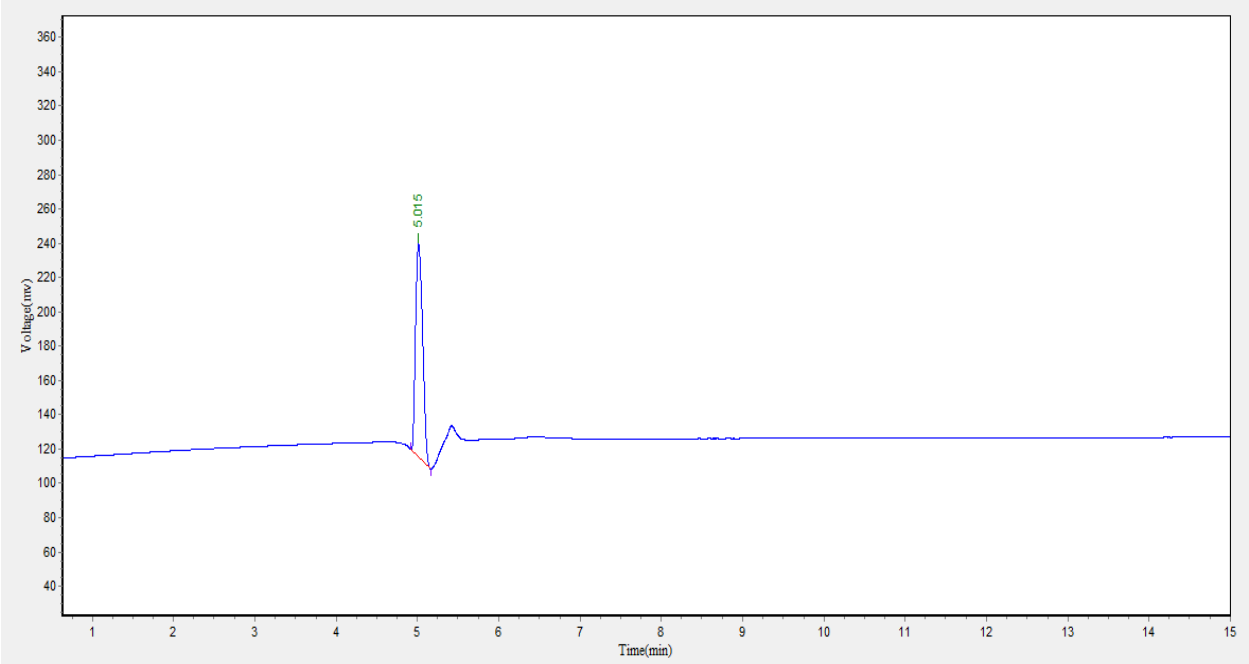

Figure 6 Benzene Extract Chromatogram of Moringa Oleifera

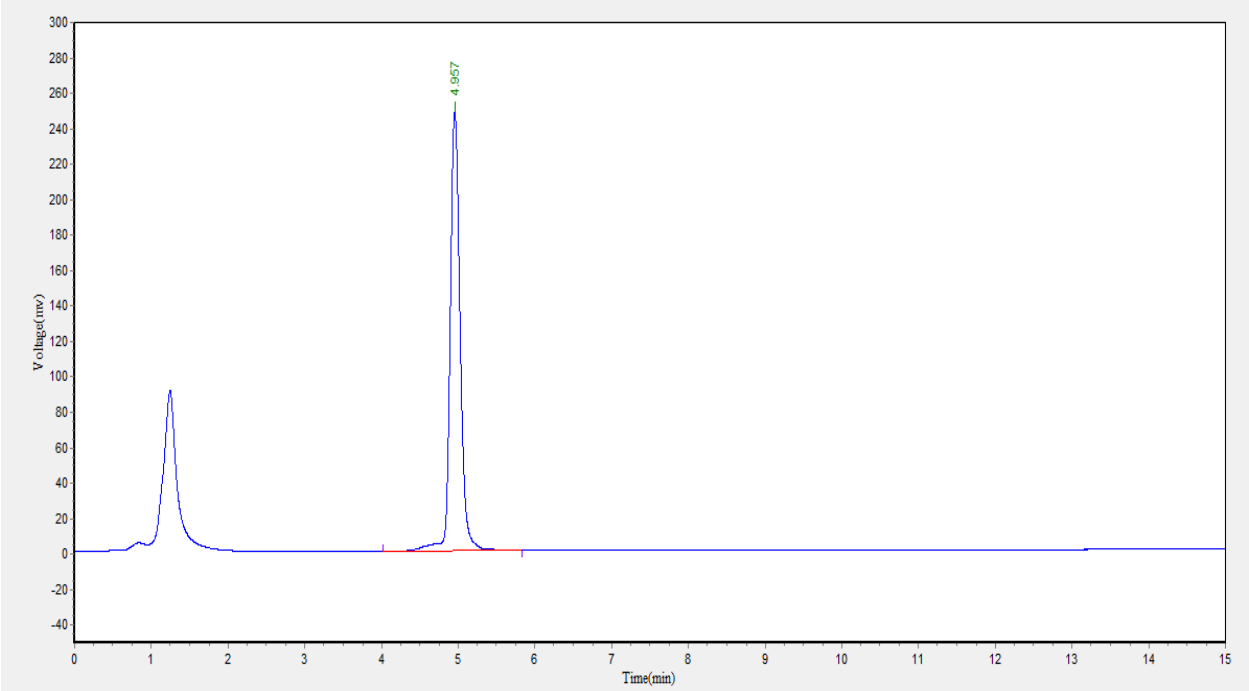

Figure 7 Chloroform Extract Chromatogram of Moringa Oleifera

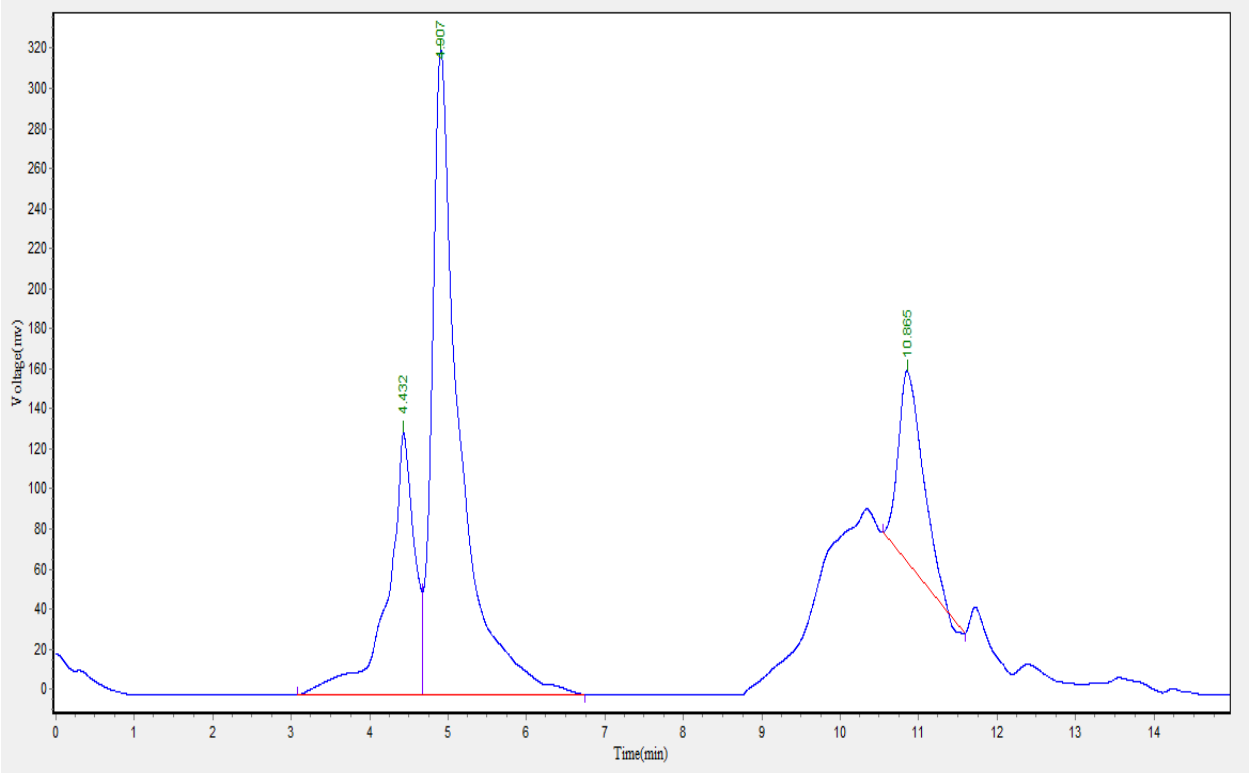

Figure 8 Ethyl Acetate Chromatogram of Moringa Oleifera

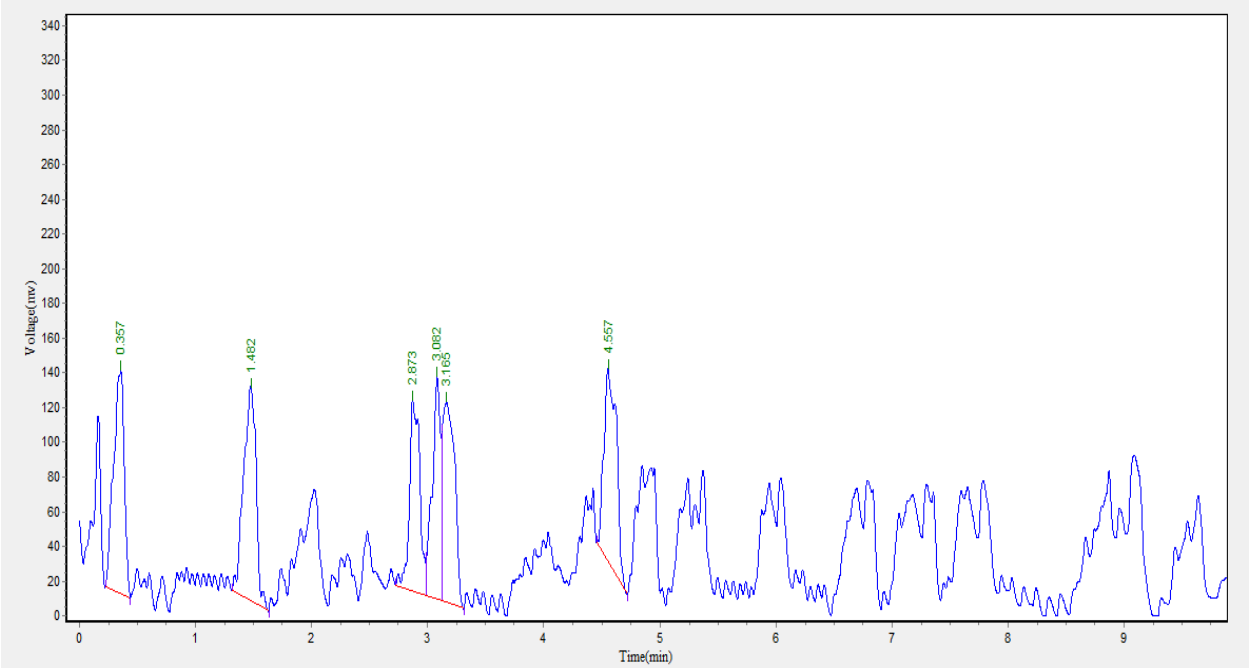

Figure 9 Hexane Extract Chromatogram of Moringa Oleifera

Conclusion –

Moringa oleifera bark is a promising aliment healer which have many therapeutic values. In the recent years a lot of work has been reported but more standardization needs to be perform. The data generated from this research is helpful for validation and novel formulation too. Because of moringa's medicinal and nutritional importance, popularity for moringa and its value-added products has so far been increasing, necessitating year-round supply. More research is required to develop the moringa. industry customising it to enhance livelihood, nutrition, and health. Much farther research into the action mechanism and constituents of the Moringa plant could provide unbelievable potential for the development of Herbal incorporated Pharmaceutical products.

