

Full Length Research Paper

Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities

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***Moringa oleifera* grown and used in many countries around the world is a multi-purpose tree with medicinal, nutritional and socio-economic values. In Senegal and Benin, *M. oleifera* leaves are dispensed as powder at health facilities to treat moderate malnutrition in children. It established the medicinal uses of *M. oleifera* leaves by local communities in Uganda and identified phytochemicals present in *M. oleifera* leaves extracts. It used quantitative and experimental methods that established the uses, and identified phytochemicals in *M. oleifera* leaves. Employed serial extractions, using ether, ethanol and water as solvents. The phytochemicals were qualitatively identified using standard chemicals and standard outcomes. Twenty-four medicinal uses of *M. oleifera* leaves were established. Phytochemicals present included: tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars. The local communities in Uganda use *M. oleifera* leaves to treat common ailments. Presence of phytochemicals in the extracts, indicate possible preventive and curative property of *M. oleifera* leaves. There is need to standardize *M. oleifera* leaves use for nutrition and herbal medicine.**

Key words: Phytochemicals, medicinal plant extracts, herbal medicine, Uganda.

INTRODUCTION

Moringa oleifera was massively grown and promoted by the local media in Uganda in the 1980s as a plant putatively able to cure a number of diseases including symptoms of HIV/AIDS.

Industrialists bought the leaves and seeds to use as raw materials and this promoted its being grown by many families. At the moment farmers have uprooted the plant

and have remained with a few trees around the compound.

Although *M. oleifera* is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan where it is used in folk medicine (Fahey, 2005), it is now widely distributed all over the world (Lockett, 2000). *M. oleifera* is referred to as a "miracle tree" or a "wonder tree" (Fuglie, 2001) of significant socio economic importance because of its several nutritional, pharmacological (Caceres et al., 1991; 1992; Fuglie, 2001) and industrial applications (Makkar and Becker, 1997; Foidl, 2001). The leaves of this plant contain a profile of important trace elements, and are a good

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source of proteins, vitamins, beta-carotene, amino acids and various phenolics (Anwar, 2007). With all those attributes to *M. oleifera* leaves we wondered why very few people and media are promoting the use of *M. oleifera* leaves in a country where malnutrition among children below 5 years stands at 15% while 45% children below 5 years are stunted.

MATERIALS AND METHODS

Over a period of two weeks, a cross-sectional study with quantitative and qualitative methods of data collection used in surveys was carried out among heads of households aged 18 years and above in four rural districts of Uganda (Arua, Kapchorwa, Mbarara and Wakiso), who had lived in the area for more than two years. They were interviewed in their homes and focus group discussions were conducted among them at the local councils' meeting places. Villages from randomly selected sub-counties were randomly selected and the list of heads of households was generated. Every fifth person was interviewed until a total of 30 respondents per district were obtained.

The experimental component extracted *M. oleifera* leaf/powder sequentially using ether, ethanol (95% v/v) and distilled water as solvent (Cowan, 1999). Medicinal phytochemicals in the extracts were determined using established methods of Ciulei (1964). The method uses the principle that different phytochemical groups, reacts with specific to give end point characteristic colour changes reagents when mixed because colour changes, froth or precipitate.

Data collection for use of *M. oleifera* leaves

A pre-tested questionnaire, translated into local languages (Luganda, Runyankore, Lugbara and Kupsabiny) and back translated to English to ensure consistence of meaning, was used by trained research assistants to collect data from the participants. Two focus group discussions per district (one for men and the other for women) were performed to complement data collected from the quantitative study. One man and one woman opinion leader from each community were interviewed to correlate the information collected from the focus group discussions. Data was also collected on respondent's social-demographic characteristics i.e. age, sex, education level, marital status, distance to the nearest public health unit and occupation. It also included knowledge and use of *M. oleifera* leaves in their homes and communities.

Consent forms which included the nature and purpose of the research translated into local languages were read to each participant. The participants consented by signing or thumbing on the consent form. Permission to conduct the study was received from the Makerere University Faculty of Medicine Research and Ethics Committee. The study was conducted in accordance with ethical standards for human experimentation established by the declaration of Helsinki (1965).

