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Research Article

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Solvent Extraction and Phytochemical Screening of Seeds, Coats, Pods and Leaves of Moringa Plant

Jacob Olalekan Arawande[*1], Adekemi Racheal Adeleke[1], Olamide Racheal Orimoloye[1], Segun Akanmu Adebisi[2], Edgar Uzezi Amuho[3] and Olajubu Ayotunde Karimu[4]

[1]*Department of Chemistry, University of Medical Sciences, PMB 536 Ondo, Ondo State, Nigeria*

[2]*Department of Pure and Applied Chemistry, Osun State University, PMB 4494 Osogbo, Osun State, Nigeria*

[3]*Department of Chemistry, Adeyemi College of Education PMB 520 Ondo, Ondo State, Nigeria*

[4]*Department of Pharmaceutical Technology, Rufus Giwa Polytechnic PMB 1019 Owo, Ondo State, Nigeria*

[*]Corresponding author
Jacob Olalekan Arawande, Department of Chemistry, University of Medical Sciences, PMB 536 Ondo, Ondo State, Nigeria

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Abstract

The potency of six different solvents in extracting phytochemicals from the seeds, coats, pods and leaves of moringa plant was investigated. The seeds, coats, pods and leaves of the plant were cut into smaller pieces, air-dried, ground into powdery sample, sieved with 40 mm mesh size and properly labelled. Each sample was individually extracted using six different solvents (methanol, ethanol, chloroform, ethyl acetate, water and acetone) at ratio 1: 10 for 72 h. Each solvent extract was screened for twelve phytochemicals (alkaloid, flavonoid, saponin, cardiac glycoside, reducing sugar, tannin, quinone, volatile oil, phenol, terpenoid, phlobatannin and steroid). It was observed that the seeds and leaves of moringa plant were richest in phytochemicals followed by moringa pods and the least was in moringa coat. In all the six solvents used, thirty-four bioactive ingredients were detected in seeds and leaves of moringa plant while twenty-eight phytochemicals were obtained in moringa pods and twenty-one bioactive ingredients were gotten from moringa coats. In all the plant samples, twenty-three bioactive ingredient were detected in ethanol extract; twenty-one were obtained in each of acetone, ethyl acetate and methanol extracts; water extract had sixteen phytochemicals and chloroform extract had fifteen bioactive ingredients. Among the solvents used for extraction for all the plant samples, ethanol ranked first while acetone, ethyl acetate and methanol ranked second, water ranked third and chloroform was the least in ranking.

Keywords: Moringa Plants, Phytochemicals, Solvent Potency, Moringa Extracts

Introduction

Phytochemicals are referred to as plant (phyto) chemicals which consist of wide variety of compounds that occur naturally in plants. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases, and they are non-nutritive compounds. Many phytochemicals are antioxidants protecting the cell of the body from oxidative damage from water, food, and the air [1, 2].

Many phytochemicals have antioxidant activity and reduce the risk of many diseases. It is crucial to know the type of phytochemical constituent, thus knowing the type of biological activity which might be exhibited by the plant [3]. The importance of medicinal plants and the contribution of phytomedicine to the well-being of a significant member of the world's population have attracted interest from diverse disciplines [4].

Moringa plant is cultivated in many tropical and sub-tropical regions worldwide, where it is known by various vernacular names as horseradish tree, drumstick tree, bean oil tree, miracle tree, and

"Mother's Best Friend" [5, 6]. Moringa plant is botanically known as Moringa oleifera [7]. Traditionally, the bark, sap, roots, leaves, seeds and flowers of moringa are used in traditional medicine [5, 8]. Moringa oleifera Lam. (Moringaceae) is a tree growing up to 5-12 m with an open umbrella shaped crown native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands [9]. It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 6.3 to 7.0), well-drained, sandy or loamy soil. In waterlogged soil, the roots tend to rot. Moringa is a sun- and heat-loving plant, and does not tolerate freezing or frost. Moringa is particularly suitable for dry regions, as it can be grown using rainwater without expensive irrigation techniques. Though there are several researches works on moringa plant but there is little or no work on the effect of solvents on extracting phytochemicals from the seeds, coats, pods and leaves of the plant [8, 10-14]. Therefore, the focus of this research work is to obtain extracts from moringa plant parts (seeds, leaves, pods and coats) using ethanol, methanol, acetone, ethyl acetate, chloroform and water as well as to investigate the different solvent-extracts of the moringa plant parts for qualitative phytochemical constituents so as to establish the part that is richest in phytochemical constituents as well as the solvent that has the highest potency for extracting phytochemicals.

