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Phytochemical profile and antioxidant capacity of leaves of *Moringa oleifera* (*Lam*) extracted using different solvent systems

Okumu MO, Mbaria JM, Kanja LW, Gakuya DW, Kiama SG and Ochola FO

Okumu Mitchel Otieno
Department of Public Health Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya.

Mbaria James Mucunu
Department of Public Health Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya.

Kanja Laetitia Wakonyu
Department of Public Health Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya.

Gakuya Daniel Waweru
Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya.

Kiama Stephen Gitahi
Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya.

Ochola Francis Okumu
Department of Pharmacology and Toxicology, Faculty of Medicine, Moi University, Eldoret, Kenya.

Correspondence
Okumu Mitchel Otieno
Department of Public Health Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya
mytchan88@gmail.com

Abstract
Traditional medicinal folklore, oral tales and histories are rife with information of the potential utility of various plant parts. However, before these claims can be validated, it is important to assess the chemical components of the plant parts. The phytochemical composition and antioxidant capacity of various parts of *Moringa oleifera* (*MO)* have not been exhaustively defined at this time. The current study evaluated the phytochemical composition and antioxidant capacity of leaves of *MO* cultivated in Kibwezi, Makueni County in Kenya. *MO* leaves were extracted using two solvent systems; water (AQ) and aqueous-methanol (AQ-ME). Preliminary qualitative phytochemical screening of AQ and AQ-ME *MO* leaf extracts revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, tannins and ascorbic acid. Antioxidant capacity of *MO* leaf extracts was expressed in terms of total phenolic, flavonoid and ascorbic acid contents. The AQ-ME *MO* leaf extract had significantly higher ($p<0.05$) antioxidant capacity than the AQ *MO* extract. The total phenolic content of the AQ and AQ-ME extracts was 35.42 ± 5.80 and 52.04 ± 3.12 milligrams of gallic acid equivalents per gram of the dry plant material (mg.GAE.g^{-1}) respectively and the total flavonoid content was 79.13 ± 13.04 and 366.09 ± 86.96 milligrams of catechin equivalents per gram of the dry plant material (mg.CE. g^{-1}) respectively while the ascorbic acid content was 2.02 ± 0.66 and 3.04 ± 2.06 milligrams of ascorbic acid equivalents per gram of dry plant material (mg.AAE.g^{-1}) respectively. The results suggest that the aqueous-methanol solvent system has better potential of extracting antioxidant components from leaves of *Moringa oleifera* than water used alone.

Keywords: Phytochemical composition, antioxidant capacity, *Moringa oleifera*, Kibwezi, aqueous-methanol, aqueous

Introduction
Health is an important aspect in the economic growth of any country because a healthy population lives longer and is more productive. In Kenya, a sedentary lifestyle adopted by a thriving middle class has resulted in increased incidences of heart diseases, cancers, arthritis, neurodegenerative and liver diseases. A relationship has been identified between these diseases and reactive oxygen species (ROS) which damage biological cells, tissues and membranes [1]. Antioxidant substances are now used in a bid to prevent this damage [2]. However, resource constraints and safety considerations have limited the use of synthetic antioxidants [3] resulting in a fuelled interest in the search for plant-derived natural antioxidants [4]. *Moringa oleifera* is the most popular plant in several species of the Moringaceae family [5]. It has a rapid rate of growth and is resistant to drought. The plant traces its origin to the Himalayan areas of the Indian sub-continent but has recently been found to thrive in the tropics and subtropics. Thus, it has several synonyms including horse radish, drumstick tree, morunga, sajna, benzolive tree among others [6]. Moringa has been associated with a variety of nutritional, medicinal and miscellaneous uses [7]. Antioxidant properties have been reported [8] but scanty scientific evidence is available to support this claim. Moreover, most antioxidant capacity studies of plants focus on single solvent systems. The present work is a comparative analysis of the phytochemicals present in two leaf extracts of Kibwezi cultivated *Moringa oleifera* as well as their antioxidant capacity in terms of phenolic, flavonoid and ascorbic acid contents.

Fig 1: Cultivation of *Moringa oleifera* plant in Kibwezi, Makueni County, Kenya

Fig 2: Aerial part of *Moringa oleifera*

Materials and Methods

Reagents and standards

Folin-Ciocalteau phenol reagent, gallic acid, catechin and ascorbic acid were purchased from Sigma chemical company (St Louis MO, USA). All other reagents and chemicals were of analytical grade and of high purity.

Plant material

Fresh aerial parts of *Moringa oleifera* were collected from the University of Nairobi field station in Kibwezi, Makueni County in Kenya in the month of October 2015 (Figure 1). The leaves were identified at the National Museums Herbarium, Nairobi Kenya and a voucher specimen deposited for future reference.

