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Choice of Solvent for Effective Extraction of Phytochemical Components in Medicinal Plant Leaves.

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ABSTRACT

The use of plant materials as natural source in the discovery and development of active compounds in drug formulation is receiving increased attention particularly in Africa because of ease of availability and access, and interest in natural drug products. *Moringa oleifera* is one of such popular medicinal plants with a wide range of therapeutic properties. However, identifying the most appropriate phytochemical bioactive components extraction solvents that will provide maximum yield from the plant materials poses a challenge. This study investigated the affinity of a choice of seven solvents in the extraction of bioactive compounds from *Moringa oleifera* leaves extract. The leaves were plucked, washed, air dried and grounded. The grounded *Moringa oleifera* leaves were soaked into the different extraction solvents including Aqueous, Ethanol, Methanol, n-Hexane, Acetone, hydroethanol and hydromethanol. Qualitative and quantitative phytochemical analysis were carried out on the solvents using standard methods of analyses. *Moringa Oleifera* leaves extract was found to contain alkaloids, saponin, tannin, phenol, flavonoids, glycoside, steroids, anthraquinone, anthrocyanin. In both qualitative and quantitative analyses of the *Moringa oleifera* leaves extract, ethanol was found to be the best solvent of choice as it had the highest extraction affinity and the highest percentage of yields for both lipophilic and hydrophilic phytochemical components of the *Moringa oleifera* plant leaf extract when compared with the other solvents.

Key Words: *Moringa oleifera*, leaves, phytochemicals, solvents.

INTRODUCTION

The use of plants products is widespread and immemorial because of its role in nutrition and maintenance of human health. Plants possess thousands of phytochemicals which are believed that when used appropriately in the treatment of diseases are safe, easily available and with no or less side effects [1]. In Africa, several attentions have been drawn to the use of these medicinal plants. These plant products are used for therapeutic purposes and more than 70% of people place a great value on it[2]

Moringa oleifera is one of these medicinal plants that are edible, easily accessible and safe. It is a fast-growing plant that is commonly known as (family: moringaceae) drumstick tree. *Moringa oleifera* is widely grown in tropical and sub-tropical regions. It is a native of sub-Himalayan region of northern India, with a wide range of therapeutic properties. It is found and cultivated in areas like Central and South America, Africa, Indonesia, Mexico, Philippines. In Nigeria, it is commonly called Moringa (English), okwe oyibo (Igbo), ewe igbale (Yoruba), Zogale (Hausa). All part of the plant is said to have medicinal properties and have been used for the treatment of various ailments and diseases[3,4]

There has been increased demand for therapeutic drugs from natural products because of their importance in the synthesis of pharmaceutical and health care products [5,6]. In the isolation of bioactive compounds from medicinal plants for pharmaceutical use, solvent extraction including water, is the most used technique. This technique has been found effective than simple water extractions, if properly prepared and extracted. However, these solvents have varying affinities for the extraction of different phytochemical compounds from medicinal plants. Getting the best bioactive compounds in terms of quality and quantity, out of these medicinal plants, is dependent on the extraction medium used. This study is aimed at investigating the appropriate solvent for the extraction of the bioactive compounds from *Moringa oleifera* leaves.

MATERIALS AND METHODS

Plant Materials: The leaves of *Moringa oleifera* was collected from various crop farms in Port Harcourt, Rivers state. The plant was identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State and deposited in the herbarium unit with an assigned voucher number UPH/P/103.

Preparation of Extract: The leaves were air dried for 4 weeks (29°c-37°c), crushed and grinded into powder with electrical machine. The powdered leaves were

extracted using different solvents which include: water (aqueous), ethanol, methanol, hexane, acetone, hydroethanol, hydromethanol.

Qualitative Phytochemical analysis: The plant extract from the *Moringa oleifera* leaves for each solvent was qualitatively tested for the presence of its chemical constituent using the method by [7] as follows:
Test for Tannins: One mililitre (1ml) of extract was boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride were added and green coloration appearance confirmed that tannin was present.

Test for Saponin: Five mililitres (5ml) of extract was boiled in distilled water of about 20ml and filtered. The filtrate (10ml) was then mixed with distilled water (5ml) and was shaken vigorously to form a steady froth. This frothing was also mixed with 3 drops of olive oil and shaken vigorously. The formation of an emulsion confirms the presence of saponin.

