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Preliminary Phytochemical Screening of Fresh and Dried *Moringa oleifera L*eaves and that of Chloroform, Ethyl-acetate and n-Butanol Fractions

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ABSTRACT

The preliminary phytochemical screening of the crude aqueous extract of fresh and dried *Moringa oleifera* leaves, of the organic solvent and residual fractions of the extract was carried out according to the standard methods. Chemical constituents of the crude aqueous extract of the fresh leaves were found to be tannins, saponins, carbohydrates, flavonoids, cardiac glycosides, alkaloids, steroids and terpenes. The crude aqueous extract of the dried leaves contain the same chemical components like that of the fresh leaves except for the absence of steroids and terpenes. Based on the organic solvent extract analysis, the chloroform portion of the extract contains saponins, carbohydrate, flavonoid, cardiac glycosides and alkaloids. The ethyl acetate fraction contains similar chemical constituents and in addtion tannins. Also the n-butanol fraction of the extract contains tannins, saponin, carbohydrate, flavonoid, and alkaloid. The residual aqueous fraction of the extract contained tannins, carbohydrate, flavonoid, and phlobatannin. Anthraquinones were completely absent from the analysis of *Moringa oleifera* leaf extracts. The observed chemical constituents have been discussed in relation to the acclaimed medicinal properties of the plant.

Key words: Phytochemistry, crude aqueous extract, organic solvent fractions, *Moringa oleifera* leaves

INTRODUCTION

For over a decade, interest has been revived in the study and use of traditional medicine in different parts of the world. As a result, countries have sought cooperation in identifying and using safe positive components of traditional medicine in their national health systems (Sofowora, 1993).

There is increasing awareness that many components of traditional medicine are beneficial while others may be harmful, hence the World Health Organisation (WHO) encourages and supports countries to identify and provide safe and effective remedies for use in the public and private health services (Sofowora, 1993).

In Nigeria, herbal medicine has become part of the people's culture with about 70% of the population relying mainly on traditional medicine (Geidam *et al.*, 2007) hence, the need to incorporate traditional medicine in our modern health care services. Compounds of herbs are said to be safe and would overcome resistance produced by pathogens (Prakash, 2006). It is thus advocated that there is the possibility of enhancing the potency of medicinal plants if further refined to obtain compounds that are more active.

Moringa oleifera Lam is said to have originated from Agra and Oudh in the Northwest region of India, south of the Himalayan Mountains. The plant is a common plant in the Northeast and Middle belt regions of Nigeria and has enormous use traditionally in these areas which earned it the name "Miracle Tree" (Fuglie, 2001).

Nutritionally, *Moringa* leaves rank as the best of all tropical vegetables and contain very high concentrations of vitamins A, B complex and C, minerals such as iron and calcium. Also the leaves contain protein and all essential amino acids (Fuglie, 2001).

Traditionally, *M. oleifera* is reported to be used in treating hypertension, diabetes, diarrhoea, dysentery, colitis, gonorrhoea, anaemia, helminthosis, and ulcer (Anon, 2000). In Northern Nigeria, the plant is used traditionally to treat arthritis, accumulation of gas in the stomach (bloat), dyspnoea in children, ulcer, skin infections as well as hypertension. The plant can also be used in alley cropping as a biogas, dye, foliar nutrient, green manure, gum, honey clarifier, honey producer, livestock feed, ornamental plant, disease prevention, pulp, rope making, tannin and

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for water purification (Fuglie, 2001).

The phytochemical screening of plants is done in order to find out their chemical constituents. Identification of the chemical constituents of *M. oleifera* leaf of the crude aqueous extrac
t of the fresh and dried leaves will provide scientific basis for the use of the plant in traditional medicine and shed more light on its possible biological activities (Bennett *et al*., 2003). The fractionation of extracts is for separation of the constituents and known to ensure better biological activity (Mclaughlin, 1991). Therefore the present study will further examine the chemical constituents of the organic solvent fractions of the extract.

