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Qualitative and Quantitative Phytochemical Constituents of Moringa Leaf

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Abstract: *Most of the traditional knowledge of medicinal plants is in the form of oral knowledge and the active ingredients of herbal remedies are unknown to the traditional healers. Herbal remedies generally lack standardization. This constitutes hindrance to acceptability by the general public and the integration into the official national health care system of many countries. The correct identification of herbal material and the active ingredients is essential to quality control, safety and efficacy, acceptability by the general public and possible integration into the national health care system. The phytochemical studies of Moringa leaves would provide information on of the therapeutic potentials of these medicinal plants, quality parameters and data about these plants. The present study is to determine the qualitative and quantitative phytochemicals constituent of Moringa leaf. Evaluation of Moringa leaf for qualitative and quantitative phytochemicals properties were conducted using standard methods. The results of the phytochemical screening revealed the presence of Tannins, Phlobatannins, Saponins, Flavonoids, Steroids, Terpenoids, Cardiac glycosides, Anthraquinones and Alkaloids. Quantitatively water leaf contained (%) Alkaloids (10.73), Saponins (18.46), Total flavonoids (8.53) and Tannins (11.43) while the estimated quantity of aqueous and ethanolic extracts are 19.28 and 14.33%. The presence of phytochemical in the Moringa suggests possible preventive and curative property of the Moringa leaf. Medically, the presence of these phytochemicals explains the use of the plant in ethno-medicine for the management of various ailments.*

Keywords: Moringa leaf; Phytochemicals; Qualitative, Quantitative

1. Introduction

Antioxidants, antimicrobial, anticancer and anti-inflammatory are some of biological activities exhibited by medicinal plants. Combinations of secondary metabolites which are bioactive compound are the basis for biological activities of medicinal plants. Alkaloids, phenolic compounds, glycosides, anthraquinones and terpenoids are major classes of plant secondary metabolites.

Exhibition of biological activities by plants through bioactive compounds present are based on parent plant relationship. Hence, the biological activities of medicinal plants signify the kind of bioactive compounds which are present in its extract. The application of medicinal plants in ethnomedicine depends on the information on the constituent of the secondary metabolites found in the plants. The ethnomedicinal plants have been the major sources of drugs and lead compounds for drug synthesis (Halilu *et al.*, 2013).

The means of identifying new sources of therapeutically and industrially important bioactive compounds from plants is referred to as Phytochemical screening (Aman *et al*., 2012). Botanical identification, extraction with suitable solvents, purification and characterization of the bioactive constituents of medicinal plants are the process involves in phytochemical screening (Bandaranayake, 2006).

Most applications of bioactive compounds in pharmaceuticals, food preservation, alternative and natural therapies are based on antimicrobial activity (Kemal *et al.*, 2013). As a result increased prevalence of microbial antibiotic resistance to the most common antibiotics (antimicrobial agents), evaluation of the antimicrobial activity of natural products has become so critical.

Moringa oleifera, (*M. oleifera*), also known as *Moringa pterygosperma* Gaertn, they belong to member of the Moringaceae family of perennial angiosperm plants, which includes 12 other species (Olson, 2002). They are cultivated throughout tropical and sub-tropical areas of the world, where it is known by various vernacular names (Ramachandran *et al*., 1980), with drumstick tree, horseradish tree, and malunggay being the most commonly found in the literature.

Moringa oleifera is an edible plant. Studies have showed that its roots, bark, leaves, flowers, fruits and seeds contains a wide variety of nutritional and medicinal properties (Kumar *et al*., 2010). Phytochemical analyses have shown that its leaves are

particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids (Amaglo *et al*., 2010).