Test for Flavonoids: Three mililitres (3ml) of Aluminium chloride solution was put into five mililitres (5ml) of extract. Yellow coloration observed confirmed the presence of flavonoids.

Test for Steroids: Two mililitres (2ml) of acetic anhydride was mixed with 2ml of the extract and 2 ml of H2SO4 was also added. A change in colour from violet to blue confirms the presence of steroids.

Test for Alkaloids: One mililitre (1ml) of extract was stirred with 5ml of 1% aqueous HCl on a steam bath and sieved while hot. Distilled water was mixed with the residue.1ml of the filtrate and some few drops of Mayer's reagent (Potassium mercuric iodide -solution) was added. Cream coloration shows the presence of alkaloids.

Test for Phenol: Ten mililitres (10ml) of distilled water was mixed with 5ml of extract in a 30ml test tube. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol was added and allowed to stand for 30min. A bluish green colour that the mixture produced confirmed that phenol was present.

Test for Cardiac Glycoside: 2ml of glacial acetic containing 1 drop of ferric chloride solution and 1ml of conc H_2SO_4 was added to the extract. Appearance of a brown ring indicates the presence of cardiac glycoside.
Test for Terpenoids: 2ml of the extract were dissolved in 10ml of methanol in a test tube by shaking. The mixture was filtered, 2ml of chloroform and 3ml sulphuric acid was added to the filterate. The appearance of a red precipitate indicates the presence of terpenoids.

Test for Anthraquinones: 2ml of ammonia solution was added to 1ml of the extract. The mixture was shaken and the appearance of a cherish-red solution indicates the presence of an oxidized form of anthraquinone.

Test for Anthocyanins: 2ml of the plant extract was added to 2ml of 2N HCL. The appearance of a pink-red solution which later turns purplish blue indicate the presence of anthocyanins.
Determination of total Phenolic contents: Total phenolics were determined using Folin-Ciocalteau reagent (FCR) as modified by [7]. FCR consist of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic cheteropolyacids. Dissociation of phenolic proton in an alkaline medium lead to phenolate anion which reduces FCR forming a blue coloured molybdenum oxide whose colour intensity is directly proportional to the phenolic contents. Briefly, 100µl of the extract dissolved in ethanol (1mg/ml) was added to 750µl of FCR (diluted 10-fold in dH2O) and allowed to stand at 220C for 5minutes; 750 µl of Na2CO3 (60g/l) solution was put into the mixture. After 90 minutes, the absorbance was measured at 725nm. Results were shown as gallic acid equivalent.

Determination of Tannin contents: This was done using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannin as described by [7]. Briefly, 1ml of extract dissolved in ethanol (1mg/ml), where the total phenolics were determined, was mixed with 100mg PVPP vortexed, left for 15 minutes at 4oC and then centrifuged for 10 minutes at 3000rpm using a Fischer Scientific centrifuge. The same method for determining total phenolics was used to determine the non-phenolics in the clear supernatant. The difference between total phenolic and non-tannin phenolic contents was used to calculate the tannin content.

Determination of Flavonoids: This was done according to the method of[8]. In this method a flavonoid-aluminium complex, which absorbs maximally at 415nm, is formed. Briefly, 100µl of extract in ethanol (10mg/ml) was added to 100µl of 20% aluminium trichloride in ethanol and a drop of acetic acid, and then diluted with ethanol to 5ml.The absorption at 415nm was read after 40 minutes. Blank samples were prepared by adding 100µl of extract and drop of acetic acid. This was diluted to 5ml with ethanol. Absorption of rutin standard solution (0.5mg/ml) in ethanol was measured. The rutin equivalents (RE) of the flavonoids content was calculated using the formula:

$$\text{Flavonoid content} = \frac{A \times mo}{Ao \times m}$$

Where 'A' is the absorption of extract solution
'Ao' is the absorption of standard rutin solution
'm' is the weight of extract in mg,
'mo' is the weight of rutin in the solution in mg.
The flavonoid content is expressed in mg rutin equivalents/mg extract.