Preparation of plant material

The leaves (Figure 2) were thinly spread on a mesh tied on racks in a well-ventilated, insect, rodent and dust free room. They were allowed to air dry for 10 days. The dried leaves were then ground into fine powder using an electric mill at the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi.

Preparation of plant extracts

The dried *Moringa oleifera* leaf powder was used to prepare two extracts using the method of [9] with modifications.

Aqueous Extract

Two hundred (200) grams of dried *Moringa oleifera* leaf powder was accurately weighed on an analytical balance and poured into a 1000ml conical flask wrapped in aluminum foil. Eight hundred (800) ml of distilled water was then gradually added to the powder with gentle shaking of the flask until slurry of uniform consistency was formed. Phytochemicals present in the leaf powder were extracted using a magnetic bar and magnetic stirrer operating at 200 revolutions per minute (RPM) for 48 hours. The resultant slurry was then centrifuged at 3000 revolutions per minute (RPM) for 5 minutes. The supernatant was then collected into light-resistant bottles and freeze-dried.

Aqueous-Methanol Extract

Two hundred (200) grams of dried *Moringa oleifera* leaf powder was accurately weighed on an analytical balance and poured into a 1000ml conical flask wrapped in aluminum foil. Eight hundred (800) ml of a methanol-water co-solvent system in the ratio 80:20 v/v was then gradually added to the powder with gentle agitation until slurry of uniform consistency was formed. The phytochemicals present in the leaf powder were then extracted using a magnetic bar and stirrer operating at 200 revolutions per minute for 48 hours. The resultant slurry was then centrifuged at 3000 revolutions per minute (RPM) for 5 minutes, the supernatant collected and subsequently transferred to a rotary evaporator set at 45 °c to remove excess solvent and concentrate the extract. Further solvent removal and concentration of the extract was done in a sand bath for 24 hours.

Phytochemical screening of *Moringa oleifera* leaf extracts

The methods of [10,11,12] were used to test for the phytoconstituents present in the plant extracts and results graded as high (++), moderate (+) or nil (-) based on the intensity of the colour produced from the reactions.

Test for alkaloids (Dragendorff test)

Procedure: Approximately 50mg of each of the extracts were dissolved in 5ml of distilled water.2M hydrochloric acid (HCL) was then added until acid reaction was noted. The resultant was then filtered and 2ml of the filtrate placed in a test tube. To this, 1ml of Dragendorff's reagent was added along the sides of the test tube.

Test for anthraquinones (Borntraggers test)

Procedure: Approximately 5 mg of the extracts were shaken with 10ml of benzene and then filtered. Five (5) ml of 10% ammonia solution (NH_3) was then added to each of the filtrates. The resultant mixture was then shaken.

Test for cardiac glycosides (Keller-Killiani test)

Procedure: 0.5gms of each of the extracts was diluted with 5ml of water. Then 2ml of glacial acetic acid was added followed by 2 drops of Ferric chloride solution ($FeCl_3$). Finally, one (1) ml of concentrated sulphuric acid (H_2S0_4) was then added along the sides of the test tube.

Test for coumarins (Fluorescence test)

Procedure: Approximately 1mg of each of the extracts were dissolved in 2ml of water. The resultant solution was then divided into 2 portions. To the first portion 0.5ml of 10% w/v of ammonia solution (NH_3) was added. The second portion acted as reference.

Test for flavonoids (alkaline reagent test)

Procedure: To 1 ml of each of the extracts 5 drops of 5% sodium hydroxide (NaOH) was added followed by 2M hydrochloric acid (HCl)

Test for phenolics (Ferric chloride test)
Procedure: To approximately 1 mg of each of the extracts 2 ml of distilled water was added followed by a few drops of 10% aqueous Ferric chloride solution ($FeCl_3$)

Test for phytosterols (Liebermann-Burchard's test)
Procedure: 2mg of each of the extracts were dissolved in acetic anhydride (CH_3-COO-CH_3) and heated to boiling, cooled and then 1ml of concentrated sulphuric acid (H_2SO_4) was added along the sides of the test tube.

Test for saponins (Foam test)
Procedure: 5 ml of the test solution of each of the extracts were taken in a test tube and shaken well for 5 minutes.

Test for tannins (Ferric chloride test)
Procedure: 2ml of the test solution of each of the extracts were added with 5% Ferric chloride solution ($FeCl_3$)

Test for triterpenes (Salkowski test)
Procedure: Approximately 2 mg of dry extracts were shaken with 1ml of chloroform ($CHCl_3$) and a few drops of concentrated sulphuric acid (H_2S0_4) were added along the sides of the test tube.

