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Original Article

Physicochemical, Phytochemical, Heavy Metal and Microbiological Analysis of *Moringa oleifera* Lam. Leaves

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ABSTRACT

Background: *Moringa oleifera* leaves is one of the highly patronized herbs on the Ghanaian market. It is used for the treatment and prevention of several diseases. It is imperative that available and effective methods can be utilized to assess the quality of samples before being consumed. **Objective:** A study of the leaves was conducted to ascertain its physicochemical, phytochemical, heavy metal and microbiological content to develop a simple but acceptable criteria which could be useful in ensuring the quality of this crude drug. **Materials and Methods:** The qualitative and quantitative morphological features, physicochemical, phytochemical, microbial load and flouresent features of the leaves of *M. oleifera* were evaluated. **Results:** *M. oleifera* leaves were found to be glaborous with opposite leaflet, leaflet tripinnate, with a micronate apex and possessing an entire margin. Microscopy showed vein islets, rosette calcium oxalate crystals, polygonal epidermal cells and unicellular trichomes. The 50 % ethanol soluble extractives of *M. oleifera* were highest, followed by the water and petroleum ether. Tannins, alkaloids, gylcosides, phenols, flavonoids, phenols, gums, and mucilage were present. **Conclusion:** The documented pharmacognostic features may be used as part of daily protocols to correctly identify and determine the quality of the the crude plant. The preliminary phytochemical, heavy metal and microbiological limits can be further used to ascertain the quality of raw materials of *M. oleifera* before they are used.

Key Words: Physicochemical, Pharmacognostic, Fluorescence, Heavy metal, Microbiological.

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INTRODUCTION

Plants continue to serve as valuable therapeutic agents, and are an integral part of both modern and traditional systems of medicine. The patronage of natural therapies in the management and treatment of diseases have been on the rise. This can be attributed to the massive contribution made by herbal medicines towards providing effective therapeutic outcomes. Poverty and limited access to modern medicine have caused a high percentage of the world's population, especially those living in developing countries to use plant medicine as their primary source of health care.[1] Evidently, most of the traditional systems of medicines are effective, but lack standardization or quality control parameters for evaluating the herbal medicines.[2] The safety and efficacy of herbal materials largely require monitoring the quality of the product from the collection, through processing, to the finished product. Standardization and quality control of herbal medicine involves physico-chemical evaluation of crude drugs whereby attention is usually given to indices such as authentication, foreign matter content, organoleptic evaluation, diagnostic tissue features present in drug powder, ash values, extractive values, moisture content determination, chromatographic and spectroscopic evaluation, determination of pesticide residues, microbial contamination, aflatoxins and radioactive contamination.[3] As such, the need for standardization is essential, given the global acceptance of herbal products as remedies for various ailments.

Moringa oleifera Lam. of the family Moringaceae is commonly known as horse radish or drumstick tree and ranges in height from 5 to 10 m. It is found mostly in Africa, India, Arabia, Southeast Asia, the Pacific and Caribbean Islands.[4-6] This plant is among the most widely patronized herbal medicines on the Ghanaian market.[7] The leaves are small and oval to ovate, with leaflets averaging 1 - 2 cm in length and 5 -10 cm in width. All parts of the plant, bark, fruit, leaves, nuts, seeds, tubers, roots and flowers have rich folkloric usage against a variety of ailments such as fever, abscess, wound, infections, diabetes, asthma, urinary tract infection, gastric ulcer, several metabolic diseases, including hypercholesterolemia, high blood pressure, non-alcoholic liver disease, cancer and inflammation Pharmacological studies have also demonstrated the ability of this plant to have corresponding biological activities which substantiate its use in the afore mentioned disease states.[7-10] Additionally the moringa is known to have analgesic, antipyretic, anticancer, hepatoprotective, antioxidant, antiepileptic, nootropic, anti-allergic, local anesthetic, anthelmintic, antimicrobial, wound healing, immunomodulatory, and antidiarrheal properties.[5,10,11] The genus Moringa consists of 13 plant species. Moringa species are well-known for their antioxidant, anticancer, anti-inflammatory, and antidiabetic properties. Their biological activities have been attributed to the high content of flavonoids, glucosinolates, and glucosides.[9] Studies have been conducted on the nutritional value and bio-active constituents of these herbs.[12] *M. oleifera* is one of the richest plant sources of nutrients,