Determination of Alkaloids: The alkaloid was extracted from the extract using the method by [7]. 100mls of 10%acetic acid was used to extract alkaloids from the extract and this was allowed to stand for 4hours. The

extract was filtered and few drops of 1%NH_4 OH was added to the filtrate until no precipitation occurred. The alkaloid was dried in an oven and the percentage yield was calculated using this formula

%Alkaloid= weight of residue × 100
weight of sample

Determination of Saponin: The method of [9] was employed. 100ml of aqueous ethanol was added to 10g of the extracts in a conical flask. The mixture was heated for 4hours on water bath with constant stirring. The mixture was filtered and 20ml of diethyl ether added and shaken vigorously. The aqueous layer of the mixture was recovered while the ether layer was discarded. 60ml of n-butanol was added to the recovered layer. This was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath and then dried in an oven. The saponin content was weighed and the percentage yield was calculated.

Determination of Cardiac Glycoside: The alkaline picrate method described of [7] was employed. 5g of the extract samples were grinded into paste and dissolved in 50ml distilled water in a corked conical flask. The samples were filtered out after staying for 12hrs overnight. 4ml of alkaline picrate was added to 1ml of the sample was collected from the filtered sample. This was incubated in a water bath for 5minutes and the absorption was read at 490nm. The cyanide content was calculated using this formula:

Cyanide (Ug) = Absorbance Gradient factor Dilution factor
g weight of sample

Determination of Terpenoids: The method of [10] was employed. 100mg of a sample of the dried extract was taken and soaked in 9ml of ethanol for 24 hours. The extract was filtrated, then extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and allowed to dry. Ether was evaporated and the yield (%) of total terpenoids contents was measured by this formula (wi-wf/wi×100).

Wi: weight of sample of plant extract
Wf: weight of extract after filtration and evaporation

Determination of Anthraquinone: The protocol of [11] was employed, 10ml of the extract was transferred to a 100ml volumetric flask which was adjusted for volume with each of the extraction solvents and analysed using ultraviolet-visible spectrophotometer. The sample were measured at an absorbance of 515nm.

Determination of Anthocyanin: The method of [12] was adopted. 0.5g of the samples were homogenised with 50ml of the extraction solution. The mixture was stirred with a glass rod for 10min. The sample was then transferred to a 100ml volumetric flask using Whatman 541 filter paper. The filtrate was analysed using spectrophotometer to determine the amounts of anthocyanin. The sample were measured at an absorbance of 520nm.

Statistical Analysis: Results were analysed and expressed as mean ± SEM.

RESULTS

The phytochemical screening of the extract using different solvent revealed the presence of the following bioactive compounds: Alkaloids, Phenols, Flavonoids, Terpenoids, Tannin, Anthocyanins, Saponin, Steroids, Anthraquinones, and Cardiac Glycosides (Table 1).

Table 1: The results of the qualitative analysis of the strength of phytochemical contents present in *Moringa oleifera* leaves extracts for each solvent

Parameters	Aqueous	Ethanol	Methanol	n-Hexane	Acetone	Hydroethanol	Hydromethanol
Alkaloids	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	-	+
Tanin	+++	+++	+	-	+	+++	++
Phenol	-	+	-	-	-	+	-
Flavonoid	++	++	+	+	+	+	-
C.Glycoside	+	+	-	-	+	+	+
Steroids	++	++	+	-	-	-	-
Anthraquinones	++	+++	+++	++	++	++	++
Terpenoids	++	++	-	-	-	+	-
Anthocyanin	+	+	-	-	-	-	-

Key: +: Sparingly seen, ++: Moderately seen, +++: Highly seen-: Not seen

Table 2: The results of the quantitative analysis of the phytochemical contents present in *Moringaoleifera* leaves extracts for each solvent.