Materials and Methods

Source of Materials

The seeds, coats, pods and leaves of Moringa oleifera were collected from a compound of a building at Ajagbale Street, Oka, Ondo City, Ondo State, Nigeria. All chemicals used were of the analytical grade with the highest purity available (<99.5%) and procured from Sigma Aldrich, USA.

Preparation and Extraction of Seeds, Coats, Pods and Leaves of Moringa Plant

The different parts of moringa plant were cut into smaller pieces for easy air-drying. The dried samples were ground separately using electric blending machine (Solitarire Mixer Grinder VTCL Heavy Duty 750 Watts) and each part was sieved with 40 mm mesh size. The powdered samples were divided into portions, packed in air tight containers labelled appropriately prior to extraction. Each sample was extracted separately with each solvent (acetone, chloroform, ethyl acetate, ethanol, methanol and water) at ratio 1:10 for 72 h during which it was intermittently shaken on a shaking orbit machine. The resulting mixture was filtered through a 0.45 µm nylon membrane filter. The extracts were desolventised to dryness under reduced pressure at 40oC by a rotary evaporator (BUCHI Rotavapor, Model R-124, Germany). The dry extracts were stored in a refrigerator (4 0C) prior to analysis [15, 16].

Phytochemical Screening of Solvent-Extracts of Seeds, Coats, Pods and Leaves of Moringa Plant

The phytochemicals were qualitatively determined using standard methods [17-19].

Test for Tannins

About 0.2 g of the extract was taken and 2 mL of 10 % ferric chloride was added. Color changes into blue black which indicates the presence of tannin.

Test for Alkaloids (Wagner's Test)

About 0.2 g of the extract was hydrolyzed by 1% hydrochloric acid; six drops of Wagner's reagent were added. Color changes into brown red/orange precipitate which indicates the presence of alkaloids.

Test for Saponin

About 0.2 g of the extract was added with 5 mL of distilled water, it was shaken for 30 seconds and the presence of foam indicates presence of saponin.

Test for Terpenoids (Salkowski Test)

About 3 mL of chloroform was added to about 0.2 g of the extract and then concentrated sulphuric acid was added from sides of the test tube. The presence of reddish-brown color appears at the interface indicates the presence of terpenoids in extract.

Test for Cardiac Glycosides (Keller - Killiani Test)

About 0.2 g of the extract was taken and then 1 mL of glacial acetic acid was added and 1 mL of 10% ferric chloride was added, then 1 mL concentrated sulphuric acid was added from the sides of test tube. Formation of green/blue precipitate indicates the presence of cardiac glycosides.

Test for Steroids (Lierbermann-Burchardt Test)

In about 0.2 g of the extract, 1 mL chloroform was added, 3 mL acetic anhydride was added from sides of the test tube, and then two drops of concentrated sulphuric acid was added. The appearance of dark green color confirms the presence of steroids.

Test for Flavonoids

About 0.2 g of the extract was taken; dilute sodium hydroxide was added to create intense yellow color, which on addition of concentrated hydrochloric acid turns into colorless which indicates the presence of flavonoids.

Test for Reducing Sugars (Fehling's Test)

About 0.2 g of the extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugar.

Test for Phlobatanins

About 0.2 g of the extract was added with distilled water then shaken and filtered, then 2 mL of 2% hydrochloric acid was added and boiled, Red colored developed which indicate the presence of phlobatannins.

Test for Phenol

2 mL of distill water followed by few drops of 10% ferric chloride was added to about 0.2 g of the extract. Formation of blue or green color occurred which indicates the presence of phenol.

Test for Volatile Oil

0.1 mL dilute sodium hydroxide and small quantity of dilute hydrochloric acid was added to about 0.5 g of the extract, the solution was shaken. White precipitate was formed which indicates the presence of volatile oil.

Test for Quinone

To about 0.2 g of the extract, 1 mL of concentrated sulphuric acid was added. Formation of red color indicates presence of quinone.