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including minerals, proteins and various vitamins such as A, B, C, D, E & K. The vital minerals present in *Moringa* include calcium, copper, iron, potassium and zinc.[6,11,13] Other active ingredients present in the leaves include; pterygospermin, spirochin, moringic acid, niazinin A and B, niazimicin, campesterol and saponins. *Moringa oleifera* leaves have been established to have a high consumption rate in Ghana. The sales of these herbs have been discovered to increase the income of some Ghanaian farmers resulting in its high demand.[5,14]

The quality parameters for plants established in other countries cannot always be directly transferred for evaluating the parameters of these herbs, due to variations in environmental conditions. The lack of criteria for quality control specifications of these plants could result in contamination or adulteration either through ignorance or fraud with other substances which can ultimately affect the final drug product.[15] Hence, the physicochemical, phytochemical, heavy metal and microbiological studies of the leaves of *M. oleifera* was carried out to develop acceptable characteristics which could be useful in ensuring the quality of these plants.

MATERIALS AND METHODS

Plant materials

The leaves of *M. oleifera* were harvested from the Botanical gardens of University of Ghana, on the 15th of October, 2018. They were identified and authenticated at the Department of Plant and Environmental Science, University of Ghana (UG), Legon and the herbarium specimen deposited at Department of Pharmacognosy and Herbal medicine, School of Pharmacy, UG, Legon. Fresh samples were kept in glycerine, and later used for the organoleptic and microscopic determinations. One kilogram leaves and sepals were air-dried for three (3) weeks, pulverised and stored in air tight containers.

Macroscopic evaluation

The organoleptic properties of this plant material were investigated by documenting both the morphological and sensory characteristics. The morphological parameters of interest include the size, nature, colour, shape, texture, odour and surface, apex, type, margin and petiole surface of the leaves, margin and shape of the plant samples. The sensory characteristic documented were colour, taste, appearance, and texture of the drug.

Microscopic evaluation

Qualitative and quantitative microscopic features were evaluated with the Leciad compound light microscope observed under x40 magnification. These features of the sample were evaluated using standard protocols of WHO Guidelines on Quality Control Methods for Herbal Materials, 2011.[3]

Physico-chemical analysis

The extractive values, total ash, water soluble ash, foreign organic matter, foaming index, swelling index, moisture content, and acid insoluble ash were determined on the dried powdered leaves by established protocols.[3,16]

Preliminary phytochemical screening

Test for saponins, tannins, alkaloids, gylcosides, anthraquinones, phenols, flavonoids, gums, and mucilage were the preliminary phytochemical screening tests conducted on both the leaves of *Moringa oleifera*.[17,18]

Fluorescence studies

Fluorescence analysis of the powdered leaves of *M. oleifera* was carried out to determine the characteristic colour that will be emitted in specific solvents. The solutions obtained were observed under visible day light, UV light of short wavelength (254 nm), and UV light of long wavelength (365 nm). The solvents used were distilled water, 95 % ethanol, chloroform, methanol, glacial acetic acid, sulphuric acid, nitric acid, 1N Hydrochloric acid, $FeCl_3$, and 1N NaOH. This protocol is widely used in pharmacognostic studies.[19,20]

Heavy metal analysis

Heavy metal analysis was performed following Frimpong-Mansah *et al*, 2016 described methods. [21] An Olympus Vanta M Portable ED-XRF (VMR) analyzer (USA) equipped with 4-Watt x-ray tube with application optimized anode material rhodium (Rh) and tungsten (W), 50kV x-ray tube and large area silicon drift detector was used to analyze the samples for heavy metals. The SARM 2711A, certified reference material and silica from the manufacturer was employed in the calibration of the XRF. The powdered sample of *M. oleifera* was passed through a stainless steel 850 µm mesh (no.20) to obtained powders of uniform particle size. Four grams of each original sample was measured using an electronic balance and mix with 0.9 grams Fluxana H Electronic BM-0002-1(Lincowax C micro powder PM-Hoechst wax) as a binder in the mortar. The pellets were placed individually under the HHED-XRF and the results recorded on a PC monitor. Measurements were done in triplicates.

