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Full Length Research Paper

Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma

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Medicinal plants are important elements of indigenous medical system that have persisted in developing countries. Many of the pharmacological principles currently used as anticancer agents were first isolated from plants. However, some important anticancer agents are still extracted from plants because they cannot be synthesized chemically on a commercial scale due to their complex structures that often contain several chiral centers. The aim of this study was to test different extracts from the leaves of Moringa or drumstick tree (*Moringa oleifera*) for activity against leukemia and hepatocarcinoma cells *in vitro*. The extracts could kill majority (70 - 86%) of the abnormal cells among primary cells harvested from 10 patients with acute lymphoblastic leukemia (ALL) and 15 with acute myeloid leukemia (AML) as well as a culture of hepatocarcinoma cells (75% death), but most significantly by the hot water and ethanol extracts. In conclusion, *M. oleifera* may have potential for use as source of natural treatment for diseases such as cancer.

Key words: *Moringa oleifera*, anti-cancer, acute lymphoblastic leukemia, acute myeloid leukemia, hepatocarcinoma.

INTRODUCTION

Herbal or 'botanical' medicines, recorded in developing countries with ancient civilizations, such as Egypt and China, provide an abundant pharmacopoeia of products that have been prescribed for many diseases over many centuries. In developed countries, people seeking alternative medicines to avoid the side effects and expenses of synthetic medications have turned back to herbs to treat a wide array of ailments (Wood, 1997).

Evidence from epidemiological and experimental studies has indicated that certain naturally occurring phytochemical phenolic compounds (e.g. alkaloids and flavonoids), particularly those ingested in the human diet, exhibit antimutagenic and anticarcinogenic properties, and play a beneficial role in the prevention of certain types of cancer (Moridani et al., 2003; Goodman, 2000; Selvendiran et al., 2004; Gao et al., 2002).

Moringa oleifera (Moringa or drumstick tree) was the most widely cultivated species of a monogeneric family, the Moringaceae. The drumstick tree was native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Oliveira et al 1999). This rapidly-growing tree was utilized by the ancient Romans, Greeks and Egyptians; hence it was widely cultivated and has become naturalized in many locations in the tropics. *M. oleifera*, a perennial softwood tree with timber of low quality, has been advocated for traditional, medicinal and industrial uses for centuries. It is an important crop in

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Abbreviations: **ALL**, Acute lymphoblastic leukemia; **AML**, acute myeloid leukemia; **DPPH**, 2,2'-diphenylpicrylhydrazyl; **HCC**, hepatocellular carcinoma; **HpG2**, hepatocellular carcinoma cell line; **DMSO**, dimethyl sulfoxide.

India, Ethiopia, the Philippines and the Sudan, and has been grown for food and phytopharmaceuticals in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands. All parts of the Moringa tree are edible and have long been consumed by humans (Fuglie, 1999). *M. oleifera* can be used as alternative to some leguminous seeds as a source of high-quality protein, oil and antioxidant compounds and a way to treat water in rural areas where appropriate water resources are not available (Ferreira et al., 2008).

Leaves of *M. oleifera* are known to have various biological activities, including hypolipidaemic, antiatherosclerotic, prevention of cardiovascular diseases and antioxidant (Chumark et al., 2008; Iqbal and Bhanger, 2006), immune boosting agent, hypotensive (Faizi et al., 1994) and tumor suppressive effect (Murakami et al., 1998).

Also, the seeds of *M. oleifera* have bioactive compounds against *Aedes aegypti* larvae and moderately toxic potential (Ferreira et al., 2009). Therefore, the aim of this study was to test the effect of natural extract of *M. oleifera* as anti-cancer agents.

MATERIALS AND METHODS

Plant material

M. olifera plant parts were collected from Botanical Garden of the Botany and Agricultural Biotechnology Department, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.

Samples preparation

The extraction used was 1 g of freeze-dried, powdered leaves suspended in 10 ml of hot water, cold water, or 80% (v/v) ethanol. Extracts were stirred mechanically for 12 h at room temperature (25°C), except the hot water extract (80°C) that was made in 30 min. Solids were removed by centrifugation (4,000 g, 10 min) and the supernatant was collected. The resulting extracts were completely dried in a rotary evaporator at 40°C and lyophilized and stored at 4°C for further process (El-Shemy et al., 2007; Khalafalla et al., 2009).