Test for ascorbic acid (Dichlorophenol Indophenol-DCPIP test)
Procedure: 50ml of the dichlorophenol indophenol was filled in a burette. Five (5) ml of the sample extracts were then poured in 100ml beakers. Titration was then performed and the volume of dichlorophenol indophenol required to decolorize each of the sample extracts was noted.

Quantitative analysis
Determination of Total Phenolic Content of crude leaf extracts of *Moringa oleifera*
The phenolic content of the sample extracts was determined by the methods of [13, 14] with modifications. Gallic acid monohydrate was used as the standard and represented the phenolic compound in the *Moringa* plant. Ten (10) mg of this standard was dissolved in 100ml of methanol to give a stock solution of 100µg/ml concentration.

Preparation of standard calibration curve of gallic acid
Aliquots of 0.25, 0.5, 1.0, 1.5 and 2.0 from the above stock solution were taken in 5 different 10ml volumetric flasks. To each flask, 2.5ml of a tenfold dilution of Folin-Ciocalteau phenol reagent (1:10 v/v with distilled water) and 2.0 ml of a 7.5% w/v sodium carbonate (Na_2CO_3) solution were added. The mixture was then made up to the mark with distilled water to make concentrations ranging from 2.5-20 µg/ml. A mixture of reagents and water was used as blank. The resultant solutions were then kept on a water bath set at 45 °C for 15 minutes. Thereafter, the absorbance was read at 765nm using a Milton Roy Spectronic 21D UV-VIS Spectrophotometer (USA). A standard calibration curve of Absorbance (y) against Concentration (x) was then plotted.

Preparation of sample extracts solution
Ten (10) mg of each of the extracts was dissolved in 10ml of methanol to get 1mg/ml solution. One (1) ml of this solution was then placed in a 10ml volumetric flask and colour development carried out in the same manner as for the standard. Absorbance of the test solutions were then measured at 765nm against a reagent blank (distilled water). The tests were done in triplicate, results averaged and expressed as mean ± standard error of the mean (SEM). The concentration of phenolics in the test samples were then determined by extrapolation from the gallic acid standard calibration curve and calculated as milligrams of gallic acid equivalents per gram of the dry plant material (mg.GAE.g^{-1}) using the formula as described by [15];

$$P = C \times V/M$$

Where;
P = Total phenolic content in milligrams per gram of the dry plant material (mg. g^{-1})
C = Concentration of gallic acid established from the calibration curve in milligram per milliliter (mg/ml)
V = Volume of the extract solution in milliliters (ml)
M = Weight of the extract in grams (g)

Determination of Total Flavonoid Content (TFC) of crude leaf extracts of *Moringa oleifera*
The flavonoid content of the sample extracts was determined according to a modified method [16]. Catechin was used as the standard and represented the flavonoid compound in the *Moringa* plant. Ten (10) mg of catechin was dissolved in 100ml of methanol to give a stock solution of 100 µg/ml concentration.

Preparation of standard calibration curve of catechin
Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml from the above solutions were taken in 5 different 10ml volumetric flasks containing 4 ml of distilled water. To these, 0.3mL of 5% w/v Sodium Nitrite ($NaNO_2$) was added. After 5 minutes, 0.3ml of 10% w/v Aluminium chloride ($AlCl_3$) was added. After a further 6 minutes, 2ml of 1M sodium hydroxide (NaOH) was added and the total volume made up to 10ml with distilled water. Absorbance was then read at 510nm using a Milton Roy Spectronic 21D UV-VIS Spectrophotometer (USA) against distilled water (reagent blank). The calibration curve was prepared by plotting a graph of Absorbance (y) against Concentration (x) (Figure 4).

Preparation of sample extracts solution
Ten (10) mg of each of the extracts was dissolved in 10ml methanol to get a solution of 1mg/ml concentration. An aliquot (1ml) of each of the extracts were added to 10ml volumetric flasks containing 4ml of distilled water. To this, 0.3ml of 5% w/v Sodium Nitrite ($NaNO_2$) was added. After 5 minutes, 0.3ml of 10% w/v Aluminium chloride ($AlCl_3$) was added. After a further 6 minutes, 2ml of 1M Sodium hydroxide (NaOH) was added and the total volume was made up to 10ml with distilled water. Absorbance was read at 510nm using a Milton Roy Spectronic 21D UV-VIS Spectrophotometer (USA). Distilled water was used as reagent blank. The tests were done in triplicate, results averaged and expressed as mean ± standard error of the mean (SEM). The concentration of flavonoids in the test samples were then determined by extrapolation from the standard calibration curve and calculated as milligrams of catechin equivalents per gram of the dry plant material (mg. CE. g^{-1}) using the formula as described by [15] with modifications;

$$F = C \times V/M$$

Where;
F = Total flavonoid content in milligrams per gram of dry plant material (mg. g^{-1})

C = Concentration of catechin established from the calibration curve in milligrams per milliliter (mg/ml)
V = Volume of the extract solution in milliliters (ml)
M = Weight of the extract in grams (g)

Determination of ascorbic acid contents of crude leaf extracts of *Moringa oleifera*
The total ascorbic acid contents of the crude leaf extracts of *Moringa oleifera* were determined by a modified method of [16].