Microbial evaluation

Determination of bacterial count

One (1) gram of the powdered leaves of *M. oleifera* was weighed into 100 mL of sterile water in a tube to form a stock concentration. After multiple serial dilutions of 1:99, one (1) mL of each content was taken and added to their corresponding petri dishes containing 15 mL of plate count agar at 45 ˚C. The dishes were inverted and incubated at 37 ˚C for 48 hours and the colonies formed were counted.[3] This experiment was performed in triplicates. Bacillus subtilis ATCC 6538-P was used as growth controls for bacteria in the nutrient agar.[3]

Determination of fungal count

One (1) gram of the powdered leaves of *M. oleifera* was weighed into 100 mL of sterile water in a tube to form a concentration. A stock concentration of 10 mg/mL of pure chloramphenicol was prepared in methanol. 0.7 mL of the stock solution was taken and added to 350 mL of the potato dextrose agar to make a concentration of 20 ug/mL. One (1) mL of each content of plant sample solution was taken and added to their corresponding petri dishes containing 15 mL of chloramphenicol potato dextrose agar at 45˚C. The petri dishes were inverted and incubated at room temperature for seven (7) days and the colonies were counted. *Candida albicans* ATCC 2091 was used as the fungal growth control for potato dextrose agar.[3] All experiments were performed in triplicates.

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). Analyses were carried out with GraphPad for Windows (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Examination on *M. oleifera* leaves as displayed in Figure 1, revealed details such as the nature, colour, shape, texture, odour and surface, apex, type, margin and petiole surface of the leaves. Deatails are provided in Table 1. The leaf of *M. oleifera* is a distinctively tripinnate compound with an entire margin.

The microscopic features of *M. oleifera* leaf section included the observation of vein islets, veinlet termination, secretory cells, unicellular trichomes and hexagonal epidermal as indicated in Figure 2.

Figure 1: Upper (**A**) and lower (**B**) surfaces of *M. oleifera* leaves.

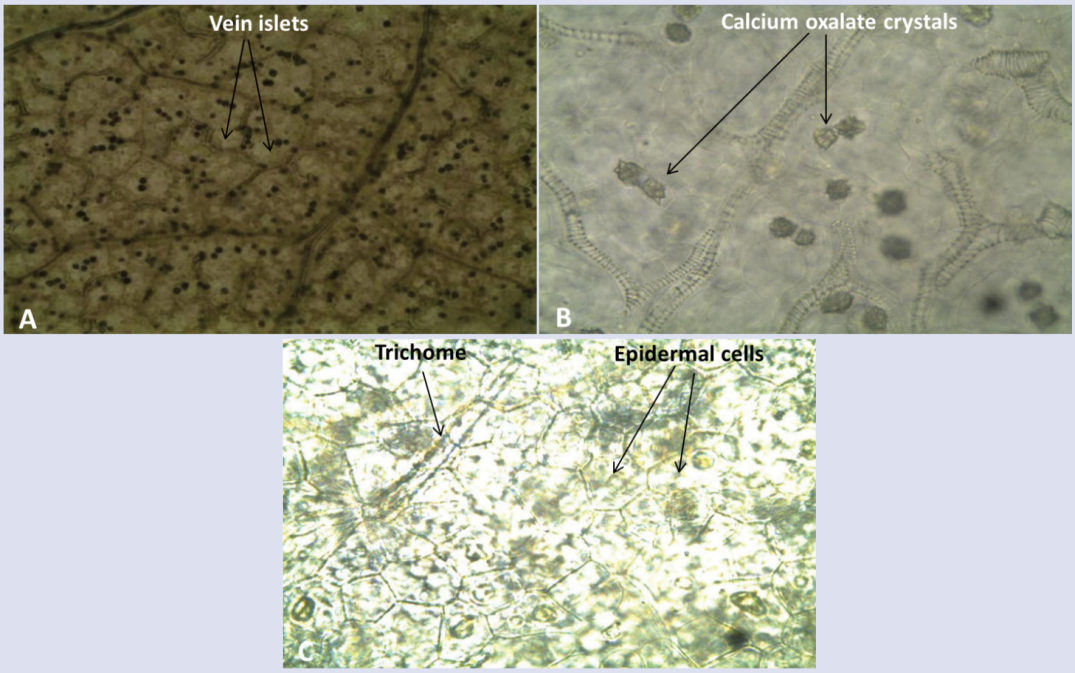

Figure 2: Surface characteristics of *M. oleifera* leaf section; (**A**) vein islets; (**B**) rosette calcium oxalate crystals; (**C**) polygonal epidermal cells and unicellular trichome.