Despite the importance of bioactive plant compounds to pharmaceuticals, only a few medicinal plants have been investigated for their phytochemicals and biological activities. The role paly by Moringa plant in treatment of several ailments is enormous and a lot of success has been recorded through local use in combating severe illness. There is needs to examine secondary phyto-constituents that are present in Moringa leaves, this will further promote the use of this plant in traditional medicine and will awake our pharmaceutical industries on importance of this plants in treatment of several health challenges. Hence, this study is focus on determination of qualitative and quantitative constituents of Moringa leaves

2. Materials and Methods

2.1 Sample collection and preparation

Moringa leaves was plucked from farms within the Osun State College of Technology, Esa Oke, Osun State, and taken to the Laboratory, Department of Science and Laboratory, Technology, Esa Oke. The leaves were washed to remove dirt, and sliced. The leaves samples were Sun-dry for 7 days and afterwards were pulverized to obtain finer ground powder, using an electric grinder.

2.2 Extraction procedure

i. Aqueous Extract

This was carried out by method described by Davis (1956), through the use of pestle and mortar, dry powder of moringa leaves was homogenized at ratio of 1:8 w/v in sterile distilled water and filtered through muslin cloth. This was followed by strained of filtrate obtained through filter paper (Whattman No. 1). The extraction procedure was done at room temperature.

ii. Ethanolic Extract

This was prepared by soaking 400g of the dry Moringa leaves in 1000ml of ethanol for 48hrs at room temperature. Thereafter, extract was filtered through a Whatmann filter paper No. 42 (125mm) and subsequently through cotton wool. The extract was then concentrated using a rotary evaporator with the water bath set at 40^{o}C was used to concentrate extract to one-tenth its original volume and finally with a freeze drier. This was followed by storage of dried residue at 4^{o}C. The crude extract residue were then weighed and dissolved in distilled water for experimental analysis.

2.3 Phytochemical Screening

Tests were carried out on the aqueous extract to identify the phyto constituents using standard procedures as described by Harborne (1973), Sofowara (1993) and Trease and Evans (1989).

a. Qualitative Analysis of the Phytochemicals

i. Test for Tannins

This was done by boiling 1g of each of the dried powdered samples (separately) in 40 ml of water in a test tube and then filtered. A brownish green or a blue-black coloration was observed after addition of a few drops of 0.1% ferric chloride.

ii. Test for Phlobatannins

An aqueous extract of the dry Moringa leaves was boiled with 1% aqueous hydrochloric acid. Appearance of red precipitate indicates the presence of phlobatannins.

iii. Test for Saponins

To 10 ml of distilled water, 1 g of the powdered dry Moringa leaves (separately) was added and boiled in a water bath. The mixture was then filtered and to resultant 5ml of filtrate, 2-3 ml of distilled water was added and shook vigorously for attainment of a stable persistent froth. Then, followed by mixture of frothing with 1-2 drops of olive oil and shook vigorously, then observed in the formation of emulsions.

iv. Test for Flavonoids

This was determined through heating of 0.5g of the dry powdered of moringa leaves extract sample (separately) with ethyl acetate (10 ml) over a steam water bath for 3 min. To 1 ml of dilute ammonia solution, 4ml of filtrate from the filtered mixture mixture was added and shook. Appearance of yellow coloration is an indication of presence of flavonoids.

v. Test for Steroids

This was carried out by addition of 4 ml of acetic anhydride to 1 g of each of the crude extract (separately) with further addition of H_2S0_4(2ml). The presence of steroids was indicated by change of colour from violet to blue or green.

vi. Test for Terpenoids
This was carried out by Salkowski's test described by Parekh and Chands (2008), To 4ml of chloroform, 10ml of the crude extract was added, followed by the careful further addition of 5ml concentrated (H_2SO_4). Formation of the reddish brown coloration at the interface is an indication of a positive result for the presence of terpenoids.

vii. Test for Cardiac Glycosides
The Keller-Killani test method described by Parekh and Chands (2008) was used for Cardiac Glycosides determination. To 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, 5 ml of the plant extract was added, this was followed by addition of 1 ml concentrated Sulfuric acid. Brown ring was formed at the interface which indicated the presence of deoxy sugar of cardenolides. A violet ring may appear below the brown ring, though in the acetic acid layer, a greenish ring may also form just progressively throughout the layer.