Preparation of standard ascorbic acid solution
Ascorbic acid was used as standard for the estimation of Vitamin C. Ten (10) mg of ascorbic acid was dissolved in 100ml of distilled water to give a stock solution containing 100 µg/ml.

Preparation of standard calibration curve of ascorbic acid
Aliquots of 0.05, 0.1, 0.25, 0.5, 1.0 and 1.5 ml of the above solution were taken in 6 different 10ml standard flasks. One hundred (100) µl of 13.3% Trichloroacetic acid (TCA) and 100µl of distilled water were then added to each of the flasks followed by 75 µl of Dinitrophenyl hydrazine (DNPH;2g Dinitrophenyl hydrazine,230mg Thiourea and 270mg $CuSO_4.5H_2O$). The reaction mixtures were subsequently incubated for 3 hours at 37 °C, then 0.5ml 65% v/v of sulphuric acid (H_2SO_4) was added to the medium followed by absorbance measurement at 520nm using a Spectronic 21D Milton Roy UV-VIS Spectrophotometer (USA).

Preparation of sample extracts solution
10mg of each of the extracts was dissolved in 10ml distilled water to get a concentration of 1mg/ml. 0.3 ml of each of the extract solutions were then mixed with 100µl of Trichloroacetic acid (TCA) and 100µl of distilled water followed by 75µl Dinitrophenyl hydrazine; DNPH (2g Dinitrophenyl hydrazine,230 mg Thiourea and 270mg $CuSO_4.5H_20$ in 100 ml of 5M H_2SO_4) and subsequently incubated for 3 hours at 37 °C .0.5ml of 65% of sulphuric acid (H_2SO_4) v/v was then added to the medium followed by absorbance measurement at 520nm using a Spectronic 21D Milton Roy UV-VIS Spectrophotometer (USA). The tests were done in triplicate, results averaged and expressed as mean ± standard error of the mean (SEM). The concentration of ascorbic acid in the extracts was then determined by extrapolation from the standard curve and calculated as ascorbic acid equivalents per gram of the dry plant material ($mg.AAE.g^{-1}$) using the formula as described by [15] with modifications;

$$V = C \times V / M$$

Where;
V = Ascorbic acid content in milligrams per gram of dry plant material ($mg.\ g^{-1}$)
C = Concentration of ascorbic acid established from the calibration curve in milligrams per milliliters (mg/ml)
V = Volume of the extract solution in milliliters (ml)
M = Weight of the extract in grams (g)

Statistical analysis
The results of all experiments were reported as mean ± standard error of the mean (SEM) of three separate determinations. Statistical analysis was performed using analysis of Variance (ANOVA) using GenStat® 4th Edition statistical software. $p<0.05$ was considered significant.

Results and Discussion

Extraction yield
Two extracting solvents, water and aqueous methanol (80% methanol v/v) were evaluated for their efficiency in extracting antioxidant compounds from *MO*. The use of aqueous methanol solvent produced 17.51grams of extract per 100grams of dry *MO* leaves which was higher than water which produced 14.23grams of extract per 100grams of dry *MO* leaves (Table 1). This compares well with the work of other authors who reported that a co-solvent system comprising of water and organic solvents gave better extraction yields of *Mentha spicata. L* [17] and *Brassica oleracea L.* [9]. In most studies on *MO,* single solvent systems using either water [18] or ethanol [19] have been used. The few authors who have investigated the influence of extraction solvents on antioxidant capacity did not include aqueous-organic solvents in their studies [20]. Based on the results of this investigation, it could be suggested that a mixture of water and organic solvents gave better recovery of antioxidant components from *MO* than a purely aqueous solvent. Thus, this co-solvent system may be given consideration in future studies involving extraction of antioxidants in this plant.

Table 1: Effect of the different solvent systems on the extraction yield (percentage weight by weight) from leaves of *Moringa oleifera*.