Table 1: Macroscopic features of *M. oleifera* leaves.

Parameters	Description
Nature	Fresh
Colour	Green
Texture	Smooth
Surface	Glaborous
Odour	Bland
Leaflet arrangement	Opposite
Apex	Micronate
Type	Tripinnate compound leaf
Margin	Entire

The average 50 % ethanol soluble extractives of *M. oleifera* were higher than that of the water and petroleum ether (Table 2). The physicochemical parameters of the leaf extract of *M. oleifera* are shown in Table 3. The foaming index, which was more than 100, indicates the presence ofsaponins in *M. oleifera* leaves. *M. oleifera* leaves also gave a swelling index of 5, which may denote the presence of constituents such

Table 2: **Extractive values of *M. oleifera*.**

Parameters	M. oleifera leaves (%w/w)
Petroleum ether	15.33 ± 1.5
50 % ethanol	41.67± 2.0
Water	36.67± 1.8

Table 3: **Physico-chemical parameters of *M. oleifera*.**

Parameters	M. oleifera leaves (% w/w)
Total Ash	9.94 ± 1
Acid insoluble ash	5.72 ± 1
Water soluble ash	9.64 ± 1
Moisture content	14.6 ± 1
Foreign organic matter	Nil
Foaming index	200 ± 20
Swelling index	5 ± 1

Table 4: **Fluorescence of *M. oleifera* leaves in different reagents**

Powdered plant sample + reagent	Daylight	Short wavelength (ʎ 254 nm)	Long wavelength (ʎ 365 nm)
Distilled water	Light green	Brown	Green
1 N HCl	Light brown	Ash	Brown
1 N NaOH	Deep brown	Pink	Ash
10 N Sulphuric acid	Light green	Pink	Brown
Methanol	Deep green	Pink	Ash
Glacial acetic acid	Brownish green	Pink	Brown
Nitric acid	Orange	Ash	Green
Chloroform	Green	Pink	Ash
50 % Ferric chloride	Brown	Pink	Brown
95 % Ethanol	Light green	Pink	Ash

as gums and mucilage.[3] The different solvent extractives also showed characteristic UV fluorescence at 254 and 365 nm. *M. oleifera* fluoresces ash in methanol, chloroform and 1N NaOH.

Phytochemical analysis showed the presence of saponins, tannins, alkaloids, glycosides, phenols, flavonoids, gums, and mucilages.

For the presence of heavy metals, *M. oleifera* showed the absence of Arsenic (As), Lead (Pb), Mercury (Hg), Chromium (Cr) and cadmium at a concentration of 0.02 ± 0.003 ppm.

Bacterial and fungi count of the powdered sepals of *M. oleifera* were 10^7 and 10^4 CFU/ g respectively. The WHO standards (World Health Organization, 2007) states that, the maximum bacteria and fungi count should be 10^7 and 10^4 CFU/ g respectively. Therefore, the results were just within the limit.

DISCUSSION/CONCLUSION

Results from the physicochemical, phytochemical, heavy metal and microbiological analysis of *Moringa oleifera* leaves are all prerequisites to the quality assurance of this plant material. These parameters provide a simple means of detecting adulteration and substitution of this plant material. In so doing, they assure the safety and efficacy of this medicinal plant. Microscopic analysis, for example is important in the establishment of the identity and purity of particularly plant samples and serves as an initial screening test for impurities.[22] Authentic *M. oleifera* leaves should therefore display rosette calcium oxalate crystals, polygonal epidermal cells and unicellular trichomes. The tripinnate description of the leaves is similar to those already published in the literature.[5]

The 50 % ethanol had an average soluble extractive of *M. oleifera* of 42 %W/W, and this is higher than that of the water and petroleum ether soluble extractives. The extractive values indicate concentrations of extractable components present in the plant materials.[23] Extractive values can also be useful as a physicochemical marker for the detection of already exhausted plant materials and possibly adulterated ones.