Antioxidant activity

The antioxidant activity of the plant extracts was evaluated by using the 2,2'diphenylpicrylhydrazyl (DPPH) assay (Cuendet et al., 1997; Burits and Bucar, 2000). The extracts (5 - 20 µg in 50 µl) were added to 5 ml of a 0.004% (w/v) of DPPH in methanol (100% v/v). After a 30 min incubation period at room temperature with absorbance at 517 nm was compared to DPPH in methanol without an extract sample (blank) and quercetin was used as positive control. The percent inhibition of free radical formation (I%) was calculated as:

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration that provided 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration (tests were carried out in triplicate (El-Shemy et al., 2007; Khalafalla et al., 2009).

Viability of tumor cells

The study was performed on cells harvested from adult leukemia patients or healthy relatives admitted to the National Cancer Institute, Cairo University. International protocols governing the ethical treatment of patient were followed.

The viability of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) cells were calculated according to MTT assay (Selvakumaran et al., 2003). The MTT assay is a test of metabolic competence based upon assessment of mitochondrial performance relying on the conversion of yellow MTT to the purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Mononuclear cells were separated from other blood cells by Ficoll hypaque density gradients (Pharmacia, Uppsala, Sweden) according to Harbeck et al. (1982). The cytotoxicity of each extract on both AML and ALL cells was determined by the MTT assay on AML (Selvakumaran et al., 2003). AML and ALL were diagnosed by peripheral blood and bone marrow examination, cytochemistry (and immunological markers in some cases). The isolated cells were washed with three changes of PBS. The cell counts were adjusted to 3×10^3 cell /well and plated in 100 µl of medium/well in 96-well plates (Costar Corning. Rochester, NY). After overnight incubation, extracts were applied at various concentrations (10 and 20 µg/ml) and compared with cytotoxicity to a human normal lymphoid cell line; 3 wells were used at each concentration. After treatment with extracts for one day, 20 µl of 5 mg/ml MTT (pH 4.7) was added per well and cultivated for another 4 h. The supernatant fluid was removed, 100 µl DMSO was added per well and shaken for 15 min. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without cells as blanks. All experiments were performed in triplicate.

Calculations

The effect of extracts on the proliferation of human AML cells was expressed as the % cytoviability, using the following formula:

$$\text{Cytoviability \%} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100.$$

Viability of HpG2 cell cultures

Hepatocellular carcinoma (HCC) cell line HpG2 was obtained from VACSERA, Egypt. The viability of HpG2 cells in culture was calculated by the MTT assay (Selvakumaran et al., 2003). As with the ALL cells, the cell counts were adjusted to 3×10^3 cell /well and plated in 100 µl of medium/well in 96-well plates (Costar Corning. Rochester, NY). After overnight incubation, extracts were in various concentrations (10 and 20 µg/ml) with cytotoxicity to human normal lymphoid cell line; 3 wells were included in each concentration. After treatment with extracts for one day, 20 µl of 5 mg/ml MTT (pH 4.7) were added per well and cultivated for another 4 h, the supernatant fluid was removed, then 100 µl DMSO were added per well and shaken for 15 min. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without cells as blanks. All experiments were performed in triplicate.

Statistical analysis

The statistical analysis was computed using analysis of variance procedure described in SAS/STAT software (SAS Institute, Cary,

Table 1 Antioxidant activity of plant extracts measured using DPPH.

Plant sample	Antioxidants activity of extract (50µl)	
	Antioxidant activity	IC_{50}
Cold water	49 ± 3.2 6	1.02
Hot water	63 ± 6.32	0.31
Ethanolic Extract	77 ± 3.89	0.26

IC_{50}, Half maximal inhibitory concentration.

Table 2. The effect of the *M. olifera* extract on the percentage of viable AML and ALL cells after 24 h of incubation.

Time	Extract concentration											
	Cold water				Hot water				Ethanolic extract			
	1µg	5 µg	10 µg	20 µg	1 µg	5 µg	10 µg	20 µg	1 µg	5 µg	10 µg	20 µg
	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)
AML(M3)												
6 h	9±1.52	32 ±2.00	39±1.40	58±3.40	18±2.20	29±2.72	53±4.50	62±5.34	22±4.30	36±2.40	59±3.47	64±3.22
18 h	26±3.22	35±0.88	47±2.14	66±4.30	15±o.79	36±3.20	63±3.80	71±3.50	31±2.14	39±4.54	68±5.32	77±2.07
24 h	37±5.23	49±3.70	53 ±3.20	72±3.50	34±3.30	55±5.17	61±4.36	75±5.51	28±3.31	51±6.40	73±2.50	82±3.20
IC_{50}	8.79				4.3				4.91			
LSD	3.68	4.50	2.65	5.34	1.56	4.57	3.89	5.91	4.32	2.47	3.59	4.39
ALL(L2)												
6 h	2±0.30	36±3.20	48±4.20	67±5.30	8±.3.42	41±4.35	56±4.37	81±2.41	19±.07	45±2.59	57±3.40	76±2.42
18 h	5±0.12	30±0.90	56±3.20	73±3.90	13±2.32	39±4.12	62±2.09	76±3.23	23±0.98	36±2.90	59±1.27	74±1.24
24 h	4±0.20	20±2.40	59±2.80	77±4.80	16±2.30	47±0.87	76±6.38	84±4.36	36±4.21	52±6.4	71±6.34	86±1.4
IC_{50}	7.52				6.31				4.59			
LSD	0.69	3.54	3.56	3.89	1.43	2.68	5.32	7.54	4.30	2.79	3.9	6.32