Parameters	Aqueous	Ethanol	Methanol	n-Hexane	Acetone	Hydroethanol	Hydromethanol
Alkaloids	2.09±0.02	2.15±0.01	1.08±0.01	0.62±0.01	0.31±0.01	1.28±0.02	1.01±0.01
Saponin	1.10±0.02	1.75±0.00	0.78±0.01	0.41±0.01	0.48±0.01	0.00±0.00	0.54±0.00
Tanin	9.03±0.01	8.58±0.01	0.19±0.01	0.00±0.00	0.54±0.01	9.42±0.02	0.11±0.01
Phenol	0.00±0.00	0.21±0.01	0.02±0.01	0.00±0.00	0.00±0.00	0.07±0.01	0.00±0.00
Flavanoids	3.13±0.01	3.61±0.01	0.95±0.01	0.65±0.00	0.61±0.01	1.44±0.01	0.00±0.00
C. Glycoside	0.12±0.01	1.16±0.01	0.00±0.00	0.00±0.00	0.15±0.01	1.18±0.01	0.22±0.01
Steroids	3.16±0.01	3.47±0.02	1.68±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Anthraquinone	6.79±0.01	12.22±0.01	10.52±0.02	4.31±0.01	4.38±0.01	8.73±0.01	9.52±0.02
Terpenoids	3.84±0.01	4.22±0.01	0.00±0.00	0.00±0.00	0.00±0.00	1.14±0.01	0.00±0.00
Anthocyanin	0.04±0.00	0.07±0.01	0.00±0.00	0.02±0.01	0.00±0.00	0.00±0.00	0.00±0.00

Values are given as mean ± SEM

The qualitative results show a range of traces of all the phytochemicals components of the *Moringaoleifera* leaves extracts in most of all the extraction solvents studied. Anthraquinone and terpenoid were more abundant, followed by tannin, in ethanol, methanol and aqueous. Flavonoids are abundant in aqueous, ethanol, and less in methanol, hexane, acetone and hydroethanol. Phenol was only present in ethanol and hydroethanol. Steroids are more in aqueous, ethanol and methanol. Alkaloids and Saponin were less in all the extracts with all the solvents.

The quantitative result indicates that a generally higher quantities of the *Moringa oleifera* leaves phytochemical component extracts studied were seen in the ethanol than all other solvents. These other solvents extracts had a range of traceable quantities. However, tanin, Phenol, Steroids, Terpenoids and Cardiac Glycoside were not detected in hexane solvent. The percentage of Tannin was higher in aqueous and hydroethanol. Phenol was absent in aqueous and acetone. Both methanol and acetone did not show the presence of Terpenoids and Anthocyanin. And the percentage of Alkaloids, Flavonoids, Saponin and Anthraquinone was highest in the ethanol solvent extracts.

DISCUSSION

The effective extraction and analysis of chemical components of plants products is the most important phase in the process of formulation and use of medicinal plant-based therapeutic measures because it helps to isolate, and in knowing the various bioactive phytochemicals constituents in the plant. These bioactive compounds are usually recovered from plant materials by extraction with solvents[13]. The choice of solvent used in the process of extraction depends on the type of active ingredients that will be isolated from the plant[14].

Generally, water has been known as a universal solvent because it is safe to use, easily accessed and used by traditional herbal medical experts to prepare medicine from medicinal plants[15]. However, not all plant bioactive phytochemicals are soluble in water. It is therefore, difficult to isolate all bioactive phytochemical compounds in medicinal plants using a particular solvent because some solvents are selective for certain bioactive compounds as demonstrated in this study. The findings from this study suggests that aqueous solvent failed to extract the presence of phenol from the *Moringa oleifera* leaves. Acetone, methanol, hydroethanol and hydromethanol have been reported as good solvents for extraction of plants phytochemical compounds[16-18]. They are used for the extraction of hydrophilic compounds. In this study, acetone, methanol and hydromethanol solvents demonstrated the highest affinity for the extraction of Anthraquinone except hydroethanol solvent which had highest affinity for both Tanin and Anthraquinone. Hexane is reported to be used for the extraction of more lipophilic compounds[6]. In this study, Hexane solvent extracted the presence of 5 out of the 10 phytochemical compounds seen in the *Moringa oleifera* leaves, namely Athraquinone, Flavanoids, Alkanoids, Saponin and Anthocyanin, and demonstrated highest affinity for Anthraquinone.

Some of these *Moringa oleifera* leaves phytochemical compounds have been reported to possess good antioxidant activity and exhibits various biological effects like antitumor, anti-inflammatory, antidiuretics, antiulcer, antihyperglycemic, antiviral, hepatoprotective and antimicrobial [4, 19-24]. Identifying the most effective solvent for the extraction of the bioactive phytochemical compounds in medicinal plants though challenging, is very crucial in their use for therapeutic measures. The results of this study suggest that water solvent had comparatively very close affinity for the extraction of most of the bioactive phytochemical compounds from *Moringa oleifera* leaves as ethanol, with the exception of absence of phenol. However, ethanol would be the recommended solvent of choice because of its highest extraction yield and affinity for almost all the phytochemical constituents in the *Moringa oleifera* leaves compared to other solvents, and could be used to extract both hydrophilic and lipophilic phytochemical compounds in plant materials.