The total ash value, averaged 10 %W/W, followed by the water and acid soluble values respectively. The values obtained in this study were considerably higher than those already published in the literature.[24,25] These differences are likely due to the differences in methods of extraction. The total ash indicates the amount of physiological ash, which is attributed to mineral components such as magnesium calcium, and potassium and non-physiological ash attributed to the presence of soil components. This value will aid in the identification of low-grade products and the establishment of the purity of the material at hand.[24,26]

The water soluble ash is attributed to water soluble components of the plant material and indicates plant materials that could have been previously extracted. The acid insoluble ash also gives an account of the inorganic components in the plant materials such as silica[27] or metals that may be present. These values will give an indication of the concentrations of various impurities that could affect the safety and efficacy of the plant material.

The presence of saponins, tannins, alkaloids, glycosides, phenols, and flavonoids as detected are already known to be present in this plant material in addition to terpenoids and glycosides.[27-29]

M. oleifera in different reagents displayed characteristic fluorescences under UV light. At 254 nm *M. oleifera* fluoresces pink in NaOH, sulphuric acid, methanol and glacial acetic acid, while it flouresces ash in NaOH and methanol at 365 nm. Results in ferric chloride, NaOH and sulphuric acid are slightly comparable to other published results.[25] The differences can be attributed to the differences in the methods used. These characteristic colours may serve as useful parameters for the identification and subsequent quality control of the powdered leaves

of *M. oleifera*. Flourescence is as a result of the presence of extended conjugated double bonds,[30,31] hence a change in this fluorescent behaviour could indicate the presence of adulteration or substandard material which has resulted in a change in chemical composition of the plant material.

Few studies have surveyed the microflora present in samples of *M. oleifera* on the Ghanaian market. Even in doing so, values need to be compared to standard samples. For the authentic plant sample, the aerobic bacteria count was an average of 10^7 CFU/g of dried plant material while the fungi count was 10^4 CFU/g. In comparison to WHO standards, the maximum aerobic bacteria and fungi count should be 10^7 and 10^4 CFU/g, respectively. Therefore, the results were within acceptable limits.[3] In conclusion, this study evaluated the morphological, physicochemical, phytochemical, heavy metal and microbiological parameters of *M. oleifera* leaves by available and straightforward methods. This is crucial for its standardization and to ensure the safe and it will ensure the safe and efficacious use of this medicinal plant.

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CONFLICTS OF INTEREST

None.

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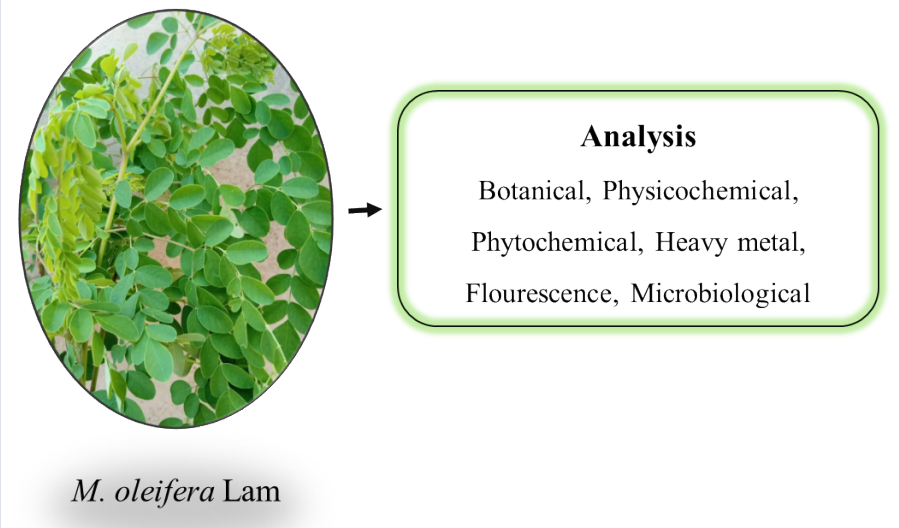
GRAPHICAL ABSTRACT



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