viii. Test for Anthroquinones
5 ml of each of the plant extracts was boiled with 10 ml of sulfuric acid (H_2SO_4) and was filtered while hot. The filtrate was shook with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes (Sofowara, 1993).

xi. Test for Alkaloids
5 ml of the Moringa leaves extracts were added to 8 ml of 1% HCl mixed, warmed and later filtered. Maeyer's and Dragendorff's reagents were added to the 2 ml of the filtrate, then alkaloids absence or presence were determined based on the turbidity or precipitate development (Parekh and Chands, 2008).

b. Quantitative Analysis of the Phytochemicals
i. Estimation of Alkaloids
This was carried out by method described by Harborne and Baxter (1983). To a 500 ml of beaker, 10 g of the dry Moringa leaves and 400 ml of 10% acetic acid in ethanol were added; the beaker was then covered and allowed to stand for 4 hours. This was then filtered, extracted and concentrated on a water bath to one-quarter of the original volume. This was preceded by dropwise addition of concentrated ammonium hydroxide to the extract until the precipitation is completed. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue is the alkaloid, which was dried and weighed to a constant mass (Trease and Evans, 1989).

Formula = B - A × 100 / S
where,
B = Weight of Whatman filter paper.
A = Weight of Whatman filter paper, after drying.
S = Sample weight.

ii. Estimation of Saponins
Saponins were estimated by method of described by Obadoni and Ochuko (2001) 50ml of 20 % aqueous ethanol was added to 10 g of the dry Moringa leaves in a conical flask. At about 55^{o}C, for 4 hours with continuous stirring, the mixture was heated using a hot water bath after which the mixture was filtered and the residue re-extracted with a further 100 ml of 20% ethanol. The combined extracts were reduced to 20 ml over a water bath at about 90°C. The concentrate was transferred into a 100 ml separatory funnel and 10 ml of diethyl ether was also be added and then shaken vigorously. The aqueous layer was recovered while the ether layer was then discarded. The purification process was repeated three times. 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 5 m1 of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the Saponin content was calculated as the percentage of the starting material (Obadoni *et al*., 2001).
Formula = B – A × 100 / S
Where,
B = Weight of Whatmann filter paper.
A = Weight of Whatmann filter paper with sample.
S = Sample weight.

iii. Estimation of Phenols

This was conducted using method described by Mallick and Singh (1980). To 0.5 ml of freshly prepared plant extracts in test tubes, 8 ml of distilled water and 0.5 ml of Folin's Ciocalteau reagent were added to all the tubes. All the tubes were kept in Biological Oxygen Demand chamber for 10 minutes at 40°C for incubation. This was followed by addition of 1 ml of sodium carbonate solution to all the test tubes, subsequently; the tubes were incubated in the dark for an hour. The colour so developed was read spectophotometrically at 660 nm. Standard curve was drawn using tannic acid as standard. Different concentrations of tannic acid were prepared and O.D was read at 660 nm in a shimadzu UV-1650 spectrophotometer. The concentrations of sample were calculated based on the standard curve.

iv. Estimation of Total Flavonoids

This was determined by method described by Osuntokun *et al*. (2014). 200 ml of 80% aqueous methanol was used for recurrent extraction of 20 g of the dry moringa leaves at room temperature. Whatman filter paper No 42 was then used for filtration of the whole solution. The filtrate was then transferred into a crucible and evaporated to dryness over a water bath; the dry content was weighed to a constant weight.

v. Estimation of Tannins

100 mg of tannic acid was dissolved in 100 ml of distilled water. 5 ml of stock solution was diluted to 100 ml with distilled water. 1 ml containing 50 µg tannic acid.

