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Original Article

A Comparative Phytochemical Characterization of *Moringa oleifera* Plant Parts by Different Solvent Extraction

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ABSTRACT

Background: *M. oleifera* is an enriched plant with a variety of rich ingredients that play a very important part in the human diet. Thus, scientists have great interest in assessing the medicinal value of the plant to promote the preparation of new and advanced drugs. Hence, the preparation of plant extracts for experimental purposes is an initial step and key to achieving a quality research outcome. **Materials and Methods:** The primary objective of this study was to evaluate the screening of *M. oleifera* bioactive compounds using various solvents (methanol, ethanol, acetone, petroleum ether, chloroform, and water) in the extraction procedure and determine the quality and quantity of bioactive constituents. **Results:** The quantitative analyses showed that crude leaf, pod, and bark extracts revealed the presence of alkaloids, phenols, terpenoids, proteins, and carbohydrates in all extracts except for petroleum ether extract. Qualitative analysis of the detected phytochemicals reveals the highest concentrations were found in leaf extracts, where a high extraction yield was recorded in the aqueous extract. **Conclusion:** This study reveals that the presence or absence of particular phytochemicals is determined by the polarity of the solvents used for extraction. Also, the justification for *M. oleifera* to contain rich phytochemicals, including alkaloids, flavonoids, phenolics, terpenoids, tannins, and saponins, that are known to have pharmacological properties, was validated, and they can be explored for biological potential.

Keywords: *Moringa oleifera*, Phytochemical, Antioxidant, Traditional medicine, Extraction yield.

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INTRODUCTION

The identification of plant secondary metabolites and their potential role as natural medicine formulations in the prevention of chronic diseases have gained popularity among researchers in recent years. This is mainly due to the fact that medicinal plants, either as pure or as standardized extracts, have unique chemical diversity, which offers vast prospects for new drug discoveries.[1] The effectiveness of the plant is solely dependent on the presence of bioactive constituents known as phytochemicals, such as phenolics, flavonoids, terpenoids, and others. Based on the World Health Organization (WHO) Bulletin, almost 25% of the modern medicines that are being employed currently are plant derivatives. Some of the successful examples of drug development from natural products are anticancer drugs (Vinca alkaloids from *Catharanthus roseus* and paclitaxel from *Taxus baccata*).[2] In fact, some of the formulations using plant extracts have been reported to be safer medicines with fewer side effects compared to synthetic drugs.[3]

Moringa is a distinguished genus in the Moringaceae family. *Moringa* is the only flowering plant genus in the family Moringaceae. The name *Moringa* originates from the Tamil word murungai, which refers to *Moringa oleifera*. *M. oleifera* is the most widely cultivated species in northwestern India, the Himalayas, and other parts of Asia, as it grows quickly in many types of environments.[4] *M. oleifera* contains numerous phytochemicals, including alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes. The leaves, pods, and bark in particular are used in traditional medication to treat

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various ailments such as skin rashes, fever, wound healing, headaches, asthma, constipation, diabetes, back pains, and muscle and stomach disorders.[5] These phytochemicals are rich sources of antioxidants, which possess the ability to protect against free radical-induced oxidative cell damage that can lead to conditions such as Alzheimer's disease, cancer, and heart disease and are also linked with chronic inflammation.[6]

The extraction method is a crucial step in the analysis of medicinal plants because it separates the pharmacologically active chemical components of the plant from the inactive or inert components. During this step, the extraction yields are always affected by the solvent system used. Hence, the selection of a suitable solvent is vital, as the rate of plant bioactive compound solubility with different chemical structures and polarities depends on the selected solvent. Hence, choosing the right solvent for extraction can maximize the phytochemical yield of the investigated plant.[7] Stimulated by the previous reports on medicinal properties of *M. oleifera* and encouraged by the usage of *M. oleifera* in various folk medicine formulations, it has been contemplated to focus this study on the phytochemicals of *M. oleifera* plant parts (leaf, pods, and bark) extract using different solvent polarities. Following, the extracts were studied for their antioxidant properties for phytochemical profiling to identify the most effective solvent extract in order to predict the active plant part of *M. oleifera*.[8]

MATERIALS AND METHODS

Plant Material Collection and Authentication

The *Moringa oleifera* plant was collected from Tiruchengode, Namakkal District, Tamil Nadu, and kept at 4°C until further processing. The family and species of *M. oleifera* were authenticated by members of the Botanical Survey of India, Coimbatore.

Plant Crude Extract Preparation

The leaves, pods, and bark were washed, separated, and ground to a fine powder using a conventional grinder before being stored in airtight bottles. Roughly 6000 g of the powdered plant parts (leaves, pods, and bark) were macerated successively with different solvents (methanol, ethanol, acetone, petroleum ether, chloroform, and water) at a ratio of 1:10 (powder/solvent) for 42 hr at 75 rpm in an orbital shaker at 37°C. The extracts were concentrated to yield crude extracts using a rotary evaporator at 45°C. The extracts obtained were stored at -20°C until further use.

Determination of Phytochemical Components

Both quantitative and qualitative chemical tests were performed on different extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) of each plant part (leaves, pods, and bark) with standard methods described for various secondary metabolites.