IC_{50}, Half maximal inhibitory concentration; LSD, least significant difference.

NC, USA). The significant differences between treatment means were separated by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Each plant extracts showed a concentration dependent scavenging activity by quenching DPPH radicals (Table 1). As judged by this assay, the *M. olifera* ethanolic extract had the highest antioxidant activity at 77% inhibition of radical formation (Table 1). This high antioxidant capacity may be due to the high concentration of phenolics and flavonoids in *M. olifera* extracts. Phenolic and flavonoid compounds are common in medicinal plants, spices, vegetables, fruits, grains, pulses and other seeds. These compounds are an important group of natural antioxidants with possible beneficial effects on human health (Meyer et al., 2003). They can participate in protection against the harmful action of reactive oxygen species, mainly oxygen free radicals. In fact, several scientific studies reported that certain phytochemicals such as flavonoids and polyphenols, have been reported as promising antioxidant compounds that might help attenuate oxidative stress (Halliwell

Table 3. The effect of the *M. olifera* extract on the percentage of viable HpG2 cells after 24 h of incubation.

Time	Extract concentration											
	Cold water				Hot water				Ethanolic extract			
	1 µg	5 µg	10 µg	20 µg	1 µg	5 µg	10 µg	20 µg	1 µg	5 µg	10 µg	20µg
	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)	Dead (%)
24 h	19±1.98	38±1.60	52± 1.15	69±2.20	35±1.82	64±1.02	73±4.55	81±4.75	23±2.23	45±3.88	61±1.31	76±2.36
IC_{50}	8.67				3.89				6.2			
LSD	4.53				3.71				3.96			

and Gutteridge, 1999: Khalafalla et al., 2009).

After 24 h incubation period of the mononuclear ALL cells with ethanolic extract, a remarkable destruction of lymphoblasts (82%) was found (Table 2). In the case of mononuclear AML cells, similar trends were observed (86%) (Table 2). The mean viability of each well of extract treated cells was 25% when compared to the control (94.6%). The extract was incubated with normal mononuclear cells as a positive control from healthy volunteers. The results showed that there was no significant difference in cell death when compared to the untreated control (negative control). Similarly, the viability of HpG2 tumor cells, after incubation with extracts were significantly affected especially ethanol extract (Table 3).

A previous report of plant derived antileukemia treatment showed that, allamandin derivatives that were extracted with water and/or ethanol from *Allamanda catharica* (Apocynaceae) had significant activity *in vivo* against the p-388 leukemia in the mouse (Kupchan et al., 1976). Here however, the major destructive effect on AML and ALL cells were obtained by the hot water and ethanol fractions. The phenolic compounds, most especially glycosides, will dissolve in water or ethanol solutions (Bravo, 1988). Therefore, these groups of compounds may contain the major active components for the destruction of leukemia and carcinoma cells (El-Shemy et al., 2007; Khalafalla et al., 2009). A number of food components have been identified that inhibit the initiation and progression of cancer or otherwise influence the potential for disease outcome (Hu et al., 2002). For example, some epidemiological studies showed a close association between low incidence of coronary heart disease and breast cancer (Renaud and Lorgeril, 1992) and moderate consumption of red wine containing natural polyphenolic compounds (Gronbaek et al., 1995).

In conclusion, the active ingredients that were easily dissolved in hot water and ethanol from Moringa or drumstick tree (*M. oleifera*) could both be used as natural antitumor medicines. They were active against leukemia and hepatocarcinoma cells *in vitro*. The metabolites within the extract and their role in killer of leukemia and hepatocarcinoma cells will be identified.

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REFERENCES

Bravo L (1988). Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev. 56: 317-333.

Burits M, Bucar F (2000). Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res 14: 323-328

Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthongngam L, Ratanachamnong P, Srisawat S, Pongrapeeporn KS (2008). The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam Leaves. J. Ethnopharmacol. 116: 439-446.

Cuendet M, Hostettmann K, Potterat O, Dyatmiko W (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helvetica Chimica Acta. 80: 1144-1152.