Extraction of the leaves

M. oleifera leaves were harvested during the dry season from trees grown on loam soil in Wakiso district, Uganda. The family and species of *M. oleifera* were confirmed by Ms. Olive Wanyanna a Makerere University botanist and leaves were kept in the University Herbarium. *M. oleifera* leaves were air-dried at room temperature in the Department of Physiology until constant weight was attained. They were kept away from high temperatures and direct sun light to avoid destroying active compounds. They were then pounded to

powder with metallic motor and pestle to ease the extraction of active compounds.

Extraction process

The process followed the already established extraction procedure of plant samples, using ether followed by ethanol and then distilled water as solvents (Cowan, 1999; Ciulei, 1964). Serial extractions were done using 200 g plant powder, in 500 ml diethyl ether (98%) in Ehlmeier flask. The mixture was shaken at two hourly intervals during day-time for 3 days. The mixture was decant and filtered using Whatman's No.1 filter paper in Buchner funnel using a suction pump. The residue was air dried at room temperature for 3 days and the same procedure repeated using ethanol (95% v/v) for 3 days. Rotary evaporator (BUCHI Rotavapor R-205) was used to recover the ether and ethanol. Finally 200 g of the dry residue was soaked into 1,000 ml distilled water at 96 °C to prevent fungal attack and cooled at room temperature. The mixture was shaken hourly to ease extraction for 12 h. The filtrate was freeze dried at pressure 32 Pa, temperature was started at -47 °C and maintained at 0 °C for 36 h to dry the extract.

Identification of phytochemical groups in the extracts

The qualitative methods already established to test for classes of compounds in plant extracts by Ciulei (1964) and Chitravadiu et al. (2009) were used. The substances that were tested for included: Alkaloids, steroids and triterpenoids, tannins, anthracenoides, reducing sugars, flavones, saponins and coumarins which are reported to have biological activities on animal tissues. The dry extracts of *M. oleifera* leaves, ether, ethanol and water extracts were used to determine the compounds.

Test for alkaloids

One milligram of dried extract was dissolved in 6 drops of 2% hydrochloric acid. The solution was divided into 3 aliquots; to the first portion which acted as a reference, 2 ml of distilled water was added. To the second test tube, 2 drops of Dragendorff's reagent whose Basic Bismuth nitrate, was purchased from Sikuda Lab distributors, P. O. Box 12553, Kampala, Uganda, and potassium iodide from Tomas Baker (Chemicals)Ltd, 4/86 Bharat, Mahal, Marine Drive, Mumbai- 400 002, India were added. A precipitate indicated presence of alkaloids. To the third portion, 2 drops of Mayer's reagent was added and a yellowish white precipitate indicated the presence of alkaloids (Raffauf, 1962).

Test for steroids and triterpenoids

One milligram of dried extracts was dissolved in 0.5 ml of acetic anhydride; 0.5 ml of chloroform from Alpha Chemicals, 18 Inman Rd, Cronner, NSW 20999 Australia, was added. The solution was pipette into a dry test tube and 1 ml of concentrated sulphuric acid added at the bottom of the tube. A brown-red ring at the interface between the two liquids and a green supernatant indicated the presence of steroids and triterpenoids.

Test for tannins

One milligram of plant extracts was dissolved in 1.5 ml of water; 3 drop of dilute ferric chloride from LOBA CHEMIE PVT. LTD. Jehangir Villa, 107, Wode House Road, Colaba, Mumai, 400 005,

India, were added. A blackish blue color indicated the presence of Gallic tannins and green blackish color indicated catechol tannins.

Test for anthraquinones

To 1 mg of the extract, 2 ml of 25% Ammonia solution from UNILAB Limited, P. O. Box 78151, Nairobi, Kenya, was added and shaken. A cherish-red solution indicates the presence of emodols (aglycones of anthracenosides in oxidized form).

Test for saponins

Three drops of dimethylsulfoxide from BDH Laboratories VWR International Ltd, 14 Media Village, Liscombe Park, Soulbury, Leighton Buzzard, LU7 0JL, UK, were added to 1 mg of plant extract, 5 ml of distilled water added and shaken. Presence of foam which persisted for more than 15 min indicated the presence of saponins.

Test for coumarins

One milligram of the extract was dissolved in 2 ml of water. The solution divided into 2 portions. To first portion, 0.5 ml of 10% ammonia solution was added. The second portion acted as a reference. The occurrence of an intense fluorescence under ultra violet light indicated the presence of coumarins and its derivatives.

