Phytochemical analysis and detection of some bioactive compound in *moringa oleifera* extract.

Ayah F. Al-Qrimli (ayah.hrhr@yahoo.com)1* ,Yousif Hussein messer 2*, Mohammed Nasser Hussein 2*,Zahraa Abass Taghi 2*, Zahraa, Mohammed Jaber 2* , Mohammed Hussein 2*

1 Department of Pharmacognosy, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.
2 Students of Pharmacy College, Al-Nahrain University, Baghdad, Iraq.

ABSTRACT

Moringa oleifera , is a natural planted species of the genus Moringa of the Moringaceae family. The cultivation of Moringa is widespread due to multiple medicinal and healing properties. It is one of the wealthiest plant sources of Vitamins A, E, B , C, D, K , in addition to proteins and minerals . Research reported that moringa can support a healthy cardiovascular system, boost normal blood-glucose levels, neutralize free radicals, provide outstanding support of the body's anti-inflammatory mechanisms, enhance anemic blood and support immune system. Moringa oleifera contains various phytoconstituents which contribute to its therapeutic uses such as alkaloids, saponins, tannins, steroids, phenolic acids, glycosides , flavonoids , and terpenoids. This study was able to identify the different phytochemical compounds present in moringa cultivated in Iraq using preliminary chemical tests and to identify the presence of Quercetin using high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) . The chromatographic procedures proved the presence of Quercetin by showing identical retention time and retention factor almost identical to that produced by quercetin standard.

INTRODUCTION

Moringa oleifera from the Moringaceae family. This family contains 13 species, distributed in several tropical areas like, Madagascar, India, Ethiopia, and Kenya. *Moringa oleifera* is the most widely cultivated genus, commonly known as “drumstick tree” or the “horseradish tree”. Moringa became popular due to its various therapeutic uses as it has been used since ancient times. Moringa has been known as one of the most economically valued harvests, due to industrial, agricultural, and medicinal uses [1]. It is one of the richest plant sources of Vitamins A, B, C, D, E and K. In addition, it is a good source of proteins and minerals. Moringa leaves are considered a storehouse of nutrients, rich in minerals such as copper, potassium, iron, magnesium, zinc, and calcium. In addition, they contain vitamins for instance beta-carotene, vitamin A, vitamin B, folic acid, nicotinic acid, pyridoxine, vitamin E, Vitamin D plus Vitamin C [1,2,3]. Moringa species contain numerous phytoconstituents, for example alkaloids, saponins, steroids, tannins, phenolic acids, flavonoids, glucosinolates, and terpenes. The multiplicity of these phytochemicals in the genus contributes to its several pharmacological uses [4]. Different types of active phytoconstituents like glycosides, fixed oil and fats are present [3,4]. Some other constituents are niazinin A, niazinin B and niazimicin A, niaziminin B which are antihypertensive agents and one of the latest natural thiocarbamates [5,6]. Many M. Oleifera plant parts gained therapeutic significance, the most important are the leaves and pods, however, all parts are used in most countries to relieve mineral and vitamin deficiencies, support cardiovascular health, blood sugar levels, deactivate free radicals, boost the anti-inflammatory pathways of the body, and enhance immunity [7,8]. The medicinal uses are numerous as it has been used to treat several illnesses, like skin infections, asthma, swelling, anemia, bronchitis, diarrhea, headache, joint tenderness, rheumatism, gout, cardiac problems, digestive disorders, diabetes [9,10]. In rural areas M. oleifera was found to remove water impurities, the seeds particularly had the ability to remove bacteria from