Extracting solvent	Weight of sample powder taken (in grams)	Yield of extract obtained (in grams)	Percentage yield of extract (%w/w)
Water	200	28.46	14.23
80% methanol	200	35.02	17.51

Preliminary phytochemical screening of *M. oleifera* leaf extracts
The results of preliminary phytochemical analysis of the extracts are as shown in Table 2. The results revealed the presence of pharmacologically active chemical compounds such as alkaloids, cardiac glycosides, flavonoids, phenolics, phytosterols, saponins, tannins and ascorbic acid.

Table 2: Preliminary phytochemical profile of *Moringa oleifera* leaf extracts

Phytoconstituent/ Metabolite	AQ	AQ-ME
Alkaloids	+	+ +
Anthraquinones	-	-
Cardiac glycosides	+	+ +
Coumarins	-	-_
Flavonoids	+	+ +
Phenolics	+	+ +
Phytosterols	+ +	-
Saponins	+	+ +
Tannins	+	+ +
Triterpenes	-	-
Vitamin C	+ +	+ +

Key;
+ low concentration (trace but detectable amounts)
++ high concentration (high amount)
-Absent

Owing to the differences in agro-climate in different regions of the world, there is an overwhelming need to perform complete phytochemical analysis of locally grown plants to ascertain their phytochemical profile. Previous work on the phytochemical profile of *MO* by various groups of researchers [21-23] revealed that both intrinsic and extrinsic factors cause variations in the secondary metabolites of the plant. In the present investigation, having used the methods of [10-12] to qualitatively screen the phytoactive chemicals in the plant extracts, the presence of alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, tannins and ascorbic acid was established in both extracts. No phytosterols were present in the AQ-ME extract which concurs with what was reported by [23]. Furthermore, both extracts tested negative for anthraquinones, coumarins and triterpenes. This observation compares well with those of earlier studies which established that not all phytochemicals are present in all the parts of a plant and for those present, their relative abundance varies depending on the source of botanical material, extraction process as well as several other factors such as temperature, and choice of solvent which also ultimately affect the extraction efficiency [24-26]. The phytochemical compounds reported in the present study are known to have biological activities in tissues and cells. Furthermore, previous workers have shown the importance of total phenolics, total flavonoids and ascorbic acid contents as parameters of antioxidant capacity of medicinal plants [27, 28].

Phenolic content of the extracts

The phenolic content of the different leaf extracts of *MO* was determined spectrophotometrically using the Folin-Ciocalteau assay method and expressed as gallic acid equivalents (GAE). There was a significant variation (*p<0.05*) in the ability of the solvents to extract phenolic compounds (Table 3). The aqueous-methanol solvent system was superior in extracting phenolics from *MO* leaves than water used on its own. The standard calibration curve of gallic acid (y=0.1311x - 0.0123) showed linearity in the range of 1-20µg/ml with a correlation coefficient (r^2) of 0.9989 (Figure 3). The AQ-ME extract contained significantly higher (*p<0.05*) contents of phenolics; 52.04 ± 3.12 mg.GAE.g^{-1} compared to 35.42 ± 5.80 mg.GAE.g^{-1} of the AQ extract (Table 3). Phenolics are one of the major secondary metabolites that are present in plant materials and have been associated with antioxidant activities [29]. The findings of this work compare well with those of other workers who established that aqueous organic solvents gave better phenolic contents than single solvents [9]. However, the values obtained were much lower than what was reported for *Malvaceae* species [30] and *Vitis vinifera* [31]. Within Moringa literature, [32] and [33] reported much lower values of phenolic content than that reported in the current studies.

Flavonoid content of the extracts

The flavonoid content of the different *MO* leaf extracts was determined spectrophotometrically and expressed as catechin equivalents (CE). There was a significant variation (*p<0.05*) in the ability of the solvents to extract phenolic compounds (Table 3). Again, the aqueous methanol solvent was superior in extracting flavonoids from *MO* leaves. The standard calibration curve of catechin (y=0.0023x + 0.0038) showed linearity in the range 10-100 µg/ml with a correlation coefficient (r^2) of 0.9995 (Figure 4). Previous studies have shown strong correlation between the total flavonoid content and antioxidant activity of medicinal plants [34]. Flavonoids are secondary metabolites naturally occurring in fruits, vegetables and other plant parts. The AQ-ME *MO* leaf extract contained 366.09 ± 86.96 mg. CE. g^{-1} which was significantly (*p<0.05*) higher than that of the AQ *MO* leaf extract; 79.13 ± 13.04 mg. CE. g^{-1} (Table 2). This may be attributable to the differences in the polarities of the solvents used. However, the values obtained from this study were much lower than those of a study on Bruckwheat groats by [35]. Literature on flavonoid content in the Moringa plant is scanty. [36] reported total flavonoid contents in Moringa that were lower than those reported presently.