Results and Discussion

Table 1: Qualitative Phytochemical Screening of Solvent Extracts of Moringa Seeds

Parameter	Solvent extract					
	Acetone	Chloroform	Ethanol	Ethyl acetate	Methanol	Water
Alkaloid	+	+	+	+	+	+
Flavonoid	-	-	+	+	-	-
Saponin	+	+	+	+	-	+
Cardiac Glycoside	-	+	-	-	-	-
Reducing Sugar	+	-	+	+	-	+
Tannin	-	-	-	-	-	-
Quinone	+	+	+	+	+	+
Volatile oil	+	+	+	+	+	-
Phenol	-	-	-	-	-	-
Terpenoid	-	-	+	-	-	+
Phlobatannin	-	-	-	-	-	-
Steroid	+	-	+	+	-	-
%Phytochemical extractable	50.00	41.67	66.67	58.33	25.00	41.67

(+): positive = present (-): negative = absent

Qualitative phytochemical screening of solvent extracts of moringa seed is presented in Table 1. Phytochemical screening of six different solvent extract of moringa seed were examined. The solvents are acetone, chloroform, ethanol, ethyl acetate, methanol and water. While the phytochemicals considered were alkaloid, flavonoid, saponin, cardiac glycoside, reducing sugar, tannin, quinone, volatile oil, phenol, terpenoid, phlobatannin and steroid. In all the solvents used for extraction of moringa seed, it was observed that alkaloid and quinone were present while tannin, phenol and phlobatannin were absent. Flavonoid was present in ethanol and ethyl acetate extracts. Saponin was present in acetone, chloroform, ethanol, ethyl acetate and water extracts. Cardiac glycoside was absent in all extracts except chloroform extract. Reducing sugar was present in acetone, ethanol, ethyl acetate and water extracts. Volatile oil was present in all extracts except water extract. Terpenoid was present in ethanol and water extracts. Steroid was present in acetone, ethanol and ethyl acetate extracts. Among the twelve phytochemicals examined for moringa seed extract, ethanol extract had eight (66.67%), ethyl acetate extract had seven (58.33%), acetone extract had six (50%), chloroform and water extracts had five (41.67%) while methanol extract had three (25%) of the phytochemicals.

Table 2: Qualitative Phytochemical Screening of Solvent Extracts of Moringa Coat

Parameter	Solvent extract					
	Acetone	Chloroform	Ethanol	Ethyl acetate	Methanol	Water
Alkaloid	+	+	+	+	+	+
Flavonoid	-	-	-	-	+	+
Saponin	-	-	-	-	+	+
Cardiac Glycoside	-	-	-	-	-	-
Reducing Sugar	+	-	-	-	-	-
Tannin	-	-	-	-	+	-
Quinone	-	+	-	-	-	-
Volatile oil	+	+	-	-	+	-
Phenol	-	-	-	-	-	-
Terpenoid	-	-	+	+	-	-
Phlobatannin	-	-	-	-	-	-
Steroid	-	+	+	+	-	-
%Phytochemical extractable	25.00	33.33	25.00	25.00	41.67	25.00

(+): positive = present (-): negative = absent

The qualitative phytochemical screening of moringa coat of six different solvent-extracts is shown in Table 2. The phytochemicals considered were alkaloid, flavonoid, saponin, cardiac glycoside, reducing sugar, tannin, quinone, volatile oil, phenol, terpenoid, phlobatannin and steroid while the solvents used were acetone, chloroform, ethanol, ethyl acetate, methanol and water. In all the solvents used for extraction of moringa coat, it was detected that alkaloid was present while cardiac glycoside, phenol and phlobatannin were absent. Flavonoid and saponin was present in methanol and water extracts. Reducing sugar was absent in all the extracts except acetone extracts. Tannin was present only in methanol extracts. Quinone was present only in chloroform extracts. Volatile oil was present in acetone, chloroform and methanol extracts. Terpenoid was present in ethanol and ethyl acetate extracts. Steroid is present chloroform, ethanol and ethyl acetate extracts. Among the twelve phytochemicals considered for moringa coat extracts, methanol extract had five (41.67%), chloroform extract had four (33.33%) and while acetone, ethanol, water and ethyl acetate extracts had three (25%) of the phytochemicals.