water [11]. The anti-inflammatory impact is attributable to flavonoids, alkaloids, tannins, glycosides and quercetin, which inhibits pro-inflammatory factors, for instance tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and others. This makes it beneficial in diseases such as diabetes, cancer, colitis, arthritis, which are linked with inflammation [12]. Polyphenols, flavonoids and ascorbic acid found in leaves are constituents that contribute to the anti-cancer effect which may protect the body from cancer, moreover glycosylated isothiocyanate, benzyl carbamate niazimycin and ß-sitosterol, which have anti-tumor properties [6,12,13]. These compounds are found in high concentrations in the leaves and seeds of the plant. The cardio protective effect results from The phytochemical compounds identified in Moringa oleifera like N, α-L rhamnopyranosyl vincosamide, isoquercetin, quercetin, and isothiocyanate, it is work as a defensive agent to cardiac mutilation and vascular dysfunction [14]. M. oleifera is rich in flavonoids, polyphenolic compounds and multiple antioxidants that scavenge free radicals that cause oxidative stress, cell damage and inflammation. which grants it a high antioxidant power. Studies showed that M. oleifera lenes and seeds had antimicrobial activities against Staphylococcus aureus, Bacillus cereus;, Klebsiella pneumonia, Escherichia coli; Enterococcus cloacae; Pseudomonas aeruginosa Salmonella typhi and Proteus vulgaris[15,16]. Interestingly, the application of moringa oil on skin helps tighten it, absorb oils and dirt, kill bacteria or viruses on the skin, and treat acne and athlete's foot. It was found that due to moringa leaves containing high levels of vitamins and antioxidants, that M. oleifera can improve brain function and produce hepatoprotective effects thus, can be beneficial in Alzheimer's disease and restoring normal liver function, respectively [17, 18]. Extensive research regarding M. oleifera medicinal uses has led to further widespread studies to identify its phytoconstituents and to produce in-vitro results that makes M. oleifera a promising area of research.

Aim of the study

To phytochemically analyze *moringa oleifera* grown in Iraqi environment and detection of some active compounds of the plant

Material and methods

The *Moringa oleifera* plant was collected from AL-Nahrain university college of pharmacy from pharmacognosy garden, 300 gm plant part collected was the leaves. The leaves were air dried under the shade for one week. The plant material was extracted using 95% aqueous ethanol by maceration which is a cold method.

General phytochemical screening was done by performing chemical tests on the crude extract to detect chemical compounds and phytochemicals in the crude extract. This chemical profiling of the crude extract facilitates the identification step.

Test for alkaloids detection:

Two milliliters of methanolic extract were put in an appropriate test container then scarce drops of Dragendorff and Mayer's reagents were added. Then test tube was agitated well, to allow disposition of a creamy-white and orange precipitate for Mayer's and Dragendorff test in that order.[19]

Test for flavonoids:

One milliliter of methanolic solution in a test tube was treated with few drops of dilute sodium hydroxide solution. A deep yellow color in the test tube. This is then followed with few 0.5 milliliters of dilute HCl, the yellow color disappears. This confirms the existence of flavonoids.

Test for saponins detection:

Foam Check, where one milliliter of extract is shaken with 20 ml distilled water in a cylinder. Creation of lather that stays for fifteen minutes implies the existence of saponins.

Test for phenolic compounds detection:

(a) Ferric chloride test

2ml of extract treated with 2ml of 5% ferric chloride solution. Development of blue or green color indicates the occurrence of phenolic compounds (tannins)

(b) Lead acetate test

Two milliliters of extract were placed in a test container where some drops of lead acetate solution were added. Founding of white precipitant suggests the occurrence of phenolics (tannins)

Test of anthraquinone detection:

The Borntrager's Test is applied where three milliliters of extract , diluted HCI solution were placed into a separatory funnel then equal volume of benzene is added. After good shaking, the organic layer was taken. Dilute ammonia solution was then added. A pinkish color denoted the occurrence of anthraquinone glycosides.

Test for terpenoids detection:

Salkowski test were five milliliters of the extract was merged with two milliliters of chloroform then 3 ml of concentrated H2O4 was added. Reddish-brown color formation indicates the presence of terpenoids.

Flavonoids identification and detection

Thin layer chromatography was used to analyze and identify flavonoids in the alcoholic extract by separation on thin analytical silica plates. The Stationary phase used is readymade silica gel plates GF254 nm of 0.25mm thickness (MERCK) were employed as the adsorbing stationary phase. Where a baseline and solvent front were designated for sample application. The mobile phase solvent system used for development is Ethyl acetate: formic acid: acetic acid: water (100:11:11:26). A few milligrams of sample and quercetin standard compound were dispersed in few drops of concentrated ethanol, then applied on the line of TLC sheets using a capillary tube and let to air dry. Recognition on TLC plates was achieved by inspection under UV light utilizing 254 nm wavelength. Chemical detection was done by spraying the plate with alcoholic KOH reagent to detect flavonoids in the sample. The Rf value (retention factor) was
calculated for the compound spot and for the corresponding standard spot using the equation below. The Rf value calculated for each compound is then used for comparison:

$$\boldsymbol{Rf\ value}=distance\ traveled\ by\ compound/\ distance\ traveled\ by\ the\ solvent\ system$$