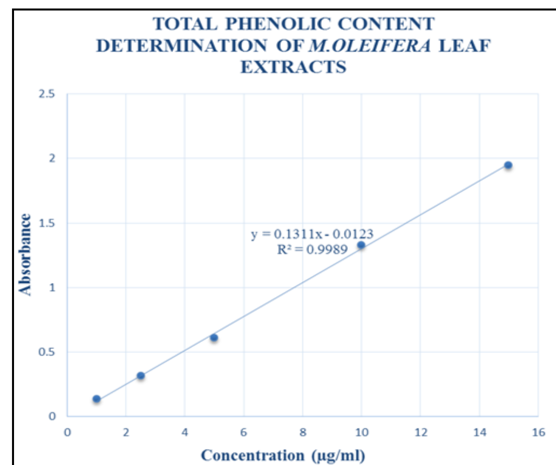

Fig 3: Standard calibration curve of absorbance against concentration of gallic acid prepared by using a UV-VIS spectrophotometer

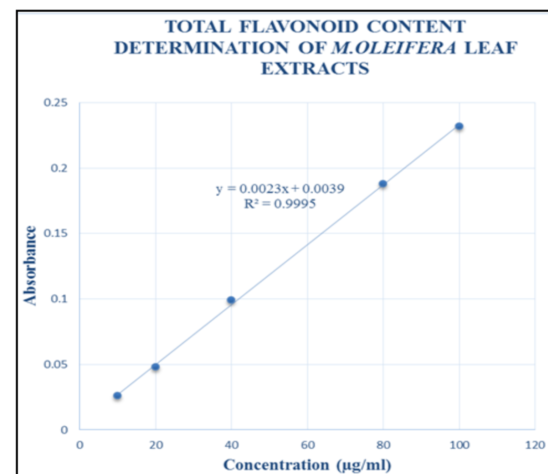

Fig 4: Standard calibration curve of absorbance against concentration of catechin prepared by using a UV-VIS spectrophotometer

Ascorbic acid content of the extracts

Quantification of ascorbic acid in the different leaf extracts of *Moringa oleifera* was determined spectrophotometrically and expressed as ascorbic acid equivalents (AAE). There was a significant variation (*p<0.05)* in the ascorbic acid contents between the two extracts. The aqueous methanol solvent extracted more ascorbic acid from Moringa leaves; 3.04 ± 2.06 mg. AAE. g^{-1} compared to 2.02 ± 0.66 mg.AAE.g^{-1} by water (Table 3). The standard calibration curve of ascorbic acid (y=1.4551x - 0.029) showed linearity in the range 0.05 to 1.0 µg/ml with a correlation coefficient (r^2) of 0.9054 (Figure 5). Ascorbic acid (vitamin C) has long been reported to

contribute to the antioxidant activity of plant materials. It also acts as a good reducing agent and exhibits antioxidant activity by donating electrons [37]. The relative ease by which ascorbic acid dissolves in various solvent systems enables it to scavenge for free radicals before they damage lipids and biological membranes [38]. The ascorbic acid contents reported presently were higher than those reported on *Moringa oleifera* leaves and *Newbuoldia laevis* cultivated in Nigeria [32].

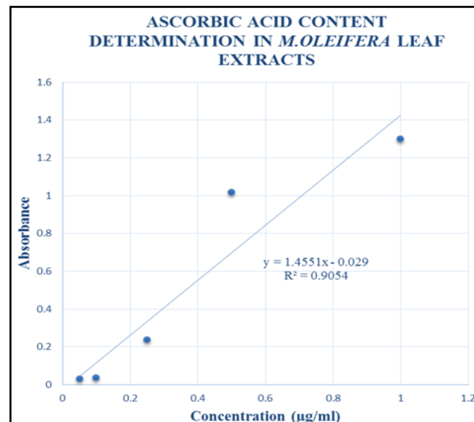

Fig 5: Standard calibration curve of absorbance against concentration of ascorbic acid prepared by using a UV-VIS spectrophotometer

Table 3: Quantification of antioxidants present in *Moringa oleifera* leaf extracts

Extract	Total phenolic contents mg.GAE.g^{-1}	Total flavonoid contents mg. CE. g^{-1}	Ascorbic acid contents mg.AAE.g^{-1}
AQ	35.42 ± 5.80	79.13 ± 13.10	2.02 ± 0.66
AQ-ME	52.04 ± 3.13	366 ± 86.96	3.04 ± 2.06

Values are expressed as mean ±standard error of three separate determinations. AQ; Aqueous, AQ-ME; Aqueous-methanol, GAE; Gallic acid equivalents, CE; Catechin equivalents, AAE; Ascorbic acid equivalents.