Duncan DB (1955). Multiple range and multiple F test. Biometrics, 11: 1-42.

El-Shemy HA, Aboul-Enein AM, Aboul-Enein KMI, Fujita K (2007). Willow leaves extracts contains anti-tumor agents effective against three cells types. PLoS ONE, 2(1): p. 178.

Faizi S, Siddiqui B, Saleem R, Saddiqui S, Aftab K (1994). Isolation and structure elucidation of new nitrile and mustard oil glycosides from Moringa oleifera and their effect on blood pressure. J. Nat. Prod. 57: 1256-1261.

Ferreira PMP, Carvalho AFFU, Farias DF, Cariolano NG, Melo VMM, Queiroz MGR, Martins AMC, Mmachado-neto JG (2009). Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals. Ann. Acad. Bras. Cienc. 81: 207-216.

Ferreira PMP, Farias, DF, Oliveira JTA, Carvalho AFFU (2008). *Moringa oleifera*: Bioactive compounds and nutritional potential. Rev. Nutr. 21: 431-437.

Fuglie LJ (1999). The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service, Dakar. Revised in 2001 and published as The Miracle Tree: The Multiple Attributes of Moringa, pp. 68,172.

Gao X, Xu YX, Divine G, Janakiraman N, Chapman RA, Gautam SC (2002). Disparate *in vitro* and *in vivo* antileukemic effects of resveratrol, a natural polyphenolic compound found in grapes. J. Nutr. 132: 2076-2081.

Goodman GE (2000) Lung cancer. 1: Prevention of lung cancer. Thorax. 57: p. 994.
Gronbaek M, Deis A, Sorensen TIA, Becker U, Schnohr P, Jensen G (1995) Mortality associated with moderate intakes of wine, beer, or spirits. Br. Med. J. 310: 1165-1169.
Halliwell B, Gutteridge JMC (1999). Free Radicals in Biology and Medicine. Third ed. Oxford Clarendon, pp. 200-783.
Harbeck RJ, Hoffman AA, Redecker S, Biundo T, Kurnick J (1982). The isolation and functional activity of polymorphonuclear leukocytes and lymphocytes separated from whole blood on a single Percoll density gradient. Clin. Jmmunol. Immunopathol. 23: p. 682.
http://www.echotech.org/bookstore/advanced_search_result.php?keywords=Miracle+Tree.
Hu H, Ahn N, Yang X, Lee Y, Kang K (2002): *Ganoderma lucidum* extract induced cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. Int. J. Cancer, 102: 250-253.
Iqbal S, Bhanger MI (2006). Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan. J. Food Compos. Anal. 19: 544-55.
Khalafalla MM, Abdellatef E, Daffalla HM, Nassrallah AA, Aboul-Enein KM, Lightfoot DA, Cocchetto A, El-Shemy HA (2009). Antileukemia activity from root cultures of *Vernonia Amygdalina.* J. Med. Plants Res. 3: 556-562.
Kupchan MS, Uchida I, Branfman AR, Caily RG, Yufei B (1976). Antileukemic principles isolated from Euphorbiaceae plants. Science, 191: 571-572.
Meyer KJ, Watkins CB, Pritts MP, Liu RH (2003). Antioxidant and antiproliferative activities of strawberries. J. Agric. Food Chem. 51: 6887-6892.
Moridani MY, Pourahmad J, Bui H, Siraki A, O'Brien PJ (2003). Dietary flavonoid iron complexes as cytoprotective superoxide radical scavengers. Free Rad. Biol. Med. 34: p. 243.
Murakami A, Kitazonz Y, Jiwajinda S, Koshimizu K, Ohigashi H (1998). Niaziminin, a thio carbamate from the leaves of *Moringa oleifera*, holds a strict structural requirement for inhibition of tumor promoter-induced Epstein-Barr virus activation. Planta Med. 64: 319-323.
Oliveira JTA, Silveira SB, Vasconcelos KM, Cavada BS, Moreira RA (1999). Compositional and nutritional attributes of seeds from the multiple purpose tree *Moringa oleifera* Lamarck. J. Sci. Food Agric. 79: 815-820.
Renaud S, Lorgeril M (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet, 339: 1523-1526.
Selvakumaran M, Pisarcik DA, Bao R, Yeung AT, Hamilton TC (2003). Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. Cancer Res. 63: 1311-1316.
Selvendiran K, Mumtaz Banu S, Sakthisekaran D (2004). Protective effect of piperine on B(a)p induced lung carcinogenesis in Swiss albino mice. Clin. Chem. Acta. 350: p. 73.
Wood M (1997). The book of herbal wisdom: Using plants as medicine: North Atlantic Books press. p. 374.