MATERIALS AND METHODS

Sample collection and identification

Fresh leaves of *Moringa oleifera* were collected from Shagari Low Cost, Maiduguri, Nigeria. The plant was identified and authenticated by a taxonomist with the Department of Biological Sciences, University of Maiduguri., Maiduguri, Nigeria.

Preparation of the extract

Fresh leaves of the plant were air-dried in the laboratory, ground into a fine powder and stored in a glass container at 4^0C. The powdered sample (800 g) was exhaustively extracted with distilled water using reflux (Trease and Evans, 1989). The resultant extract was concentrated *in-vacuo,* properly labeled and stored in glass bottles in the refrigerator at 4^0C. Also, the fresh leaves were ground using pestle and mortar, processed in a similar manner and then stored in the refrigerator at 4^0C (Trease and Evans, 1989) until required.

Fractionation of the aqueous extract

The crude aqueous extract obtained (268 g) was suspended in cool distilled water and then filtered. The filtrate was successively fractionated using chloroform, ethyl acetate and n-butanol in sequence as described by earlier workers (Cho *et al.,* 2003; Motohashi *et al.,* 2004). The resultant extract was then oven-dried.

Phytochemical analysis

The qualitative phytochemical screening of all extracts including the soluble fractions were carried out to identify the various classes of active chemical constituents such as carbohydrates, tannins, phlobatannins, saponins, cardiac glycosides, steroids/terpenes, flavonoids, anthraquinones and alkaloids. The phytochemical analysis was performed according to standard methods as described by Trease and Evans (1997).

RESULTS

The crude aqueous extract obtained was a fine powder brown in color and yielded about 33.5% (w/w).

Phytochemical screening

The results obtained from the phytochemical screening of the fresh and dried leaves crude aqueous extracts, the organic solvents and the residual fractions of *M. oleifera* leaves is shown in Table 1. The crude extract of the fresh leaves contains carbohydrates, tannins, flavonoids, alkaloids, steroids and terpenes and cardiac glycosides. The dried leaf crude aqueous extract also contains all these except tannins, steroids and terpenes. In addition to these the dried leaf crude also contains saponins and phlobatannin (Table 1). Nevertheless carbohydrates, flavonoids, alkaloids and cardiac glycosides were detected (Table 1). The ethyl acetate fraction of the extract contains tannins, carbohydrates, flavonoids, alkaloids (low concentration) and cardiac glycosides. The normal butanol fraction of the crude aqueous extract contains tannins, carbohydrates, flavonoids and alkaloids. Present in the residual portion of the aqueous extract are tannins, carbohydrates, flavonoids and phlobatannins.

DISCUSSION

This study has demonstrated the presence of chemical constituents in *M. oleifera* leaves that are of medicinal value. This finding is in consonance with reports of other workers from different parts of the world (Asres, 1995; Pal *et al*., 1995; Dahot, 1998). The medicinal significance of the compounds include for instance tannins which have been shown to have pronounced physiological astringent properties that promote healing of wounds and ameliorate inflamed mucus membrane (Tyler *et al.*, 1988). It is used in treating diarrhoea, irritation of the throat, mouth, gums, burns, skin wounds and infections of skin (Anon, 2000). The presence of these compounds in *Moringa oleifera* leaf is very significant and brightens its prospects for medicinal use especially in the repair of damaged tissues. The study also demonstrated that all the extracts and fractions except the chloroform fraction contain these secondary metabolites.