Qualitative analysis

Test for Alkaloids

Mayer's test

Mayer's reagent (2-3 drops) was mixed with 2 mL of each plant part's (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water). The appearance of a green precipitate in the solution indicated the presence of alkaloids.[9]

Wagner's test

Wagner's reagent (2-3 drops) was mixed with 2 mL of each plant part's (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water). The appearance of brick-colored precipitate in the solution indicated the presence of alkaloids.[10]

Dargendroff's test

Dargendroff's reagent (2-3 drops) was mixed with 2 mL of each plant part's (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water). The appearance of a reddish-brown precipitate in the solution indicated the presence of alkaloids.[10]

Test for flavonoids

Alkaline test

An equal amount of 2.0% NaOH mixture was added to plant parts (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water), forming a yellow-colored mixture. Color changes to colorless upon addition of 2-3 drops of diluted acid, indicating the presence of flavonoids. The formation of a pink-to-crimson colored solution indicated the presence of flavonal glycosides.[11]

Shinod's test

Fragments of magnesium ribbon with concentrated Hydrochloric acid (HCl) (2-3 drops) were mixed with 5 mL of plant extracts (dissolved in alcohol). The formation of a pink to crimson-colored solution indicated the presence of flavone.[12]

Test for saponin

Lead acetate test

An equal amount of 1% lead acetate solution and plant part (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were mixed. The development of a white precipitate suggests the presence of saponins.[13]

Foam formation test

The plant parts (leaves, pods, and bark) and extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were

dissolved in 20 mL of distilled water in a measuring jar subjected to continuous agitation for at least 15 min. The formation of stable forms (1-2 cm) indicated the presence of saponins.[14]

Test for protein

Biuret test

The plant extracts (leaves, pods, and bark) (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were dissolved in 2 mL of water and filtered. Then, the filtrate was mixed with 1 drop of 2% copper sulfate solution, 1 mL of 95% ethanol and Potassium Hydroxide (KOH) pellets. A pink-colored appearance indicated the presence of protein.[15]

Millon's test

The plant extracts (leaves, pods, and bark) (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were dissolved in 2 mL of water and filtered. The 2 mL of filtrate were mixed with Millon's reagent (2-3 drops). A white precipitate in the solution indicated the presence of protein.[15]

Test for carbohydrate

Molish's test

An equal amount of alcoholic alpha-naphthol was used to treat the plant extracts (leaves, pods, and bark) (methanol, ethanol, acetone, petroleum ether, chloroform, and water). Following, 1 mL of conc. sulfuric acid (H_2SO_4) was slowly mixed along the sides of the test tube. The appearance of violet ring colorization at the junction of the test tube indicated the presence of carbohydrate.[16]

Test for tannins

Ferric chloride test

Filtered plant parts (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were used to mix with Ferric Chloride solution ($FeCl_3$). The coloration of blue, green, or blue-black indicated the presence of tannins.[16]

Test for Terpenoids

Libermann Burchart test

The plant part (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were dissolved in a few drops of acetic anhydride and concentrated Sulfuric acid (H_2SO_4) along the side of the tube. Color changes from blue to blood red in appearance indicated the presence of terpenoids.[17]

Test for phenols

Ferric chloride test

A few drops of Ferric Chloride ($FeCl_3$) solution (5%) were mixed with 2 mL of plant parts (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water). Colorization to a dark green or bluish black appearance indicated the presence of phenol.[18]

Glycoside's test

An equal amount of chloroform and acetic acid was added to plant extracts (leaves, pods, and bark) (methanol, ethanol, acetone, petroleum ether, chloroform, and water). The mixture was kept at 4°C for 1 hr, followed by the addition of concentrated Sulfuric acid (H_2SO_4). The color change from blue to green indicated the presence of glycosides.[18]

Phytosteroids test

An equal amount of chloroform and acetic acid was added to plant extracts (leaves, pods, and bark) (methanol, ethanol, acetone, petroleum ether, chloroform, and water) with a few drops of concentrated Sulfuric acid (H_2SO_4). The appearance of a bluish-brown ring indicates the presence of phytosteroids.[18]

Quantitative Analysis

Test for Alkaloids Content

The powdered form of plant parts (leaves, pods, and bark) was dissolved in 10% acetic acid (diluted in 20 mL of ethanol). The solution was incubated for 4 hr at room temperature. Following, the filtrate from the solution was collected and kept in a water bath to reduce its volume by ¼. A concentrated Ammonium Hydroxide (NH_4OH) was added to the reduced mixture for precipitation. The settled residue is the alkaloid, which was filtered, dried, and weighed.[18]

Test for Flavanoids Content

The powdered form of plant parts (leaves, pods, and bark) was dissolved in 80% methanol and left at room temperature for at least 2 hr. Then, the solution was filtrated using 42 No. Whatman filter paper. The resulting filtrate was subjected to complete evaporation and weighted for flavonol estimation.[18]

Test for Saponins Content

The powdered form of plant parts (leaves, pods, and bark) was macerated using 10% aqueous ethanol in a hot water bath (55°C) for 2 hr with continuous agitation. The formed extract was filtered and the maceration process was repeated again. The extracts from two filtrates were combined in a conical flask and heated over a water bath (90°C). The mixture was added to diethyl ether with constant agitation. The diethyl ether layer was discarded before adding n-butanol. The mixture was washed with 5% sodium

chloride and heated to dryness. The dried samples were weighted for saponin estimation.[19]

Test for Terpenoids Content

The plant part (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were diluted in alcohol and left for 24 hr. After incubation, the extract was filtered with petroleum ether. The ether extract was filtered again before estimating the total terpene content.