Test for flavones aglycones

One milligram of dry plant extract was dissolved in 1 ml of methanol at 50°C. Metallic magnesium from BDH Lab Supplies (U) Ltd. Plot 7. Bombo Road, Kampala, Uganda and 5 drops of concentrated hydrochloric acid were added. A red or orange color indicates the presence of flavones aglycones (Shibata's reaction or Cyanidin test).

Test for reducing sugars

One milligram of the extract dissolved in 2 ml of water and 1 ml of Fehling's reagent which contained a mixture of Fehlings solution I and II purchased from Sikuda Lab distributors, P. O. Box 12553, Kampala, Uganda was added and the mixture heated. A brick red precipitate denoted the presence of reducing sugars.

Data analysis

Data from the quantitative study was entered into Microsoft Excel 2007 and exported to SPSS 13.0 statistical program for analysis. The qualitative data was manually analyzed by grouping the ailments mentioned in the group discussions and in the key informant interviews.

RESULTS

The findings revealed that most of the participants were aged 50 - 55 years (78; 65%), there were more males (93; 77.5%) than females (27; 22.5%), the majority were subsistence farmers (110; 91.6%) with primary education level (88; 73.3%). Majority of the participants (109; 90.8%) were

in some form of marital union and those that had divorced or widowed had remarried or cohabiting. Most respondents (63; 52.5%) lived more than 5 km from the nearest public health unit.

It also found out that there are twenty-four uses of *M. oleifera* leaves by rural communities in Uganda, Table 1. The highest percentage of respondents (108; 90%) use *M. oleifera* leaves to treat hypertension and diabetes. A small number (28; 23.3%) had never used the leaves for treating any condition and 19 (15.8%) had no knowledge of its use. There were no traces coumarins in ether, ethanol or water extracts. However, steroids and triterpenoids, flavonoids, anthraquinones and saponins were extracted by all the solvents. Ether and ethanol did not extract alkaloids while water extracted all the other phytochemicals (Table 2).

DISCUSSION

This study has established the fact *M. oleifera* leaves in Uganda are used for treatment of twenty-four medical conditions as shown in Table 1. The respondents are of low income and live more than 5 km from a public health facility which makes them susceptible to use of local herbs as a first line of illness management. They believe that *M. oleifera* leaves cure the ailments mentioned and many of them use it at primary health care level before seeking help at health facilities. Reports reveal that there are 43 uses of *M. oleifera* leaves around the globe (Fahey, 2005).

Yet in Uganda the leaves are known to treat twenty-four medical conditions. Out of the twenty-four ailments six including: impotence, heartburn, bone setting, asthma, flu, syphilis are only mentioned by the Ugandan rural communities and not reported by other communities. This could be due to the different naming of ailments in different countries and communities for example: in many of the Ugandan local languages it is difficult to get different words distinguishing cough, flu, pneumonia and common cold or malaria and fever. Despite the documented nutrition attributes to *M. oleifera* leaves, very few respondents (13 (10.8%)) appreciated its use in treatment or prevention of malnutrition. There is inadequate knowledge about the nutritional and medicinal values of *M. oleifera* leaves among the Ugandan rural communities. The communities need to be educated on the attributes of the leaves in order to prevent malnutrition among the children. The respondents were not able to tell whether the leaves cured the ailments or just caused the relief symptoms. Unlike in the developed world where herbal medicines are used because they are considered to be safer than the orthodox medicine, the Ugandan rural communities use them due to inadequate access to medical care.

Having used the methods of Ciulei (1964) and latter adopted by Cowan (1999); Ogwal-okeng (1998) and Waako (1996) to qualitatively establish phytochemicals in

Table 1. Reported differential uses of *M. oleifera* leaves in Ugandan rural communities.

Use	Percentage (%)
HIV/AIDS -related symptoms	60 (50.0)
Bronchiasis	12 (10.0)
External sores/ulcers	13 (10.8)
Malaria/Fever	15 (12.5)
Anti-hypertensive	108 (90)
Diabetes mellitus	108 (90)
Colitis	13 (10.8)
Gastritis/ulcers	12 (10.0)
Impotence	13 (10.8)
Syphilis	13 (10.8)
Flu	13 (10.8)
Asthma	12 (10.0)
Heart burn	12 (10.0)
Bone setting	12 (10.0)
Worms in people and cattle	70 (58.3)
Skin disease	13 (10.8)
Stress	12 (10.0)
Lactation enhancer	70 (58.3)
Protein energy malnutrition	13 (10.8)
Energy	30 (25.0)
Anti-septic	12 (10.0)
Soap	30 (25.0)
Tea spices	30 (25.0)
Vegetables	30 (25.0)
Never used the plant	28 (23.3)
No knowledge of the use	19 (15.8)

A number of respondents knew more than one use of *M. oleifera* leaves.