REFERENCES

1. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Compl Altern Med*. 2006; 6:35-41.
2. Tijani AY, Oguru MO, Salawu OA, Abubakar A, Onyekwelu NO, Akingbasote JA. Effect of Faidherbia albida on some biochemical parameters of rats infected with Trypanosoma brucei brucei. *Afr J Pharm Pharmacol*. 2009; 3(1):26-30.
3. Fuglie L.J. The miracle tree*: Moringa oleifera*: natural nutrition for the tropics. Church World Service, Dakar, Senegal. 1999.
4. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytother Res*2007; 21: 17-25.
5. Ivanova D, Gerova D, Chervenkov T, Tankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *J Ethnopharmacol*.2005; 96:145-150.
6. Cosa P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger in vitro' proof-of-concept'.*J Ethnopharmacol.*2006; 106:290-302.
7. Onwuka GI. Food analysis and instrumentation: theory and practice. Naphathali prints, Nigeria. 2005; 143-146.
8. Kumaran A, Karunakaran RJ. Invitro antioxidant activities of methanol extracts of five Phyllanthus species from India.*Lebensm Wiss Technology*.2006; 40:344-352.
9. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of crude extract of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science*.2001;86:203-208.
10. Indumathi C, Durgadevi G, Nithyavani S and Gayathri PK. Estimation of terpenoid content and its antimicrobial property in Enicostemma litorrale. *Intern J Chem Tech Res*, 2014; 6 (9): 4264 – 4267
11. Sakulpanich A and Gritsanapan W. Extraction method for high content of anthraquinone from Cassia fistula pods. *J Healt Res*. 2008; 22(4):167-172.
12. Sakar D, Karaoglan GK, Gumrukcu G, Ozgur MU.Determination of anthocyanins in some vegetables and fruits by derivative spectrophotometric method.*Rev Analy Chem*.2008; 27:1-15.
13. Sultana B, Farooq A, Muhammad A. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecule*.2009; 14(6):2167-2180.
14. Masoko P, Mokogotho MP, Mbazima VG, Mampuru L J. Biological activity of Typha Capensis (Typhaceae) from Limpopo Province (South Africa). *Afri J Biotechnol*. 2008; 20:3743-3748
15. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agents: current method and future trends. *J Med Pla Res.*2010; 4(2):104-111.
16. Masoko P, Eloff JN. Screening of twenty-four south species Combretum and six Terminalia species (Combretaceae) for antioxidant activities.*Afric J Trad Compl Altern Med.*2006; 4(2):231-9.
17. Njume C, Afolayan AJ and Ndip RN. An overview of antimicrobial resistance and the future of medicinal plants in the treatment of Helocobacter pylori infections.*Afr J Pharmharmacol*. 2009; 3(13): 685-699
18. Sharida F, Syazana S, Palanisamy A. *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. *Molecules*.2012; 17(7):8334-50.
19. Pal SK, Mukherjee PK, Saha P. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats*. Phytotherapy Res*. 1995; 9(6):463-465.
20. Napolean P, Anitha J and Renitta RE. Isolation, Analysis and identification of phytochemicals of Antimicrobial Activity of *Moringa oleifera. Lam.Current Biot*2009; 3(1):33-39.
21. Iqbal S, Bhanger MI. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J Food Comp Analysis*.2006; 19(6-7):544-551.
22. Asare GA, Gyan B, Bugyei K, Adjei S, Mahama R, Addo P, Otu-Nyarko L, Wiredu EK, Nyarko A. Toxicity potentials of the nutraceutical *Moringa oleifera* at supra-supplementation levels. *J Ethnopharmacol*.2012; 139:265-272.
23. Fakarazi S, Sharifudin S A, Arulselvan P. *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen induced hepatotoxicity in experimental rats through their antioxidant nature.*Molecules*.2012;17(7):8334-8350
24. GhadaZAS. Anti-diabetic activity of dried *Moringa oleifera* leaves in normal and streptozotocin-induced diabetic male rats. *Ind J Appl Res*.2013;3(9):18-23.