Test for Tannins Content

The plant part (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were dissolved in 50 mL of distilled water (dh20) with constant agitation for an hour. The resulting extract was filtrated into a test tube and mixed with Iron (III) Chloride ($FeCl_3$) in Hydrochloric acid (HCl) and potassium ferrocyanate ($K_4[Fe(CN)_6]\cdot 3H_2O$). The mixture was subjected to tannin content estimation using a spectrophotometer against the blank at 730 nm.[19]

Test for Phenolics Content

The phenolic content was determined using Folin-Ciocalteu's Reagent (FCR). The plant part (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were diluted in FCR (diluted 1:10 v/v), followed by the addition of Sodium Carbonate (Na_2CO_3) solution, and allowed to stand for 90 min at room temperature. The total phenolic content was measured using a spectrophotometer against the blank at 750 nm.[19]

Test for Nutritional Composition

The plant part (leaves, pods, and bark) extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) were tested for their nutritional components (proteins, carbohydrates, fats, fiber, and ash).[20]

Statistical Evaluation

The data was examined with SPSS Version 20.0. Results were determined as the mean±SE of three repeat determinations.

RESULTS

Qualitative Phytoconstituents of *M. oleifera* Plant Parts (leaves, pods and bark) Different Solvent Extract

M. oleifera leaf extracts

From the qualitative results outlined in Table 1, all the extracts of *M. oleifera* leaf indicated the presence of alkaloids, phenolics (flavonoids, lignin, tannins, phenols), terpenoids (terpinoids, sterols, glycosides, saponins), protein, and carbohydrate. In alkaloid determination, the Dragendroff and iodine tests varied among extracts. Alkaloids were absent when tested using the Dragendroff reagent in acetone, and in the iodine test, alkaloids were also absent in chloroform, petroleum ether, and water. The phenolic content was indicated by the presence of flavonoids, lignin, tannins, and phenols. Flavonoids were determined by alkaline, pews, and Shinoda test reagents. Petroleum ether extracts did not show the ability to extract flavonoids in the Pews and Shinoda tests. Lignin was determined by the Lignin and Labat Test reagents. The petroleum ether extracts did not show the ability to extract lignin in both tests. As for tannin content, the ferric chloride and gelatin test reagents showed positive results for all extracts except for petroleum ether extracts (Lignin test) and chloroform and petroleum ether extracts (Labat test). The presence of phenols was seen to vary among extracts. In the ferric chloride, elagic, and phenolic tests, phenols were absent in petroleum ether, chloroform, and methanolic extracts,

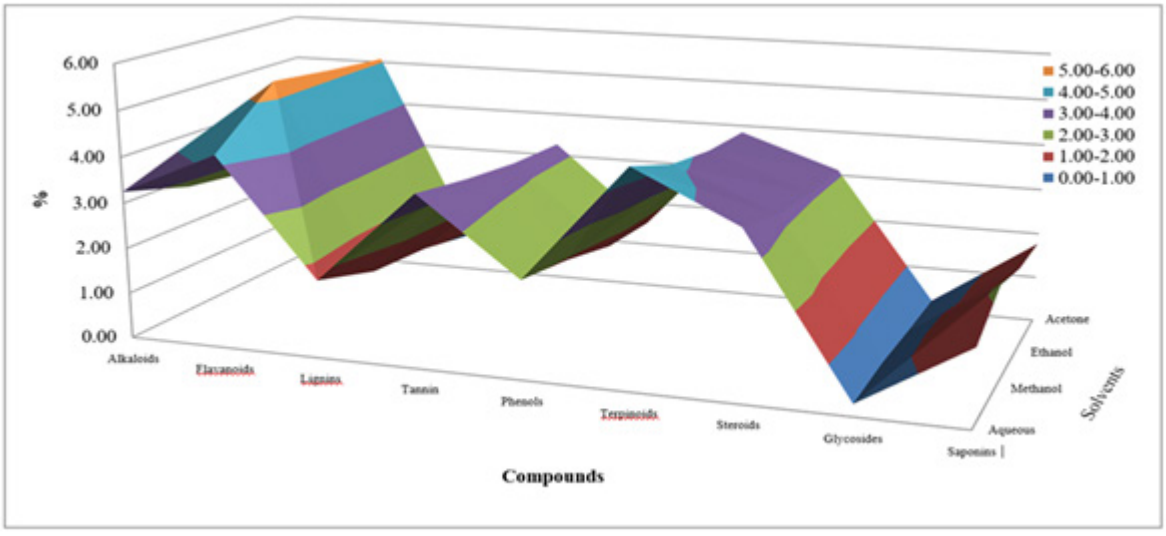

Figure 1: Secondary metabolites concentration in crude extract of *M. oleifera* leaf in different solvent.

Table 1: Phytochemical test of *M. oleifera* leaf crude extract with different solvents.