The sample was further analyzed by HPLC with the same quercetin standard compound used in TLC. HPLC is an extremely sensitive and accurate technique which can detect even trace amounts of compounds present in the extract. The retention time (Rt) in minutes in which each compound in sample appears in the HPLC chromatogram is then compared to that of standard.
Conditions used in analysis by HPLC: [20,21]

- Mobile phase: 40% methanol 30% 0.1% H3PO4
- Particle size: 5µm
- Column: ODS C18 (150 x 4.6 Id mm)
- Flow rate: 1 ml/ min.
- Column temperature: room temp.
- Injection volume: 20 µl.
- Injection concentration: 10 ppm of quercetin standard.
- Detection wavelength: UV detector at λ 254 nm.

Results

Phyto-chemical screening tests were performed on the crude extracts; this step gives a general idea about the Phyto-chemical compound classes present in the crude extract. The results of these tests are illustrated in Table1.

Table 1:- Phytochemical screening profile of ethanolic crude extract of *moringa oleifera* cultivated in Iraq.

Test	Result
1. Alkaloid (Dragendroff/May er)	+
2. Cardiac glycosides	-
3. Flavonoids	+
4. Phenols	+
5. Anthraquinone	-
6. Saponins	-
7. Terpenoids (Salkowski)	-

Examination and separation

Thin layer chromatography (TLC) Using ethyl acetate: formic acid: acetic acid: water (100:11:11:26) solvent system, the extract showed spots that have almost identical Rf (0.6) values as that of standard quercetin compound used. The figures show the chromatograms detected under UV and visualized by alcoholic KOH.

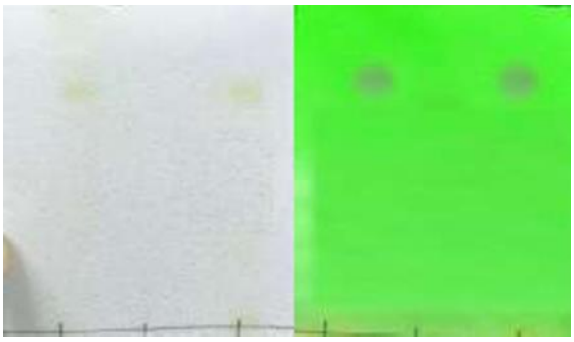

Figure 1 :- TLC chromatogram of moringa olifera extract with quercetin standard detected under A: alcoholic KOH spray and B: UV-254 nm

High performance liquid chromatography (HPLC)

The phytochemical screening and TLC appeared to contain quercetin flavonoid thus, this fraction was further analyzed by HPLC to confirm the TLC results. The data confirms that HPLC was able to detect quercetin in the extract since it appeared at retention time (3.698 min) almost identical to those by the sample (3.645 min) as seen in the Figures. This proves that HPLC is more efficient, sensitive, and accurate method. This makes moringa olifera a good candidate for preparative isolation of phenolic compounds and for evaluating its pharmacological activity

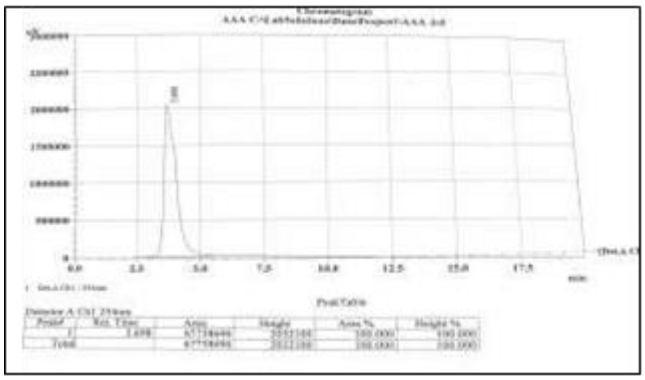

Figure 3 :- HPLC chromatogram of standard compounds

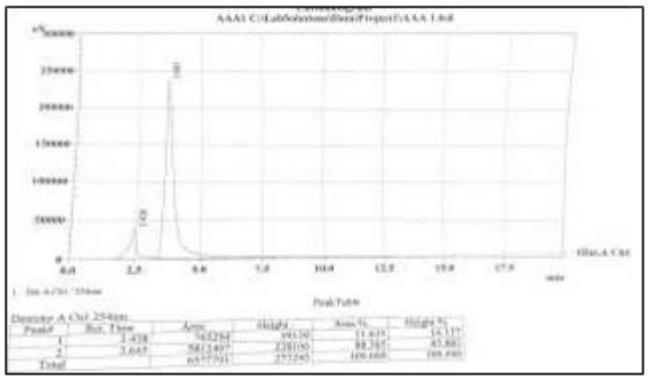

Figure 4 :- HPLC chromatogram of ethyl acetate fraction.