Table 1. Phytochemistry of the crude aqueous extract of fresh, dried, organic solvents and residual fractions of *Moringa oleifera* leaves

Phytochemical contents	Type of test	Crude aqueous extract of fresh leaves	crude aqueous extract of dried leaves	Chloroform	Ethyl acetate	n-butanol	Residual aqueous
	Ferric chloride	+	+	-	+	+	+
Tannin	Lead acetate	-	-	-	-	-	-
	Formaldehyde	-	-	-	-	-	-
Saponin	Froth test	+	+	-	-	-	-
carbohydrate	Molisch's test (General test)	+	+	+	+	+	+
	Free reducing sugar	+	+	+	-	-	+
	Combined reducing sugar	+	+	-	-	-	+
	Barfoed's test (for monosaccharides)	-	-	-	-	-	-
	Test for ketoses	+	+	+	-	+	-
	Test for pentoses	+	+	+	+	+	+
Flavonoid	Lead acetate test	+	+	+	+	+	+
	Sodium hydroxide	+	+	+	+	+	-
	Ferric chloride test	+	+	-	+	-	-
	Pew test	+	+	+	+	+	+
Phlobatannin	Hydrochloric acid test	-	-	-	-	-	-
	Lime water test	-	+	-	-	-	+
Steroids and Terpenes	Lieberman Buchard's test	+	-	-	-	-	-
	Salkowski's test	-	-	-	-	-	-
Cardiac glycosides	General test	+	+	+	+	-	-
Anthraquinone	Bontrigger's test (Free anthraquinone)	-	-	-	-	-	-
	Combined anthraquinone	-	-	-	-	-	-
	Anthraquinone derivative in a reduced form which are not easily hydrolized	-	-	-	-	-	-
Alkaloids	Dragendroff's test (General test)	+	0	0	0	0	-
	Mayer's test (General test)	0	0	0	0	0	-

Key : + = Present ; - = Absent

Alkaloids also contained in the leaves points at the possibility of analgesic effects of the plant. Colchicine which is an alkaloid derived from the plant has been found to be effective in relieving joint pain and inflammation caused by gout (Anon, 2000).

Another important constituent found in the extract in this study are cardiac glycosides, which represents a group of chemical compounds which when taken orally slows down the heart rate and regulate the rhythm of the heart beat as well as strengthen the cardiac muscle (Anon,2000). Generally glycosides, apart from exerting pronounced physiological effects are also known to have antiseptic properties (Robbinson, 1967). This study demonstrates the presence of the chemical agent in most forms of the extract. Since the leaf is mostly consumed orally, the possible effects of extract on heart rate may be more important. It is also apparent that the extract contains saponin which is an indication that it may have antibacterial activities. Saponins generally act by lowering the surface tension and posses emulsifying activities. They tend to alter the permeability of the cell wall and hence exert a general toxicity on all organized tissues. Saponins combine with cell membrane sterol to produce changes in cell morphology leading to lysis of the cells (Birk and Petri, 1980). They are known to posses some antibacterial activity. Flavonoids also found in the leaf extracts are group of phenolic compounds which possess antioxidant activity (Shabidi and

Wanasundara, 1992; Rice-Evans *et al.*, 1995; Heinonen *et al.*, 1998). They also possess antiallergic properties (Berg and Daniel, 1988). Pharmacologically flavonoids have been found to have CNS, cardiotonic, lipid lowering, antiulcer, hepatoprotective, antioxidant, anti-inflammatory and antimicrobial activities (Narayana *et al.*, 2001). Most of the extracts investigated in this study contain flavonoids. The type of flavonoids contained in the plant needs further investigation. This will add credence to the medicinal values of *Moringa oleifera* plant and its name the "Miracle Tree" as it is called in some parts of Nigeria.

REFERENCES

Anon, 2000). Herbs, www.herbs2000.com

Asres K (1995) The major constituents of the acetone fraction of Ethiopian *Moringa stenopetala* leaves. *Mansoura J. Pharmacol. Sci.* 11(1): 55-64.

Bennett RN, FA Mellon, N Foidl, JH Pratt, MS DuPont, L Perkins and PA Kroon (2003) Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (Horseradish tree) and *Moringa stenopetala* L. *J. Agric. Food Chem.* 51: 3546-3553.

Berg, P. A. and Daniel, P.T. (1988). *Plant flavonoids in Biology and Medicine* II. *Progress in Clinical and Biological Research.* Alan R.L.S.S. Publication, New York. pp. 157-280.