Table 2. Phytochemicals present in *M. oleifera* leaves.

Phytochemical	Ether extract	Ethanol extract	Water extract
Gallic tannins	+	+	++
Catechol tannins	+	—	++
Coumarins	—	—	—
Steroids and triterpenoids	+++	++	++
Flavonoids	++	++	++
Saponins	+	+	++
Anthraquinones	+	++	+++
Alkaloids	+	—	++
Reducing sugars	—	++	++

Key -: not detected; +: present in low concentration; ++: present in moderate concentration; +++ present in high concentrations.

different plant extracts, this study established that ether; ethanol and water *M. oleifera* leaves extracts contained: catechol tannins, gallic tannins, steroids and

triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars which have been identified by other researchers in various plants and in different parts of plants (Devbhuti et al., 2009; Tijjan et al., 2009; Hassan et al., 2007; Bennett et al., 2003). None of the extracts contained coumarins. The findings in this study agree with earlier studies which also found that, not all phytochemicals are present in all plant parts and that those present differ according to the type of the extracting solvent used (Tijjan et al., 2009; Ayinde et al., 2007).

Flavonoids, which are many in number (Ramo-Tejada, 2002), are strong antioxidants, also found to be effective antimicrobial substances *in vitro* against a wide array of microorganisms by inhibiting the membrane bound enzymes (Cowan, 1999). They have been reported to possess substantial anti-carcinogenic and anti-mutagenic activities due to their anti-oxidant and -inflammatory properties (Li-Weber, 2009; Nandakumar et al., 2008; Hausteen, 2002). They are also active in reducing high blood pressure (Ayinde et al., 2007; Dhawan and Jain, 2005).

Tannins are a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution (Scalbert, 1991), causing local tumors (Kapadia et al., 1978), inactivating and killing microorganisms (Cowan, 1999; Hausteen, 2005).

On the other hand anthraquinones (9,10-dioxoanthracene) which are a group of naturally occurring phenolic compounds are found in *M. oleifera* leaves and tend to have laxative effects. Terpenoids and steroids present in *M. oleifera* leaves are described as being active against bacteria such as *Staphylococcus aureus* (Cowan, 1999), capable of preventing cancer (Raju et al., 2004), having anti-carcinogenic effects (Yun, 1996). Rausch et al. (2006) reported Ginseng saponins to have antioxidant, anti-inflammatory, anti-apoptosis and immunostimulant properties, which raised speculation that these compounds could positively affect neurodegenerative disorders and delay neural aging. The local communities use *M. oleifera* leaves as soap due to the presence of saponins which form froth and act as soap.

M. oleifera leaves also contain alkaloids which are nitrogen-containing naturally occurring compound, commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms. The presence of glucosinolates in *Moringa stenopetala* (Bennett et al., 2003) and that of hypotensive thiocarbamate glycosides in *M. oleifera*, (Faizi et al., 1995) contributes to the use of the plants in hypertension. On the other hand, they are also reported to modify tumorigenesis (Ueno et al., 2009), able to inhibit carbohydrate-mediated tumor growth (Nangia-Makker et al., 2002), induced a stress response and apoptosis in human breast cancer cells.

It is also documented that phytochemicals in plant-based foods can improve glucose metabolism as well as enhance the overall health of diabetic patients by improving

lipid metabolism, antioxidant status, improving capillary function, and lowering blood pressure and cholesterol (Kelble, 2006; Broadhurst et al., 2000). *M. oleifera* leaves having these phytochemicals are able to treat the ailments mentioned by the heads of households in Ugandan rural communities.

Conclusion

The rural community in Uganda use *Moringa oleifera* leaves to treat common medical conditions but a few use it for preventing and treating malnutrition. Presence of phytochemicals indicates possible preventive and curative properties of *M. oleifera* leaves. There is need to carry out more pharmacological studies to support the use of *M. oleifera* as a medicinal plant.

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