Conclusions

In conclusion, results of this present investigation indicate that the choice of extracting solvent has a significant influence on the extraction yield and phytochemical composition of the leaves of *Moringa oleifera*. When the aqueous-methanol solvent was used, extraction yield and antioxidant capacity parameters were consistently higher than when water was used on its own. Thus, a mixture of water and methanol may be considered for future extraction of antioxidant compounds from the leaves of plants. However, further studies are needed to elucidate the structure of the particular molecules responsible for the antioxidant properties of this plant as are *in vivo* studies to confirm the utility of these extracts in the mitigation of free radical related diseases.

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References

1. Vuillaume M. Reduced Oxygen Species. Mutation, induction and Cancer initiation. Mut Res. 1987; 186:43-72.
2. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39:44-84.
3. Bonilla F, Mayen M, Merida J, Medina M. Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. Food Chemistry. 1999; 66:209.
4. Sharma SK, Agrawal PR, Sharma BS, Chadha MK, Khosla A, Saxena K. Cytotoxic and apoptotic activity of essential oil from Ocimum viride towards COLO 205 cells. Food Chem. Toxicol. 2010; 48:336-344.
5. Jahn SAA, Musnad HA, Burgstaller H. The tree that purifies water-cultivating multipurpose Moringaceae in the Sudan. Unasylva 1986; 152:23-28.
6. Jed W Fahey. Moringa oleifera: A Review of the Medical Evidence for its nutritional, therapeutic and prophylactic properties, Part 1. Trees for Life Journal. 2005; 1:5.
7. Dharmendra Singh, Priya Vrat Arya, Ved Prakash Aggarwal and Radhey Shyam Gupta: Evaluation of Antioxidant and Hepatoprotective Activities of Moringa oleifera Leaves in Carbon-Tetrachloride Intoxicated Rats. Antioxidants. 2014; 3:569-591. 2014.ISSN 2076-3921.
8. Santos AF, Argolo AC, Paiva PM, Coelho LC. Antioxidant activity of Moringa oleifera tissue extracts. Phytother Res. 2012; 26(9):1366-1370.
9. Anwar F, Kalsoom U, Sultana B, Mushtaq M, Mehmood T, Arshad HA. Effect of drying method on the Total Phenolics and Antioxidant Activity of Cauliflower (Brassica oleraceae L) extracts. International Food Research Journal. 2013; 20(2):653-659.
10. Harborne JB. `Phytochemical methods: A guide to modern techniques of plant analysis, 'Chapman and Hall, London. 1998.
11. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan, Delhi, ED. 2005; III:107-121.
12. William Charles Evans. Trease and Evans Pharmacognosy.16th Edition, Saunders Ltd, Edinburgh. 2009, 585-589.
13. Harnafi H, Caid HS, Bounani HS, Aziz M, Armani S. Hypolipemic activity of polyphenol rich extracts from Ocimum basilicum in Triton WR-induced hyperlipidemic mice, Food Chem. 2008; 108:205-211.
14. Singleton VL, Orthofer R, Lamueia-Raventos RM. Analysis of antioxidants by means of Folin-Ciocalteau reagent. Methods Enzymol. 1999; 299:152-17.
15. Sandra Gouveia and Paula Castilho. Antioxidant potential of *Artemisia argentea* L'Her alcoholic extract and its relation with the phenolic composition. Food Research International.2011. Vol.44(6):1620-1631
16. Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological

contaminants in medicinal herbs. Journal of the University of Chemical Technology and Metallurgy. 2011; 46(1):81-88.