Sl. No.	Phytochemical test		Solvents					
			Chloroform	Methanol	Ethanol	Petroleum Ether	Acetone	Water
1	Alkaloids	Dragendroff	+	+	+	+	-	+
		Iodine	-	+	+	-	+	-
		Mayers	+	+	+	+	+	+
		Wagners	+	+	+	+	+	+
2	Flavonoids	Alkaline	+	+	+	+	+	+
		Pews	+	+	+	-	+	+
		Shinoda	+	+	+	-	+	+
3	Lignin	Lignin	+	+	+	-	+	+
		Labat Test	-	+	+	-	+	+
4	Tannins	Ferric chloride	+	+	+	+	-	+
		Gelatin	+	+	+	-	+	+
5	Phenols	Ferric chloride	+	+	+	+	-	+
		Ellagic	-	+	+	+	+	+
		Phenol	+	-	+	+	+	+
6	Terpinoids	Libermann-Burchard	+	+	-	+	-	+
7	Sterols	Libermann- Burchard	-	-	-	-	-	-
		Salkowski	+	+	+	+	+	-
8	Glycosides	Legals	+	+	+	+	+	+
		Keller Killani Test	-	+	+	+	+	+
		Glycosides Test	-	+	+	+	+	+
		Conc. H_2SO_4	-	+	+	+	+	+
		Molisch's	-	-	+	-	+	+
9	Saponins	Lead acetate	+	+	+	+	-	+
		Foam	+	+	+	+	-	+
		Haemolysis Test	-	+	+	+	+	+
10	Protein	Millons	+	+	+	+	+	+
11		Biuret	-	-	-	+	+	-
12	Carbohydrate	Molichs	+	+	+	+	+	+

respectively. Terpenoids were indicated by the presence of terpenoids, steroids, glycosides, and saponins. For terpenoids, the Libermann-Burchard test showed positive results for all extracts except for ethanol and acetone extracts. The sterol content was determined by using Libermann-Burchard and Salkowski testing reagents. Unfortunately, Libermann-Burchard showed negative results for all the extracts, while in the Salkowski test, only the water extract showed a negative result for sterol. The presence of glycosides was determined by Legals, Keller Killani, glycosides, concentrated hydrochloric acid, and Molisch's test. The legal test showed positive results for all extracts: Keller Killani, glycosides, concentrated hydrochloric acid, and Molisch's test showed negative results for chloroform extracts, while the Salkowski test showed negative results for water extracts. The content of saponin was tested using the lead acetate and foam haemolysis methods. Both the lead acetate and foam tests showed negative saponins in acetone extract and the haemolysis test showed negative for chloroform extract. For protein and carbohydrates, all tests showed positive results except for the Biuret test, which showed poor ability to extract protein from chloroform, methanol, ethanol, and water extracts.

M. oleifera pod extracts

The result of the qualitative phytoconstituents test on the *M. oleifera* pod extracts, as shown in Table 2, revealed the following: All the extracts showed positive results for all the phytoconstituents except for petroleum ether extracts, which revealed negative results in all tests conducted for alkaloid and phenolic compounds.

Table 2: Phytochemical test of *M. oleifera* pod crude extract with different solvents.

Sl. No.	Phytochemical test		Solvents					
			Chloroform	Methanol	Ethanol	Petroleum Ether	Acetone	Water
1	Alkaloids	Dragendroff	+	+	+	-	+	+
		Iodine	-	+	+	-	+	+
		Mayers	+	+	+	-	+	+
		Wagners	+	+	+	-	+	+
2	Flavonoids	Alkaline	+	+	+	-	+	+
		Pews	+	+	+	-	+	+
		Shinoda	+	+	+	-	+	+
3	Lignin	Lignin	-	+	+	-	+	+
		Labat Test	-	+	+	-	+	+
4	Tannins	Ferric chloride	-	-	-	-	-	-
		Gelatin	-	+	-	-	+	+
5	Phenols	Ferric chloride	+	-	-	-	+	+
		Ellagic	-	+	-	-	+	+
		Phenol	-	-	+	-	+	+
6	Terpinoids	Libermann-Burchard	+	+	+	-	+	+
7	Sterols	Libermann- Burchard	-	-	-	-	-	-
		Salkowski	+	+	+	-	+	+
8	Glycosides	Legals	+	+	+	+	+	+
		Keller Killani Test	-	+	+	-	+	+
		Glycosides Test	-	+	+	+	+	+
		Conc. H_2SO_4	-	+	+	-	+	+
		Molisch's	-	-	+	-	+	+
9	Saponins	Lead acetate	+	+	+	-	+	+
		Foam	+	+	+	-	-	+
		Haemolysis Test	-	+	+	-	+	+
10	Protein	Millons	+	+	-	-	+	+
11		Biuret	-	-	-	+	+	+
12	Carbohydrate	Molichs	+	+	+	+	+	+

In addition, alkaloids were absent in chloroform extracts of the alkaloid test. For phenolic determination, flavonoids were present in all extracts. Lignin was absent in all two tests (Lignin and Labat Test) for chloroform extracts. Tannin, on the other hand, showed complete absenteeism in the ferric chloride test for all extracts and chlorofoam and methanol extracts in the gelatin test. In the Ferric Chloride, Ellagic, and Phenol tests, phenols were absent in methanol and ethanol extracts (Ferric Chloride test), petroleum ether and ethanol extracts (Ellagic test), and petroleum ether and methanolic extracts (Phenol test). For terpenoids, the Libermann-Burchard test showed positive results for all extracts except for petroleum ether extract. As for sterol determination, Libermann-Burchard and Salkowski testing reagents were employed. Unfortunately, Libermann-Burchard showed negative results for all the extracts, while in the Salkowski test, all extracts showed the ability to extract lignin except for petroleum ether extract. The results for glycosides (Legals, Keller Killani, Glycosides, Concentrated Hydrochloric Acid, and Molisch's test) showed variability, where all extracts showed positive in the legal test, chloroform and petroleum ether extracts were negative in the Keller Killani test, negative in the chloroform extract for the glycosides test, negative for chloroform and petroleum ether extracts in the hydrochloric acid test, and for Molish's test, chlorofoam, methanol, and petroleum ether extracts showed negative. For saponin, both the lead acetate and foam tests showed negative results in acetone extracts, while the hemolytic tests were negative results in chloroform extract. The presence of protein (Millons and Biuret tests) was inconsistent, where ethanol and

Table 3: Phytochemical test of *M. oleifera* bark crude extract with different solvents.