References

1. Abarikwu, S. O., Benjamin, S., Ebah, S. G., Obilor, G., and Agbam, G. (2017). Protective effect of Moringa oleifera oil against HgCl2-induced hepatoand nephro-toxicity in rats. J. Basic Clin. Physiol. Pharmacol. 28, 337–345. doi: 10.1515/jbcpp-2016-0033
2. Agrawal, N. D., Nirala, S. K., Shukla, S., and Mathur, R. (2015). Co-administration of adjuvants along with Moringa oleifera attenuates beryllium-induced oxidative stress and histopathological alterations in rats. Pharm. Biol. 53, 1465–1473. doi: 10.3109/13880209.2014.986685
Akanni, E. O., Adedeji, A. L., Adedosu, O. T., Olaniran, O. I., and Oloke, J. K. (2014). Abd El Baky, H. H., and El-Baroty, G. S. (2013). Characterization of Egyptian Moringa peregrine seed oil and its bioactivities. Int. J. Manage. Sci. Bus. Res. 2, 98–108.
3. Chemopreventive and anti-leukemic effects of ethanol extracts of Moringa oleifera leaves on wistar rats bearing benzene induced leukemia. Curr. Pharm. Biotechnol. 15, 563–568. doi: 10.2174/1389201015666140717090755
4. Ahmad S, Sharma R, Mahajan S and Gupta A (2012). Antibacterial activity of Celtis australis by in vitro study. Int. J. Pharm. Pharm. Sci., 4(2): 629-631.
5. Rao PK, Rao DB, Ravi CK, Nadh MR, Madhavi Y and Rao TR (2011). In vitro antibacterial activity of Moringa oleifera against dental plaque bacteria. J. Pharmacol. Res., 4: 3
6. Ayyari, M., Salehi, P., Ebrahimi, S. N., Zimmermann, S., Portmann, L., Krauth-Siegel, R. L., et al. (2013). Antitrypanosomal isothiocyanate

and thiocarbamate glycosides from Moringa peregrina. Planta Med. 80, 86–89. doi: 10.1055/s-0033-1351102.

7. Chen, K. H., Chen, Y. J., Yang, C. H., Liu, K. W., Chang, J. L., Pan, S. F., et al. (2012). Attenuation of the extract from Moringa oleifera on monocrotaline-induced pulmonary hypertension in rats. Chin. J. Physiol. 55, 22–30.
8. Awodele, O., Oreagba, I. A., Odoma, S., Texeira Da Silva, J. A., and Osunkalu, V. O. (2012). Toxicological evaluation of the aqueous leaf extract of Moringa oleifera Lam. J. Ethnopharmacol. 139, 330–336. doi: 10.1016/j.jep.2011.10.008.
9. Dewangan G, Koley KM, Vadlamudi VP, Mishra A, Poddar A and Hirpurkar SD (2010). Antibacterial activity of Moringa oleifera (drumstick) root bark. J. Chem. Pharm. Res., 2(6): 424-428.
10. Tende JA, Ezekiel I, Dikko AAU (2011). Effect of ethanolic Moringa oleifera on blood glucose levels of streptozocin-induced diabetics and normoglycemic Wistar rats. British J Pharmacol Toxicol, 2, 1-4.
11. De-Heer NEA. Formulation and sensory evaluation of herb tea from Moringa oleifera, Hibiscus sabdariffa and Cymbopogon citratus. M.Sc. Thesis (Ghana: Kwame Nkrumah University of Science and Technology), 2011.
12. Thurber MD, Fahey JW. Adoption of Moringa oleifera to combat under-nutrition viewed through the lens of the Diffusion of Innovations theory. Ecology of food and nutrition. 2009; 48(3):212-225.
13. Khan FW, Gul P, Malik MN. Chemical composition of oil from Moringa oleifera. Pakistan Journal of Forestry. 1975; 25(2):100-102.
14. Gbeassor M, Kedjagni AY, Koumaglo K, De Souza C, Agbo K, Aklikokou K, et al. In vitro antimalarial activity of six medicinal plants. Phytotherapy Research. 1990; 4(3):115-117.
15. Nautiyal BP, Venhataraman KG. Moringa (drumstick)- an ideal tree for social forestry: growing conditions and Uses-Part I. Myforest. 1987; 23(1):53-58.
16. Anwar A, Ruffenach G, Mahajan A, Eghbali M, Umar S. 2016. Novel biomarkers for pulmonary arterial hypertension. Respiratory research 17(1), p 88.
17. Kou X, Li B, Olayanju B, Drake JM, Chen N. 2018. Nutraceutical or pharmacological potential of Moringa oleifera Lam. Nutrients 10(3), 343.
18. Ramachandran C, Peter KVG, Opalakrishnan PK. Drumstick (Moringa oleifera a multipurpose

Indian vegetable. Econ. Bot. 1980; 34(3):276-283.