Birk , Y. and Petri, I (1980). Saponins. In: *Toxic Constituents of Plant Foodstuff*, 2nd ed. Academic Press, New York. pp. 161-182.

Cho, E. J., Yokozawa, T., Rhyu, D. Y. Kim, S.C., Shibahara, N., and Park, J.C. (2003). Study on the inhibitory effects of Korean Medical Plants and their main components on the 1.1-diphenyl-2-picrylhydrazl radical, *Phytomed.* 10: 544-554.

Dahot MU (1998) Antimicrobial activity of small protein of *Moringa oleifera* leaves. *J. Islamic Acad. Sci.* 11(1): 6 pp.

Fuglie, L.J. (2001). *The Miracle Tree*: *The Multiple Attributes of Moringa.* Netherlands: Technical Centre for Agricultural and Rural Cooperation (CTA).

Geidam, Y.A., Ambali, A.G; Onyeyili, P.A, (2007). Preliminary phytochemical and antibacterial evaluation of crude aqueous extract of *Psidium quajava* leaf. *J. Appl. Sci.* 7 (4): 511-519.

Heinonen, I. M ; Mayer, A. S and Fankel, E. N. (1998). Antioxidant activity of berry phenolics in human low density lipoprotein and liposome oxidation. *J. Agric. Food Chem.* 46(10): 4107-4112.

McLaughlin, J. L. (1991). Crown gall tumours on potato disc and brine shrimplethargy: Two simple bioassays for higher plant screening and fractionation. *In: Assays for Bioactivity.* (K. Hostettmann, ed.). London: Academic Press. pp. 1-32.

Motohashi, N., Wakabayashi, H.; Kurihara, T., Fukushima, H., Yamada, T., Kawase, M., Sohara, Y., Tarira, S., Shimtaki, Y., Sakagami, H., Satoh, K., Nakashima, H., Molnar, A., Spengler, G., Gyemant, N., Ugoesai, K. and Molnar, J. (2004). Biological activity of Barbados Cherry (Acerola fruits, fruit of malpighia emarginata DC) extracts and tractions, *Phytother. Res.* 18: 212-223.

Narayana, K. R.; Reddy, M. S.; Chaluvadi, M. R. and Krishna, D. R. (2001). Bioflavonoids classification, pharmacological, biological effects and therapeuticpotential. *Indian J. Pharmacol.* 33: 2-16.

Pal SK, PK Mukherjee, K Saha, M Pal and BP Saha (1995) Antimicrobial action of the leaf extract of *Moringa oleifera* Lam. *Ancient Sci. Life* 14(3): 197-199.

Prakash, S.A. (2006). Effects of herbal extracts towards microbicidal activity against Pathogenic Escherichia coli in poultry. *Int. J. Poult. Sci*. 5: 259-261.

Shabidi, F and Wanasundara, P.K.P. (1992). Phenolic antioxidant. *Crit Rev.Food Sci. Nutri.* 32(1): 67-103.

Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. 2nd ed. Spectrum Books Ltd., Ibadan, Nigeria.

Rice –Evans, C.A.; Miller, N. A.; Bolwell, P. G. *et al.* (1995). The relative antioxidant activity of plant-derived poly phenolic flavonoids. *Free Rad. Res.* 22(4):375-383

Robbinson, J. (1967). *Porphyrine, Organic Constituents of Higher Plants*. 1st ed. USA: Burgress Publications. 20.

Trease, G.E. and Evans, W.C. (1989). *Pharmacognosy,* 13th ed. Billiare Tindall,. London. pp. 61-62.

Trease, G.E. and Evans, W.C. (1997). *Textbook of* Pharmacoqnosy,. 14th ed. W.B. Saunders, London. pp.13-53.

Tyler, V.E.; Braddy, L. R. and Roberts, J.E. (1988). *Pharmacognosy*. Lea and Febriger, Philadelphia, USA. pp. 85-909.