Sl. No.	Phytochemical test		Solvents					
			Chloroform	Methanol	Ethanol	Petroleum Ether	Acetone	Water
1	Alkaloids	Dragendroff	+	+	+	-	+	+
		Iodine	-	+	+	-	-	+
		Mayers	+	+	+	-	+	+
		Wagners	+	+	+	-	-	+
2	Flavonoids	Alkaline	+	+	+	-	+	+
		Pews	+	+	+	-	+	+
		Shinoda	+	+	+	-	-	+
3	Lignin	Lignin	-	+	+	-	+	+
		Labat Test	-	+	+	-	-	+
4	Tannins	Ferric chloride	-	-	-	-	-	-
		Gelatin	-	+	-	-	-	+
5	Phenols	Ferric chloride	+	-	-	-	-	+
		Ellagic	-	+	-	-	-	+
		Phenol	-	-	+	-	-	+
6	Terpinoids	Libermann-Burchard	+	+	+	-	+	+
7	Sterols	Libermann- Burchard	-	-	-	-	-	-
		Salkowski	+	+	+	-	-	+
8	Glycosides	Legals	+	+	+	+	+	+
		Keller Killani Test	-	+	+	-	+	+
		Glycosides Test	-	+	+	+	+	+
		Conc. H_2SO_4	-	+	+	-	-	+
		Molisch's	-	-	+	-	+	+
9	Saponins	Lead acetate	+	+	+	-	+	+
		Foam	+	+	+	-	-	+
		Haemolysis Test	-	+	+	-	+	+
10	Protein	Millons	+	+	-	-	+	+
11		Biuret	-	-	-	-	+	+
12	Carbohydrate	Molichs	+	+	+	+	+	+

petroleum ether extracts showed negative results in the Millon test while the Biuret test showed negative results for chloroform, methanol, and ethanol extracts. Carbohydrate (Molichs test), on the other hand, was present in all extracts.

M. oleifera bark extracts

From the qualitative findings presented in Table 3, it is observed that the *M. oleifera* bark of different extracts confirmed the presence of alkaloids, phenolics, terpenoids, proteins, and carbohydrates. Similar to *M. oleifera* pod extracts, petroleum ether solvent showed an inability to extract all the phytoconstituents in varying degrees, especially alkaloids and phenolic compounds. In addition, alkaloids were not present in chloroform extract in the iodine test. The presence of phenolic compounds was indicated through various constituents. Flavonoids were absent in the acetone extract of Wagner's test. Lignin was absent in the chloroform extract of both tests and in the acetone extract of the Labat test. All the extracts were negative for tannin when tested with the ferric chloride test, while in the gelatin test, only methanol and water solvent were able to extract tannin. In terpenoid, terpenoids were present in all extracts except petroleum ether extract and sterol was absent in all extracts when tested using the Libermann-Burchard reagent, while Salkowski showed negative results for petroleum ether and acetone extracts. For glycosides, the legal test was positive for all extracts. Chloroform and petroleum ether extracts were negative. Keller's killani test and glycosides test were positive for all extracts except chloroform extracts, while concentrated hydrosulfuric acid and Molisch's test

Table 4: Concentration of different secondary metabolites in *M. oleifera* Leaf crud extract with different solvents.

Compounds	Aqueous	Methanol	Ethanol	Acetone	*p* value	*f* value
Alkaloids	3.25±0.09	2.82±0.03	2.63±0.06	2.76±0.09	<0.0001	41.836
Flavanoids	4.20±0.07	5.35±0.03	5.20±0.07	5.08±0.04	<0.0001	260.71
Lignins	1.70±0.06	1.70±0.48	1.13±0.05	0.86±0.04	<0.0001	76.467
Tannin	3.72±0.14	3.49±0.09	3.32±0.05	3.27±0.05	<0.0151	9.137
Phenols	2.12±0.04	1.89±0.06	1.57±0.04	1.52±0.06	<0.0003	41.216
Terpinoids	4.60±0.06	4.12±0.06	3.91±0.04	3.85±0.03	<0.0008	29.656
Steroids	3.57±0.06	3.23±0.05	3.12±0.04	3.12±0.07	<0.0001	43.238
Glycosides	0.26±0.02	0.32±0.04	0.26±0.02	0.24±0.03	<0.0459	5.379
Saponins	1.64±0.04	2.17±0.06	1.86±0.04	1.76±0.02	<0.0001	73.446
p value	<0.0001	<0.0001	<0.0001	<0.0001		
f value	1171.1	2501.7	3074.6	2593.8		

Table 5: Concentration of different secondary metabolites in *M. oleifera* pod crude extract with different solvents.