Extraction of Tannin: 0.5 gm of the powdered material was weighed and transferred to a 250 ml conical flask and 75 ml water was added. The flask was heat gently and boiled for 30 min centrifuge at 2,000 rpm for 20 min and the supernatant was collected in 100 ml volumetric flask and make up the volume. 1 ml of the sample extract was transferred to 100 ml volumetric flask containing 75 ml water. 5 ml of folin denis reagent, 10 ml of sodium carbonate solution were added and diluted to 100 ml with water. Shake well. The absorbance was read at 700 nm after 30 min. If absorbance is greater than 0.7 make a 1 + 4 dilution of the sample. A blank was prepared with water instead of the sample. A standard graph was prepared by using 100 mg tannic acid.

The tannins content of the sample was calculated as tannic acid equivalents from the standard graph.

vi. Statistical analysis

Data are expressed as mean ± standard deviation (SD) of triplicates.

3. Results and Discussion

3.1 Qualitative phytochemical screening of *Moringa* leaf extracts

The results in this study for both aqueous and ethanolic extracts revealed the presence of the following phytochemical constituents saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids (aqueous extract) and tannins, saponins, flavonoid, steroids, cardiac glycosides, anthroquinones and alkaloids (ethanolic extract) in moringa leaves (Table 1). This is an indication that the Moringa leaves contained tannins, saponins, flavonoid, steroids, terpenoids, cardiac glycosides, anthroquinones and alkaloids as secondary metabolites.

Several functions and roles are attributed to flavonoids in human and animals; this includes protection and fight against inflammatory disorders, allergies, diarrhea, microbes' invasion, platelet aggregation, ulcers, hepatotoxins, viruses, and tumors (Kumar *et al.,* 2010). Flavonoids were able to achieved the aforementioned properties because of their antipyretic (fever-reducing), antioxidant, analgesic (pain-relieving), and spasmolytic (spasm-inhibiting) activities (Krishnaiah *et al.,* 2009). The presence of epicatechin, quercetin and luteolin in flavonoids plays pivotal roles in inhibition of fluids that is responsible for diarrhea (Krishnaiah *et al.,* 2009).

The presence of flavonoids in Moringa leaves is responsible for it's used in acceleration of labour in southwestern Nigeria during birth and this might be linked to high content of flavonoids and phenolic compounds (Okwu *et al.,* 2009).

Phenolic compounds are responsible for blockage of specific enzymes that causes inflammatory disorders. The also protect platelets from clumping through modification of the prostaglandin pathways (Okwu *et al.,* 2009). As a result of the presence of phenolic compound in Moringa leaves, this is a signal that the Moringa leaves could act as antioxidants, anti-clothing agents, immune enhancers, antioxidants, and hormone modulators (Okwu *et al.,* 2009). Moringa leaves can also be used as aphrodisiac because they are rich in phenolic compound which acts as stimulating agents (Kenner and Requena, 2009).

Antibacterial, antifungal, antiviral, cytotoxic, analgesic, and anticancer are arrays of biological properties linked to Triterpenes/triterpenoids. Triterpenoids produced an acid (pentacyclic triterpenoid betulinic acid) which inhibit HIV (Cowan, 1999). Increase the concentration of antioxidants in wounds, strengthening of the skin and restore inflamed tissues by increasing blood supply are few of the fuctions attributed to terpenoids in clinical studies (Krishnaiah *et al.,* 2009). Several others functions and vital roles of terpenoids in herbal medicine includes treatment of burns, psoriasis, prevention of scar formation following

surgery, recovery from an epistomy following vaginal delivery of a newborn, and treatment of external fistulas (Krishnaiah *et al.*, 2009).

Moringa leaves are used as rejuvenating agents and have been found to be a very useful for anti-aging and overall beauty enhancement as a result of the presence of terpenoids (Krishnaiah *et al.*, 2009). Studies have shown that terpenoids reduces insulin requirement in diabetics by between 30 to 50 %, they also reduces complications associated with diabetes and lower sugar level in blood (Krishnaiah *et al.*, 2009).