19. Miltonprabu S, Tomczyk M, Skalicka-Woźniak K, Rastrelli L, Daglia M, Nabavi SF, Nabavi SM. 2017. Hepatoprotective effect of quercetin: From chemistry to medicine. Food and Chemical Toxicology 108, 365-374.
20. Helmy SA, Morsy NF, Elaby SM, Ghaly MA. 2017. Hypolipidemic effect of Moringa oleifera Lam leaf powder and its extract in diet-induced hypercholesterolemic rats. Journal of medicinal Food 20(8), 755-762.
21. . Mahatab SN, Ali A, Asaduzzaman AHM. Nutritional potential of sajna leaves in goats. Livestock Advisor. 1987; 12(12):9-12.
22. Jaja-Chimedza A, Graf BL, Simmler C, Kim Y, Kuhn P, Pauli GF, Raskin I. 2017. Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (Moringa oleifera) seed extract. PloS one 12(8), 14.
23. Sadek KM, Abouzed TK, Abouelkhair R, Nasr S. 2017. The chemo-prophylactic efficacy of an ethanol Moringa oleifera leaf extract against hepatocellular carcinoma in rats. Pharmaceutical Biology 55(1), 1458-66.
24. Xiong B, Piechowicz B, Wang Z, Marinaro R, Clement E, Carlin T, Velegol SB. 2018. Moringa oleifera f-sand filters for sustainable water purification. Environmental Science & Technology Letters 5(1), 38-42.
25. Tiloke A, Phulukdaree AA. 2016. Chuturgoon The antiproliferative effect of Moringa oleifera crude aqueous leaf extract on human esophageal cancer cells Journal of Medical Food 19, 398-403.
26. Nambiar S, Guin P, Parnami S, Daniel M. Impact of antioxidants from drumstick leaves on the lipid profile of hyperlipidemics. Journal of Herb Medical Toxicology. 2010; 4:165-172.
27. Gupta, K., & Shrivastava, P. (2022). Development and validation of UV spectrophotometric method for trimethoprim in pure and marketed formulation. International Journal of Health Sciences, 6(S2), 10829²10839. https://doi.org/10.53730/ijhs.v6nS2.7860.
28. Khushboo Gupta*, Pranjul Shrivastava. (2022). MODERN SOPHISTICATED INSTRUMENTS USED IN PHARMACEUTICAL SCIENCE FOR DRUG DISCOVERY. Journal of East China University of Science and Technology, 65(2), 292–302. Retrieved from http://hdlgdxxb.info/index.php/JE_CUST/article/view/69.

29. Awanish P, Rishabh DP, Poonam TP, Gupta JH, Saumya BA. Moringa oleifera Lam. (Sahijan)—a plant with a plethora of diverse. Therapeutic benefits: an updated retrospection. Med Arom Plants. 2012;1:1–8.
30. aini RK, Shetty NP, Prakash M, Giridhar P. Effect of dehydration methods on retention of carotenoids, tocopherols, ascorbic acid and antioxidant activity in Moringa oleifera leaves and preparation of a RTE product. J Food Sci Technol. 2014;51:2176–82.
31. Bhattacharya A, Ghosh G, Agrawal D, Sahu PK, Kumar S, Mishra SS. GC-MS profiling of ethanolic extract of Moringa oleifera leaf. Int J Pharm Bio Sci. 2014;5:263–75.
32. Kanchan PU, Vinod DR, Vijay BM. Antimigraine activity study of Moringa oleifera leaf juice. Int J Green Pharm. 2012;6:204–7.
33. Bhattacharya A, Agrawal D, Sahu PK, Swain TR, Kumar S, Mishra SS. Anti-inflammatory effect of ethanolic extract of Moringa oleifera leaves on albino rats. RJPBCS. 2014;5:540–4.
34. Kumar S. Medicinal importance of Moringa oleifera: drumstick plant. Indian J Sci Res. 2017;16:129–32.