Compounds	Aqueous	Methanol	Ethanol	Acetone	*p* value	*f* value
Alkaloids	5.59 ±0.19	4.31±0.05	4.16±0.05	4.23±0.02	<0.0001	134.14
Flavanoids	1.55 ±0.07	2.55±0.03	2.38±0.03	2.35±0.04	<0.0001	195.02
Lignins	0.81 ±0.04	0.68±0.03	0.53±0.04	0.45±0.03	<0.0001	61.340
Tannin	3.83 ±0.05	3.56±0.02	3.32±0.04	3.32±0.04	<0.0001	116.11
Phenols	0.65 ±0.03	0.55±0.03	0.43±0.03	0.35±0.03	<0.0001	58.111
Terpinoids	1.82 ±0.04	1.55±0.03	1.26±0.03	1.33±0.02	<0.0001	201.05
Steroids	1.24 ±0.04	1.12±0.05	0.86±0.03	0.88±0.03	<0.0001	70.169
Glycosides	0.27 ±0.02	0.35±0.03	0.32±0.04	0.37±0.02	<0.0132	6.879
Saponins	0.82 ±0.04	1.28±0.04	1.18±0.04	1.16±0.03	<0.0001	84.491
p value	<0.0001	<0.0001	<0.0001	<0.0001		
f value	1611.7	4598.2	4080.2	6589.9		

were negative for chloroform and petroleum ether extracts. In addition, Molisch's test was also negative for methanol extract. For saponin and carbohydrate, the test results were similar to those of *M. oleifera* pod extracts. Finally, in protein investigation, the Millons test was negative for ethanol and petroleum ether extracts, while the biuret test was negative for all extracts except for acetone and water extracts.

Qualitative and qualitative evaluation of *M. oleifera* leaf crude extracts secondary metabolite concentration in different solvents

As illustrated in the standard graph (Figure 1), flavonoid content was highest, followed by terpenoids, steroids, tannin, alkaloids, saponins, lignins, and glycosides, respectively, in *M. oleifera* leaf crude extract. In terms of solvent efficacy, aqueous extract gave the highest yield for terpenes, flavonoids, tannins, steroids, phenols, lignins, and glycosides. In methanol extract, flavanoid content was highest, followed by terpenoids, tannins, steroids, alkaloids, saponins, phenols, lignin, and glycosides. For ethanol and acetone, the content displayed a similar pattern of yield: flavonoids, terpenoids, tannins, steroids, alkaloids, phenols, saponins, lignins, and glycosides. The qualitative estimation is tabulated in Table 4, together with the *p* and *f* values.

Qualitative and qualitative evaluation of *M. oleifera* pod crude extracts secondary metabolite concentration in different solvents

As illustrated in the standard graph (Figure 2), alkaloid concentration was highest, followed by tannin, flavonoids, terpenoids, steroids, saponins, lignins, phenols, and glycosides, respectively, in *M. oleifera* pod crude extract. In terms of solvent efficacy, aqueous extract gave the highest yield for alkaloids, followed by tannin, terpenoids, flavonoids, steroids, saponins, lignins, phenols, and glycosides. In methanol extract, alkaloids were found to have the highest concentration, followed by tannin, flavonoid, terpenoids, saponins, steroids, lignin, phenols, and glycosides. For ethanol and acetone, the content displayed a similar pattern of yield: alkaloids, tannins, flavonoids, terpenoids, steroids, saponins, lignins, phenols, and glycosides. The qualitative estimation is tabulated in Table 5, together with the *p* and f values.

Table 6: Concentration of different secondary metabolites in *M. oleifera* pod crude extract with different solvents

Compounds	Aqueous	Methanol	Ethanol	Acetone	*p* value	*f* value
Alkaloids	2.91±0.04	2.56±0.03	2.38±0.04	2.25±0.02	<0.0001	218.76
Flavonoids	1.35±0.03	2.48±0.04	2.35±0.02	2.31±0.03	<0.0001	854.18
Lignins	1.38±0.04	1.14±0.03	0.96±0.02	0.78±0.02	<0.0001	467.03
Tannin	6.25±0.03	5.78±0.04	5.66±0.02	5.54±0.02	<0.0001	351.36
Phenols	1.28±0.03	1.18±0.03	1.08±0.03	1.17±0.02	<0.0002	25.903
Terpenoids	2.87±0.02	2.57±0.02	2.35±0.03	2.49±0.03	<0.0001	360.71
Steroids	2.52±0.04	2.38±0.03	2.24±0.03	2.25±0.02	<0.0001	54.605
Glycosides	0.24±0.03	0.38±0.03	0.34±0.03	0.36±0.02	<0.0012	14.968
Saponins	0.48±0.04	0.84±0.02	0.76±0.02	0.66±0.02	<0.0001	103.29
p value	<0.0001	<0.0001	<0.0001	<0.0001		
f value	8589.8	8136.9	9957.3	14418		

