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Study on phytochemical availability, variability and density of *Moringa oleifera*

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Abstract
The increasing awareness of the presence of bioactive components in *Moringa oleifera* plant and their increasing application in management of various disease conditions has prompted the need for further study and validation. Using various quantitative and qualitative methods with spectroscopic determination we evaluated the presence of alkaloids, tannins, saponins, flavonoids, glycosides, terpenoids and phenol. There was a preponderance of alkaloids (13.3%). Result of triplicate proximate analysis show 87.66% Dry matter and 89.48% Nitrogen free extracts. The highest concentration 35.78 mg/L and 30.45 mg/L were obtained for calcium and sodium respectively. Other components identified were moisture, Ash, Protein, lipids and fibre at various concentration. It is concluded that the components of phytochemicals present in *Moringa oleifera* confer on it the ability to ameliorate some disease condition and enhance health.

Keywords: Phytochemicals, qualitative, quantitative, variability, density, *Moringa oleifera*

Introduction
A large number of people living in developing countries have taken advantage of the abundance of several species of flora to leverage advantage as herbal medicine for the management of many ailments. A variety of communicable and non-communicable diseases ranging from acute to chronic and end stage have been applied with herbal preparations that have yielded significant result, there is however still a knowledge gap on the available phytochemicals of these herbal drugs that need further enquiry and scientific scrutiny that can lead to the validation of their potential claim as herbal medicine.

Moringa oleifera, also referred to as the Miracle tree, drumstick or horseradish is a drought resistant and fast-growing tree cultivated across the tropic worldwide for therapeutic and nutritional uses [1]. It is a cheap and readily available plant source whose different parts are locally and traditionally used in the treatment and management of various diseases like prostate enlargement [2]. In southwest Nigeria, the immersion of *Moringa oleifera* leaves in alcohol is widely used by traditional healers for the treatment of prostate and bladder problems.

Some earlier studies on *Moringa oleifera* has reported the presence of phytochemicals [3-5], which includes alkaloids, amines, vitamins, sterols, glycosides, amino acids and flavonoids. These phytochemicals have been identified from various parts of the plant such as the seeds and dried leaves. Moreover, other parts of the plant such as roots, flowers, barks and fruits have been applied in diverse conditions. Its application is not only restricted to medicine but is also widely applied in water treatment, food preparation and as biodiesel. Its diverse biological activities include protecting DNA damage, anti-inflammatory, anti-artherosclerotic, cardio-protective and antiperoxidative. Moreover, its use in water coagulation and as proteins and fatty acid esters have been documented.

To a large extent, the multifunctionality of bioactive products of *Moringa oleifera* have been considered safe [6, 7]. Previous works by [8, 9, 10] have elucidated *Moringa oleifera* as exquisite source of glucosinolates such as 4-0-(a-l-rhamnopyranosyloxy)-benzyl-gluccosinate and benzyl glucosinolate (glucotropaeolin). There is a variation in the bioactive phytochemicals present in various parts of the plant. Several ions and trace elements such as potassium, magnesium and calcium and iron are also present at various concentration.

In this work, we have done qualitative, and quantitative determination and proximate analysis of the plant.

Materials and Methods

Moringa oleifera leaves and stems were used for the study after identification. Fresh leaves were collected, washed and allowed to air dry for 3 weeks. Using an electric blender, the air- dried leaves were grounded to powder. 300g of the powdered leaves were soaked in 1.5L of ethanol for 72 hours for ethanol extraction using a rotary vacuum evaporator at 5ºC and 40rpm.

Fresh stems of *Moringa oleifera* were collected, washed and air dried for 3 weeks. The dried stems were grounded to powder using electric blender. About 600g of the powder was then soaked in 2.5L of ethanol for 72 hours for extraction. The extract was collected, filtered with cheese cloth and then concentrated under decreased pressure using rotary evaporator at 50ºC and 40rpm.

Qualitative analysis for identification of active ingredients were carried out for alkaloids, flavonoids, saponins, tannins and phenols using the methods of (Harbone 1973) [11].

Qualitative determination of terpenoids and steroids were carried out by the methods of Salkowski and Kokate 2001 [12] respectively.

Quantitative determination was done for alkaloids, tannins, saponins and flavonoids using the method of Abodoni and Achuko (2001) [13], cardiac glycosides Botello *et al*., 2019 [14].

Proximate analysis was done for % moisture, % crude protein, % crude lipid, % crude fibre, % dry matter and % Nitrogen free-extract, using the method of Oetzel *et al*. 1993 [15].

Results

Results of qualitative and quantitative determination of the various bioactive present in *Moringa oleifera* are shown in tables 1 and 2. Results of tests done for proximate analysis of the plant extract is shown in Table 3 while that for mineral content is shown in Table 4.

Table 1: Quantitative phytochemical screening of M. oleifera

Sample code	ALK	TAN	SAP	FLAV	GLY	TERP	PHEN
i	++	++	++	++	+	+	+
ii	+++	+	+	+	+	+	+
iii	++	++	+	++	+	+	+

Triplicate determinations of various phytochemical of *Moringa oleifera* leaves revealed the presence of alkaloids (ALK), Tannins (TAN), Saponins (SAP), Flavonoids (FLAV), Cardiac Glycosides (GLY), Terpenoids (TERP).