17. Benderritter M, Maupoli V, Vergely C, Dalloz F, Briot F, Rochette L. Studies by electron paramagnetic resonance of the importance of iron in the hydroxyl scavenging properties of ascorbic acid in plasma: Effect of iron chelators. Fundamentals of Clinical Pharmacology. 1998; 12:510-516.
18. Brahmi Fatiha, Madani Khodir, Dahmoune Farid, Rahmani Tiziri, Bousbaa Karima, Oukmanou Sonia *et al.* Optimization of Solvent Extraction of Antioxidants (Phenolic Compounds) From Algerian Mint (Mentha spicata L.). Pharmacognosy Communications. 2012; 2(4).
19. Anang Otoluwa, Abdul Salam, Yasmin Syauki, Mur Nurhasan, Yusiyanty Monoarfa, Suryani A *et al.* Effect of Moringa oleifera Leaf extract supplementation in preventing maternal DNA damage. International Journal of Scientific and Research Publications. 2004; 4(11).
20. Kasolo JN, Bimenya GS, Ojok L, Ochieng J, Ogwal-Okeng JW. Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities. J Med Plant Res. 2010; 4:753-757.
21. Forster N, Ulrich C, Schreiner M, Arndt N, Schmidt R, Mewis I. Ecotype Variability in growth and Secondary Metabolite Profile in Moringa oleifera. Impact of water and sulphur availability. Journal of Agriculture and Food Chemistry. 2015; 63:2852-2861.
22. Fratianni F, Tucci M, De Palma M, Pepe R, Nazarro F. Food Chem. 2007.
23. Joshi P, Mehta D. Effect of Dehydration on The Nutritive Value of Drumstick leaves. J Metabol Syst Biol. 2010; 1:5-9.
24. Raghavendra M, Madhusudhana Reddy, Pulala Raghuveer Yadav, Sudharshan Raju A, Sivakumar L. Comparative studies on the in vitro antioxidant properties of methanolic leafy extracts from six leafy vegetables of India. Asian Journal of Pharmaceutical and Clinical Research. 2013; 6(3).
25. Brahmi Fatiha, Madani Khodir, Dahmoune Farid, Rahmani Tiziri, Bousbaa Karima, Oukmanou Sonia, Chibane Mohamed.Optimisation of Solvent Extraction of Antioxidants (Phenolic Compounds) From Algerian Mint (Mentha spicata L.). Pharmacognosy Communications. 2012; 2(4).
26. Tijjaini M, Bello I, Aluyu A, Olurishe T, Maidawa S, Habila J *et al.* Phytochemical and antibacterial studies of root extract of Cochlospermum tinctorium. A rich (Cochlospermaceae). Res Journal of Medicinal Plants. 2009; 3:16-22.
27. Ayinde Ba, Onwukaeme DN, Omogbai Eki. Isolation and characterization of two phenolic compounds from the stem bark of Musanga cecropoides R. Brown (Moraceae). Acta. Pol. Pharm. 2007; 64:183-185.
28. Rumit Shah, Heena Kathad, Rajal Sheth, Naveen Sheth. *In vitro* Antioxidant Activity of roots of Tephrosia purpurea Linn. International Journal of Pharmacy Sciences. 2010; 2:30-33.
29. Saswati Roy, Dutta Chodhury M, Paul SR. Antioxidant Potential of Rhizome of Alocasia decipiens Schott. Asian Journal of Pharmacy and Clinical Research. 2013; 120-122.
30. Holasova M, Fiedlerova V, Smcinova H, Orsak M, Lachman J, Varreinova S. Buck Wheat-The source of antioxidant activity in functional foods. Food Res Intl. 2002; 35:207-211.
31. Adriana Maria Fernandes de Oliveira, William Sousa pinheiro, Charlane Kelly Souto Pereira, Wemerson and Otemberg Souza Chavez *et al*. Total Phenolic Content and Antioxidant Activity of some Malvaceae Family species. Antioxidants. 2012; 1:33-43.
32. Cassaza AA, Aliakbarian B, Mantegna S, Cravotti G, Perejo. Extraction of Phenolics from Vitis vinifera wastes using non-conventional techniques. Journal of Food Engineering. 2010; 100:50-55.
33. Seun F Akomolafe, Ganiyo oboh, Afolabi A Akindhahunsi, Ayodele J Akinyemi, Olusola Adeyangu. Inhibitory Effect of Aqueous Extract of Moringa oleifera and Newbuoldia leaves on Ferrous Sulphate and Sodium Nitroprusside Induced Oxidative Stress in Rat's Testes in vitro. Open Journal of Medicinal Chemistry. 2012; 2:119-128.
34. Sarabjout Kaur, Poonam Mondal. Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial properties of Medicinal Plants. Journal of Microbiology and Experimentation. 2014; 1(1).
35. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and Total phenolics in selected fruits, vegetables and grain products. Journal of Agriculture and Food Chemistry. 1998; 46:4113-4117.
36. Marzanna Hes, Krzysztof Dziedzic, Danuta Gorecka, Agnieska Drodzynska and Elzbieta Gujika. Effect of Boiling Water of Barley and Bruckwheat Groats on the Antioxidant Properties and Dietary Fibre Composition. Plants Food for Human Nutrition. September 2014; 69(3):276-282.
37. Adisakwattana S, Chanathong B. Alpha-glucosidase inhibitory activity and lipid lowering mechanisms of Moringa oleifera leaf extract. European Review Medical Pharmacological Sciences. 2011; 15(7):803-8.
38. Oboh G. Effect of Blanching on the Antioxidant Property of Some Tropical Green Leafy Vegetables. Lebenmittel Wissenschaft and Technologie, 2005; 38(5):513-517.
39. Kronhausen, Eerhard and Kronhausen, Phylles with Demopoulos, Harry B, Formula for Life. William Morrow and Co., New York. 1989.