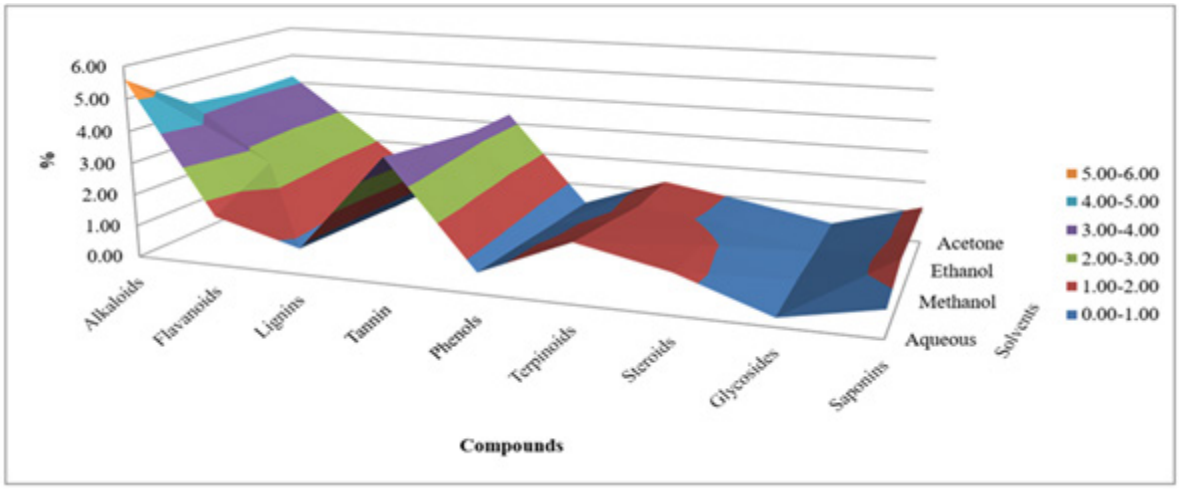

Figure 2: Secondary metabolites concentration in crude extract of *M. oleifera* pod in different solvent.

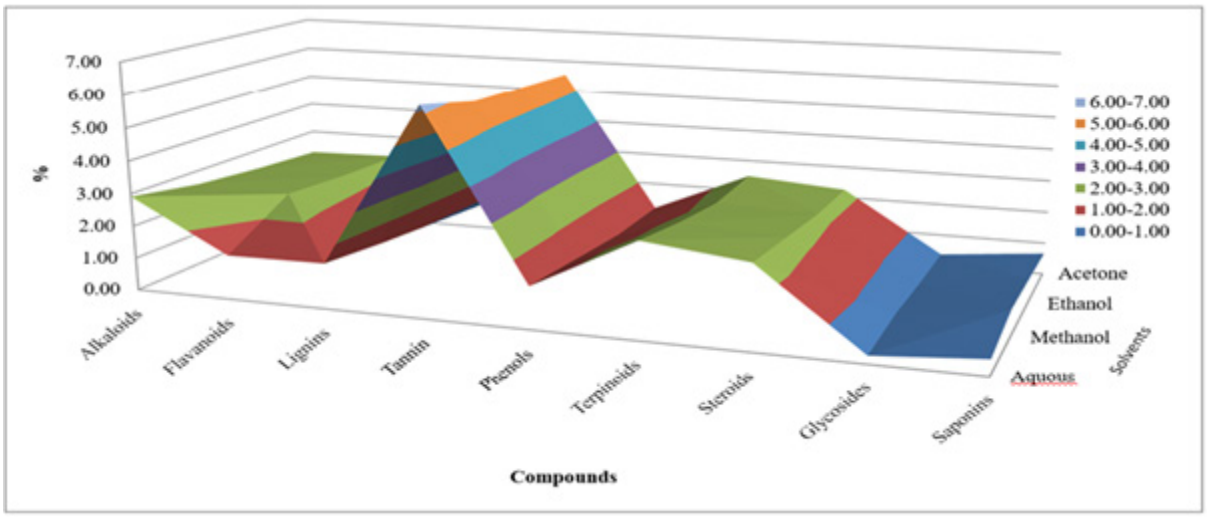

Figure 3: Secondary metabolites concentration in crude extract of *M. oleifera* bark in different solvent.

Qualitative and qualitative evaluation of *M. oleifera* bark crude extract secondary metabolite concentration in different solvents

As illustrated in Figure 3 and Table 6, alkaloid concentration was highest, followed by tannin, flavonoids, terpenoids, steroids, saponins, lignins, phenols, and glycosides, respectively, in *M. oleifera* pod crude extract. In terms of solvent efficacy, aqueous extract gave the highest yield for alkaloids, followed by tannin, terpenoids, flavonoids, steroids, saponins, lignins, phenols, and glycosides. In methanol extract, alkaloids were found to have the highest concentration, followed by tannin, flavonoid, terpenoids, saponins, steroids, lignin, phenols, and glycosides. For ethanol and acetone, the content displayed a similar pattern of yield: alkaloids, tannins, flavonoids, terpenoids, steroids, saponins, lignins, phenols, and glycosides. The qualitative estimation is tabulated in Table 6, together with the *p* and *f* values.

DISCUSSION

Primary and secondary metabolites are two categories of organic compounds produced by plants. Primary metabolites, such as proteins, carbohydrates, amino acids, and lipids, are involved in plants' growth and developmental processes.[21] Secondary metabolites are non-nutritive components that are not essential for plant survival but play an important role in the interaction of plants with their environments, ensuring the sustained existence of plants in their ecosystems. For example, they are associated with plant responses to stress such as temperature, drought, UV light, and infections. The accumulation of secondary metabolites is highly dependent on stress factors. Hence, a change in an individual stress factor can differ from the content of secondary metabolites with complexity in structure and more restriction in distribution than the primary metabolites, even if other factors remain constant.[22]

Secondary metabolites are classified based on their biosynthetic pathways because metabolites are small molecules, intermediates, and products of metabolism. They are classified into four groups of large molecules: flavonoids, steroids, and alkaloids.[23] Alkaloids are derivates of primary metabolites, amino acids, which are heterocyclic nitrogen compounds.[24] In plants, alkaloids are defensive against herbivores and pathogens. Based on their potent biological activity, almost 12,000 alkaloids have been exploited as narcotics, poisons, pharmaceuticals, and stimulants. Flavonoids belong to the polyphenolic compounds group. In plants, flavonoids are responsible for aroma color and fruit pollination.[25] Sterols can be characterized into several major groups based on the location of the hydroxyl group. In plants, they are membrane constituents and are the starting material for the biosynthesis of plants.[26]