Table 3: Qualitative Phytochemical Screening of Solvent Extracts of Moringa Pod

Parameter	Solvent extract					
	Acetone	Chloroform	Ethanol	Ethyl acetate	Methanol	Water
Alkaloid	+	+	+	+	+	+
Flavonoid	+	-	-	-	-	-
Saponin	+	-	+	-	+	
Cardiac Glycoside	-	-	-	-	-	-
Reducing Sugar	-	+	+	-	+	+
Tannin	-	-	-	-	-	-
Quinone	-	-	-	-	+	-
Volatile oil	+	-	+	+	-	-
Phenol	-	-	-	-	-	-
Terpenoid	+	-	+	+	+	+
Phlobatannin	-	-	-	-	-	-
Steroid	+	+	+	+	+	-
%Phytochemical extractable	50.00	25.00	50.00	33.33	50.00	25.00

(+): positive = present (-): negative = absent

Qualitative phytochemical screening of six different solvent extracts of moringa pod were examined and shown in Table 3. The solvents used are acetone, chloroform, ethanol, ethyl acetate, methanol and water. The phytochemicals considered were alkaloid, flavonoid, saponin, cardiac glycoside, reducing sugar, tannin, quinone, volatile oil, phenol, terpenoid, phlobatannin and steroid. Alkaloid was detected in all the solvents used for extraction of moringa pod while cardiac glycoside, tannin, phenol and phlobatannin were absent. Flavonoid was absent in all the solvent extracts except acetone extract. Saponin was present in acetone, ethanol and methanol extracts. Reducing sugar was present in all the solvent extracts except acetone and ethyl acetate extracts. It was methanol extracts among all the solvent extracts that contained quinone. Volatile oil was present acetone, ethanol and ethyl acetate extracts. Terpenoid was present in all the six extracts except chloroform extract. Steroid was present in all the six solvent extracts except water extract. Amidst the twelve phytochemicals observed, acetone, ethanol and methanol extracts had six (50%); ethyl acetate extract had four (33.33%) while chloroform and water had three (25%) of the phytochemicals.

Table 4: Qualitative Phytochemical Screening of Solvent Extracts of Moringa Leaf

Parameter	Solvent extract					
	Acetone	Chloroform	Ethanol	Ethyl acetate	Methanol	Water
Alkaloid	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+
Saponin	-	-	-	+	+	+
Cardiac Glycoside	+	-	+	+	+	-
Reducing Sugar	-	-	+	+	+	+
Tannin	+	-	+	-	-	-
Quinone	-	-	-	-	-	-
Volatile oil	-	-	-	-	-	-
Phenol	+	-	+	+	+	-
Terpenoid	-	-	-	-	-	+
Phlobatannin	-	-	-	-	-	-
Steroid	+	+	-	+	+	-
%Phytochemical extractable	50.00	25.00	50.00	58.33	58.33	41.67

(+): positive = present (-): negative = absent

Qualitative phytochemical screening of solvent extracts of moringa leaf is presented in Table 4. Phytochemical screening of six different solvent extracts of moringa leaf was examined. The solvents used for extraction were acetone, chloroform, ethanol, ethyl acetate, methanol and water; and the phytochemicals considered were alkaloid, flavonoid, saponin, cardiac glycoside, reducing sugar, tannin, quinone, volatile oil, phenol, terpenoid, phlobatannin and steroid. It was detected that alkaloid and flavonoid were present in all the solvents used for extraction of moringa leaf while quinone, phlobatannin and volatile oil were absent in all the solvent extracts. Saponin was present in ethyl acetate, methanol and water extracts. Cardiac glycoside and phenol were present in acetone, ethanol, ethyl acetate and methanol extracts. Reducing sugar was present in ethanol, ethyl acetate, methanol and water extracts. Tannin was present in acetone and ethanol extracts. Terpenoid was detected only in water extract. Steroid was present in acetone, chloroform, ethyl acetate and methanol extracts. Among all the phytochemicals examined for moringa leaf extract, ethyl acetate and methanol extracts had seven (58.67%), acetone and ethanol extracts had six (50%), water had five (41.67%) while chloroform had three (25%) of the phytochemicals [20].

Conclusion

The seeds and leaves of moringa plant are richest in bioactive ingredients followed by moringa pods and the least was in moringa coats. Ethanol had the highest potency in obtaining bioactive ingredients from all the parts of moringa examined and the next in ranking was acetone, methanol and ethyl acetate while water and chloroform ranked the third and fourth respectively. Further investigation can be carried out by considering the antioxidant activity of ethanol, acetone, methanol and ethyl acetate extracts of the seeds and leaves of moringa plant with a view of using them as preservatives/additives or antioxidants in foods especially in edible oils.

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