Discussion

The phytochemical analysis of moringa oleifera revealed the presence of various bioactive compounds, including alkaloids, cardiac glycosides , flavonoids , anthraquinone , terpenoids , saponin and phenols .

Through our work, we seek to collect a sufficient quantity of moringa oleifera, and we extracted it in order to reveal the bioactive ingredients contained in moringa oleifera grown in Iraqi environment , which are the reason for Moringa's many uses.

During the process of discovering the active substances present in Moringa oleifera, we demonstrated the presence of flavonoids, which contribute to the use of Moringa oleifera as an anti-oxidant agent (by reducing the production of free radicals).
The detection of quercetin, a flavonoid with potent antioxidant activity, further supports the medicinal potential of moringa oleifera. Also We have proven the presence of alkaloids in Moringa oleifera , which is responsible for the use of moringa as an antibacterial, analgesic, and anti- inflammatory drug . The presence of phenols, specifically tannins, indicates astringent properties and potential benefits in wound healing and gastrointestinal health. Tannins are also known for their antioxidant and antimicrobial activities, which contribute to the overall therapeutic potential of Moringa moringa oleifera.
The results obtained from TLC and HPLC analysis confirm the presence of quercetin in the extract, validating the initial findings from the phytochemical screening. HPLC, being a more sensitive and accurate method, provides further evidence of the presence of quercetin in moringa oleifera extract. This compound, along with other identified phytochemicals, underscores the medicinal significance of moringa oleifera in traditional and modern medicine. The phytochemical analysis for the presence of Terpenoids, Saponins, cardiac glycosides and Anthraquinone give a negative result , so moringa oleifera grown in Iraqi environment specifically in al- nahrain university at pharmacognosy garden of college of pharmacy lack these phytochemicals and pharmacological uses of them. The absence of these phytochemicals in moringa grown in Iraqi environment compared to other countries or environment may be due to several reasons like : change in soil , change in climate , change in the extraction process or change in place where extraction done .

Conclusion

Phytochemical analysis of moringa oleifera extract indicate the presence of phenolic compounds , flavonoids which is quercetin and alkaloids , these phytochemicals composition of Moringa oleifera grown in the Iraqi environment contribute to its potential pharmacological uses, so moringa oleifera is an important medicinal plant and have numerous pharmacological uses , and according to these results we have achieve our objective of study

Future work

We discovered in our research the phytochemical constituents of moringa oleifera and pharmacological uses of them , but further in vitro studies of the plant is an important future work of our research

Acknowledgments

We would like to express our sincere gratitude, grateful and truthful thanks to our academic supervisor Dr . Ayah Al-Qrimli for her continuous support and valuable comments during the planning, designing and implementation of this study.

References

1- Mallenakuppe R, Homabalegowda H, Gouri MD, Basavaraju PS, Chandrashekharaiah UB. History, taxonomy and propagation of Moringa oleifera–a review. crops.
2015;3(3.28):3-15.
2- Meireles D, Gomes J, Lopes L, Hinzmann M, Machado J. A review of properties, nutritional and pharmaceutical applications of Moringa oleifera: integrative approach on conventional and traditional Asian medicine. Advances in Traditional Medicine. 2020 Dec;20(4):495-515.
3- Paikra BK, Gidwani B. Phytochemistry and pharmacology of Moringa oleifera Lam. Journal of pharmacopuncture. 2017 Sep;20(3):194.
4- Bhattacharya A, Tiwari P, Sahu PK, Kumar S. A review of the phytochemical and pharmacological characteristics of Moringa oleifera. Journal of Pharmacy and Bioallied Sciences. 2018 Oct 1;10(4):181-91.
5- Atolani A, Oe O, Priyanka B, Osin O, Preissner R, Aa N. Isolation, characterisation and in silico toxicity evaluations of thiocarbamates, isothiocyanates, nitrile, glucosinolate and lipids from Moringa oleifera Lam. seed. Journal of the Turkish Chemical Society Section A: Chemistry. 2020 Feb 2;7(1):233-42.
6- ShaheenáSiddiqui B. Isolation and structure elucidation of novel hypotensive agents, niazinin A, niazinin B, niazimicin and niaziminin A+ B from Moringa oleifera: the first naturally occurring thiocarbamates. Journal of the Chemical Society, Perkin Transactions 1. 1992(23):3237-41.