Moringa leaves are used in the treatment of inflammations, due to the fact that they are rich in saponins, which showed anti-inflammatory activity (Kenner and Requena, 1996). Antimicrobial activity of Moringa leaves was as a result of the presence of saponins (Osborn, 2003). Saponin is used in medicine as gentle blood cleanser (Kenner and Requena, 1996). Saponins are essential in treatment of hypercholesterolemia by preventing cholesterol reabsorption and results in low serum cholesterol by binding to cholesterol to form insoluble complexes excreted via the bile (Olaleye, 2007). Saponin reduces the cholesterol in blood and blood pressure. Thus, saponins reduce the risk of cardiovascular diseases such as hypertension.

Cardiac glycosides, are also known as cardenolides, are cardioactive. They include digitoxin and digoxin which are from foxglove. The major function of plant contains this compound is in the treatment of heart disease. Saponins and cardiac glycosides are cardioactve glycosides and the active component is the steroidal aglycone. Contraction of heart muscle and treatment of various forms of heart failure are few of roles of cardioactive glycosides in human.

Some steroids have cardiotonic activity and some are sex hormones. For example, the steroid hormones, progestogens (e.g, progesterone) and the estrogens (e.g, estradiol) are female hormones responsible for female sexual characteristics, for the maintenance of pregnancy and for the control of menstral cycle. Modified estrogens and progestogens are used in oral contraceptives. Some breast cancers are estrogen dependent. Androgens (e.g, testosterones) are male sexual hormones. Among other properties is that they have a stimulating effect on the development of muscle (anabolic effect). Anabolic steroids are used in stimulating the growth of beef cattle. The cortical steroids (e.g, cortisone) are produced by the adrenal cortex and the two main functions are regulation of mineral balance and conversion of protein to carbohydrate and its storage as glycogens. Cortical steroids have immunosuppressive activity and reduce inflammation. They are used in the treatment of rheumatoid arthritis, asthma, and in creams for reducing inflammation. The cardenolides and bufadienolides groups of steroids are important for their cardiotonic activity and they possess insecticidal and antimicrobial properties. Plants rich in steroids (e.g, *Cheoma rutidosperma*) are used as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as starting material for the synthesis of steroid hormones (Edeoga *et al*., 2005).

Table 1: Qualitative analysis of Aqueous and ethanolics extracts of *Moringa* leaves

Constituents	Aqueous Extract	Ethanol Extract
Tannins	-	++
Phlobatannins	--	--
Saponins	++	+++
Flavonoids	++	+++
Steroids	-	+++
Terpenoids	+	-
Cardiac Glycosides	+	+
Anthroquinones	-	+
Alkaloids	+	+++

Legend;
+++ = very much
++ = much
+ = little
– = nil

3.2 Quantitative analysis of *Moringa* crude extracts

The quantitative analysis of Moringa leaves extracts showed below percentage yield of the chemical constituents, 19.28 and 14.33% for aqueous and ethanolic crude extracts respectively, while the 10.73, 18.46, 8.53, 9.31 and 11.43% each for Alkaloids, Saponins, Total flavonoids, and Tannins Table 2.

Table 2: Quantitative analysis of crude extracts of *Moringa leaves*

Chemical components	Percentage Yields (%)
Aqueous extract	19.28
Ethanolic extract	14.33
Alkaloids	10.73
Saponins	18.46
Phenols	8.53
Total flavonoids	9.31
Tannis	11.43

4. Conclusion

The results obtained from qualitative and quantitative phytochemical analysis of Moringa leaves showed various secondary metabolites that are beneficial in treatment of various human ailments. Most of the constituents found are indication of appraisal in traditional medicine where Moringa formed parts of leaves used in curing of several human diseases. The presence of tannins, saponins, flavonoid, steroids, cardiac glycosides, anthroquinones and alkaloids in moringa leaves is another assertion and confirmation of its used in the traditional medicine to treat diabetes, hypertension, malaria, gonorrhea, dysentery, trypanosomiasis and leprosy.

Further work should focus on antimicrobial properties of Moringa leaves extracts to ascertain the spectrum of activity against antibiotic resistant microbial strains. Further study should be extended to isolation of the phyto-constituents in n-hexane and methanol to fully understand the metabolite profile of the plant.

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