Plant chemistry forms the basis for many commercial pharmaceutical drugs, as the different chemical constituents in plants possess biological activities that can improve human disorders through dietary intake and therapeutic use (pharmaceutical and food industries).[27] In addition, they also play an important role in the cosmetic and agrochemical industries. Many secondary metabolites, such as alkaloids, terpenoids, and phenylpropanoids, are being considered for drug development; however, limited knowledge of the chemical composition of plants leads to a restricted understanding of their possible medicinal value.[28] Most studies investigating the bioactive properties of medicinal plants rarely progress to studies at the molecular level due to limited preliminary screening for further chemical characterization. Hence, data collection on the chemical constituents of a plant through phytochemical screening is necessary for the synthesis of complex chemical substances.[29] In the present study, qualitative and quantitative phytochemical analysis was carried out for *M. oleifera* plant parts using different solvents. This preliminary screening is crucial in the identification of various classes of bioactive compounds present in the extract. Prior to the analysis, the extraction procedure is a vital step to ensure the desired compounds from the plant are isolated.[30] This is done by using various solvents of different polarities. Hence, in this study, six different solvents from various polarities were used to maximize the isolation of the chemical compounds from *Moringa oleifera* different plant parts.

Chloroform, methanol, ethanol, petroleum ether, acetone, and water were used as the solvent systems for quantitative analysis, as they ranged from non-polar to polar solvents. An ideal solvent system provides quality preliminary information on the chemical composition of the plant.[31] The leaves showed the most diversity in secondary metabolites compared to the pod and bark of the *M. oleifera* plant. This is probably due to extensive exposure to environmental conditions, such as temperature. A differential distribution of these classes of compounds is based on their polarity in polar and nonpolar solvent extracts.[32] In this context, ethanolic extract was shown to comprise a significant amount of secondary metabolites, while petroleum ether presented the lowest contents of secondary metabolites, probably due to the low hydro-solubility of the solvent, which was able to dissolve lipophilic compounds including sterols, several terpenoids, and alkaloids and therefore extract fewer secondary metabolites. The high polarity index of ethanol was able to extract flavonoid glycosides, tannins, and several alkaloids that had polar properties. Also, this solvent was effective in extracting phenolic compounds with low molecular weights and medium levels of polarity, such as flavonoids, saponins, and polyphenolic compounds.[33]

The diverse compositions of these secondary metabolites may be responsible for the pharmacological activities of *M. oleifera* extracts. Almost all phytochemicals can be used as natural antibiotics to assist the body in fighting infections. For instance, alkaloids consist of nitrogen-based chemical constituents that occur naturally in plants.[34] Apart from their antioxidant properties, alkaloids have a wide range

of pharmacological activities, including protection against microorganisms, cancer, platelet aggregation, hepatotoxins, ulcers, inflammation, free radicals, and allergies.[35,36] Phenols are antioxidants capable of ameliorating human disorders, which can be achieved by improving the dietary intake of nutrients with antioxidant properties. Terpenoids are the most abundant compounds in plants that possess anti-inflammatory, antitumor, antibacterial, antiviral, and antimalarial effects, prevent and treat cardiovascular diseases, promote transdermal absorption, and have hypoglycemic activities.[37]

The phytochemical screening and solvent extraction analysis give a good guide to the phytochemicals present in *M. oleifera* extracts as well as suitable extraction solvents. Overall, aqueous is the best solvent to use for extraction, as the extracts are more soluble in polar solvents and aqueous has more polar organic properties.[38,39] The results obtained on the phytochemical contents of*M. oleifera* different plant parts are in agreement with the study done previously done to use solvents of different polarity.[40,41] Despite the well-documented traditional uses of *M. oleifera*, its phytochemical quality and quantity still require complete scientific validation. In addition, this finding served as further justification to validate *M. oleifera* as a medicinal plant and a potential source of phytochemicals that could be used in drug discovery and development. Since aqueous solvent extraction yields the most phytochemicals compared to the other solvents, it is therefore recommended for the extraction of these plant materials.

CONCLUSION

Conclusively, this work was chosen for the study of the phytochemical screening of various phytochemical compounds in *Moringa oleifera*. The data proved that the use of solvents of different polarities in extraction had a big influence ($p<0.05$) on the richness of *M. oleifera* in secondary metabolites. These results of the preliminary scientific evidence ensured its unequivocal recommendation for use in the pharmaceutical and nutraceutical sectors of *M. oleifera*. Furthermore, biological tests are needed to characterize the eventual activities and assess toxicity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; **NaOH:** Sodium hydroxide; **HCL:** Hydrochloric acid; **$FeCl_3$:** Ferric chloride; **H_2SO_4:** Sulfuric acid; **NH_4OH:** Ammonium hydroxide; **$K_4[Fe(CN)_6]\cdot 3H_2O$:** Potassium ferrocyanate; **FCR:** Folin-Ciocalteu's reagent; **SPSS:** Statistical Package for the Social Sciences.

SUMMARY

The present study found the presence of various phytochemicals in different parts of *M. oleifera*, such as leaf, pod, and bark. The major phytoconstituents present in the *M. oleifera* plant are alkaloids, phenols, terpenoids, proteins, and carbohydrates. These preliminary scientific findings ensured its unequivocal recommendation for use in *M. oleifera's* pharmaceutical and nutraceutical sectors.

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