7- Alia F, Putri M, Anggraeni N, Syamsunarno MR. The potency of Moringa oleifera Lam. as protective agent in cardiac damage and vascular dysfunction. Frontiers in Pharmacology. 2022 Jan 24;12:724439.

8- Xiao X, Wang J, Meng C, Liang W, Wang T, Zhou B, Wang Y, Luo X, Gao L, Zhang L. Moringa oleifera Lam and its therapeutic effects in immune disorders. Frontiers in Pharmacology. 2020 Dec 17;11:566783.

9- Kamran M, Hussain S, Abid MA, Syed SK, Suleman M, Riaz M, Iqbal M, Mahmood S, Saba I, Qadir R. Phytochemical composition of moringa oleifera its nutritional and pharmacological importance. Postepy Biologii Komorki. 2020 Jan 1;47(3):321-34.

10- Meireles D, Gomes J, Lopes L, Hinzmann M, Machado J. A review of properties, nutritional and pharmaceutical applications of Moringa oleifera: integrative approach on conventional and traditional Asian medicine. Advances in Traditional Medicine. 2020 Dec;20(4):495-515.

11- Abiyu A, Yan D, Girma A, Song X, Wang H. Wastewater treatment potential of Moringa stenopetala over Moringa olifera as a natural coagulant, antimicrobial agent and heavy metal removals. Cogent Environmental Science. 2018 Jan 1;4(1):1433507.

12- Cuellar-Núñez ML, De Mejia EG, Loarca-Piña G. Moringa oleifera leaves alleviated inflammation through downregulation of IL-2, IL-6, and TNF-α in a colitis-associated colorectal cancer model. Food Research International. 2021 Jun 1;144:110318.

13- Araújo LC, Aguiar JS, Napoleão TH, Mota FV, Barros AL, Moura MC, Coriolano MC, Coelho LC, Silva TG, Paiva PM. Evaluation of cytotoxic and anti-inflammatory activities of extracts and lectins from Moringa oleifera seeds. PloS one. 2013 Dec 9;8(12):e81973.

14- Alia F, Putri M, Anggraeni N, Syamsunarno MR. The potency of Moringa oleifera Lam. as protective agent in cardiac damage and vascular dysfunction. Frontiers in Pharmacology. 2022 Jan 24;12:724439.

15- Abalaka ME, Daniyan SY, Oyeleke SB, Adeyemo SO. The antibacterial evaluation of Moringa oleifera leaf extracts on selected bacterial pathogens. Journal of Microbiology research. 2012;2(2):1-4.

16- van den Berg J, Kuipers S. The antibacterial action of Moringa oleifera: A systematic review. South African Journal of Botany. 2022 Dec 1;151:224-33.

17- Toppo R, Roy BK, Gora RH, Baxla SL, Kumar P. Hepatoprotective activity of Moringa oleifera against cadmium toxicity in rats. Veterinary world. 2015 Apr;8(4):537.

18- Ganguly R, Hazra R, Ray K, Guha D. Effect of Moringa oleifera in experimental model of Alzheimer's disease: Role of antioxidants. Annals of Neurosciences. 2010 Mar 22;12(3):33-6.

19-Gupta P, Jharia V. Preliminary Standardization, Extraction and Toxicity study of Moringa Oleifera Lam. Stem Bark. Current Research in Pharmaceutical Sciences. 2014 Sep 30:77-83.

20-Abo El-Fadl S, Osman A, Al-Zohairy AM, Dahab AA, Abo El Kheir ZA. Assessment of total phenolic, flavonoid content, antioxidant potential and hplc profile of three moringa species leaf extracts. Scientific Journal of Flowers and Ornamental Plants. 2020 Mar 1;7(1):53-70.

21-Ramesh CJ. Antimicrobial, phytochemical and quantitative HPLC analysis of Moringa oleifera root. Innoriginal: International Journal of Sciences. 2018 Oct 17:25-8.