Table 2: Quantitative phytochemical Determination of *Moringa oleifera*

Sample code	%ALK	%TAN	%SAP	%FLAV	%GLY	%TERP	%PHEN
i	13.52	6.23	3.16	0.64	0.11	0.28	1.24
ii	13.08	6.28	3.24	0.72	0.12	0.24	1.20
iii	12.78	6.32	3.18	0.68	0.11	0.31	1.26

Quantitative determination of *Moringa oleifera* extracts reveals the percentage average yield of the chemicals, Alkaloid (13.13%) Tannins (6.3%), Saponins (3.2%), Flavonoids (0.68%), glycosides (0.11%), Terpenoids (0.28%) and phenol (1.2%).

Table 3: Proximate analysis of *Moringa oleifera* plant extract

Sample code	%Moist	%Ash	%Prot	%Lipid	%FIB	%DM	%NFE
i	12.32	0.54	7.65	0.72	1.62	87.68	89.47
ii	12.36	0.52	7.63	0.74	1.60	87.64	89.51
iii	12.34	0.53	7.67	0.70	1.63	87.66	89.46

Results of Triplicate determinations of Proximate analysis are as shown on the table 12.34% moisture, 0.53% Ash, 7.65% protein, 0.72% Lipids, 1.62% Fibre, 87.66% Dry matter and 89.48% Nitrogen free extract.

Table 4: Mineral content of Moringer oleifera

Sample code	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	Fe mg/L	Mn mg/L	Cu mg/L	Zn Mg/L	Po_4 mg/L
i	35.76	18.50	30.42	17.14	2.214	0.34	0.13	0.075	0.56
ii	35.80	18.48	30.46	17.18	2.216	0.38	0.15	0.077	0.54
iii	35.78	18.46	30.48	17.16	2.212	0.32	0.17	0.073	0.58

Results of triplicate determination of various mineral content of *Moringa oleifera* are shown on table 4.

Discussion

Studies on phytochemistry of plants have elucidated evidence of the present of a variety of bioactive components that have enhanced their use in medication and industrial applications. There is now a plethora of evidence to support the fact that products of most plants and herbs are a source of multifunctional curing agents and most are considered to be safe for human use as drugs [16, 17].

Exhaustive study of M. oleifera in-vivo and in-vitro has been used to confirm the rich phytoconstituents and advantages has been taken of these for the rationalization of this plant as a unique source of bioavailability and biological potency both of which are strong attributes for adequate recommendation of phytochemicals [18]. Several areas of application have been identified such as in cosmetics manufacturing, the food industries and in the production of biodiesels. Moreover, they have found application in insulin homeostasis, as antioxidants and both as anti-cancer and anti-tumour applications [19].

In this work, we have used methanolic extracts possessing high polarity that enhances the solubility of the various molecules which are known to confer unique bioactive properties which include hepatoprotective, anti-inflammatory, antiperoxidative and anti-proleiferative potentials.

Our quantitative and qualitative analysis revealed the presence of functional bioactive compounds such as phytosterols, natural sugars, phenolic acid, flavonoids, alkaloids, organic acids, vitamins and minerals. We identified low molecular weight proteins. Studies have revealed that cationic proteins present in M. oleifera are useful as ideal substance for water and waste water treatment that function to eliminate turbidity, noxious toxic ions and a range of microbial species involved in water pollution, coagulation efficiency that are in tandem with chemical coagulants found in raised turbidity water [20]. Previous studies have given credence to the fact that compounds such as isocthocynates play a role in glucose regulation by virtue of its ability to improve insulin sensitivity with a resultant increase in glucose uptake by the cells.

We have observed in our quantitative determination Alkaloids, Tannins, Phenols and flavonoids with percentages of 10.73%, 11.43%, 8.3% and 5.2% respectively. Values obtained are in agreement with [21]. These bioactive components have been known to play active role in management of various diseases. In addition, saponin is popular for its use as adjuvant in the production process of vaccines and in addition as anti-inflammatory and immune stimulant [22]. The role of flavonoids in cancer management, allergy and platelet aggregation are available in literature [23].

Terpenoids are known to produce pentacylic triterpenoid betulinic acid a chemical that has antiviral property by their

ability to inhibit viral invasion.

Proximate analysis of the plant extract show the presence of moisture (0.53%), Ash (7.65%) Protein (0.72%), Fibre (1.6%), Lipid (1.6%), Dry matter (87.66%) and Nitrogen free extract 89.48%. while some other works have been able to isolate these components there is however variation from values obtained, a factor that could be attributed to the ecological niche from which the plants were derived.

Evaluation of mineral content revealed the presence of calcium, magnesium, sodium, manganese, copper, zinc, and phosphates existing at varied concentration with a preponderance of calcium and sodium 35.78mg/L and 30.45mg respectively with copper sowing the least concentration of 0.075mg/L. These minerals act in diverse ways in several reaction pathways and as components of enzymes and proteins to maintain homeostasis.

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