Investigation of Proximate Compositions, Minerals Content and Acute Toxicity Study of *Moringa oleifera* Lam

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Abstract

Moringa oleifera Lam., popularly known as Drumstick tree, Dan-tha-lon in Myanmar,belongs to family Moringaceae. This study aimed to scientifically investigatephytochemicalconstituents and acute toxicity studyof *M. oleifera* leaves. The proximate compositions were done by Association of Official Analytical Chemist (1990). Mineral elements were measured by atomic absorption spectrophotometer. Qualitative phytochemical analysis was carried outby Raaman (2006). Acute toxicity study was done by Organization for Economic Co-operation and Development, 425 Guideline. According to proximate compositions, the percentage of moisture, ash, fibre, fat, protein and carbohydrate in *M. oleifera*leaves was 6.69, 11.97, 10.13, 4.84, 23.88 and 42.49%. Macrominerals (Ca, Mg, K, Na) content were 1603.89±10.42, 66.99±0.25, 205.63±0.37 and 51.78±0.31 ppm. Microminerals (Cr, Cu, Fe, Mn, Zn) content were 2.05±0.28,13.71±0.76, 57.38±0.53, 17.71 ± 0.05 and 13.83±1.26 ppm. Toxic minerals,Cd was not detectedand Pb content was4.19±1.00 ppm.All minerals content were within the permissible limit.Inphytochemical analysis,the leaves contained alkaloids, α amino acid, flavonoids, glycosides, phenols, reducing sugar, saponins and tannins.Acute toxicity study revealed that, no toxic sign and lethality at dose 5000 mg/kg (LD_{50}>5000 mg/kg). These findings indicated that, *M. oleifera*leavesprovide good source ofmany nutrients for human health andused as food supplement for community.

Key Words: Proximate Compositions, Minerals Content, Acute Toxicity, and *Moringa oleifera*

Introduction

Medicinal plants are potential sources of natural compounds with biological activities and therefore many researchers have studied for its nutritional potentials worldwide [1]. In the developing world, there is about 3.4 billion people depend on plant-based traditional medicines. World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe[2]. Plants are basic source of human diet, cheap, essential, remunerative source of proximate nutrients, minerals, vitamins for many economical suffered community[3].Nowadays, consumer demand herbal products as dietary supplements and scientifically interest upon herbal medicine[4]. Plants proximate analysis gives valuable information on moisture, ash, fat, fibre, carbohydrate, protein and provides the quality of sample [5].

The human body requires a number of minerals for their growth and other activities which are obtained from plants. Plants absorb and accumulate minerals from environment [6.] Mineral composition of a plant plays significant role its nutritional, medicinal andtherapeutic values [7].Macro and micro minerals influence biochemical

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processes in the human organism. Several attempts have been made to determine of the macro and micronutrient contents of medicinal plants from many countries all over the world [8].

Moringa oleifera Lam., popularly known as Drumstick tree, Dan-tha-lon in Myanmar, belongs to family Moringaceae. It is native to India, Pakistan, Bangladesh and distributed in Myanmar, Asian, African, Latin America and Caribbean countries. It is used as anti-inflammatory, anti-hypertensive, antioxidant, hepato-protective, anti-diabetic and antimicrobial activities[5,9].However, scientific evaluation of mineral contents by measuring of atomic absorption spectrophotometer (AAS) from *M. oleifera* Lam.has not been published yet. Thus, the present study aims to scientificallyinvestigateproximate compositions, minerals content, phytochemical constituents and acute toxicity studyof *M. oleifera* leaves.

General objective

To investigateproximate compositions, minerals content, phytochemical constituents and acute toxicity studyof *M. oleifera* leaves.

Specific objectives

1. To find out phytochemical constituents of *M.oleifera* leaves
2. To investigate the proximate composition of *M.oleifera* leaves by AOAC method
3. To determine the minerals content of *M.oleifera* leaves
4. To evaluate LD_{50} value of aqueous extract by acute oral toxicity test in ICR (Institutional Cancer Research) mice

Materials

Chemicals and Reagents

Analytical grade standard of calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), Lead (Pb), zinc (Zn), 70% nitric acid (HNO_3), 69% hydrochloric acid (HCl), sulphuric acid (H_2SO_4), pet ether (60-80°C), boric acid, sodium hydroxide (NaOH), methyl red indicator from Merck and distilled water (DDW) were used inthis study.

Instruments and Apparatus

Kjeldahl digestion system (KT85) distillation unit, soxhlet 6 places, atomic absorption spectrophotometer (AA 6650), muffle furnaces (LEF 1035) and rotary evaporator were used.

Methods

Study design

The present study performed was laboratory based experimental study.

Study place and Period

This study was conducted at Biochemistry Research Division, Department of Medical Research (Pyin Oo Lwin Branch) and Biology Department, Sagaing University of Education.

Plant authenticity

The selected *M.oleifera*leaves with their inflorescenceswereidentified and confirmed for its specific botanical name by competent taxonomist from Biology Department, Sagaing University of Education[10].

Sample Collection

*M.oleifera*leaves were collected from Mandalay city within 2019. These fresh leaves werewashed, air dried in shade, ground to powder andkept in sampling bags for further analysis.

Proximate investigation

The proximate compositions (moisture, ash, fat, fiber, carbohydrate and protein) were done by Association of Official Analytical Chemist(AOAC), 1990guideline[11].

Determination of moisture content

Fresh sample 2 g was taken in petridish and kept in a hot air oven at 110°C for 3hours. The loss in weight was regarded as moisture content.

Calculation;

$$\text{Moisture\%} = \frac{\text{Weight of moisture (g)}}{\text{Weight of sample (g)}} \times 100$$

Determination of total ash

About 2g of samples in porcelain basin was weighed and heated at 110°C in an oven. Then, it was ashed with muffle furnace at 550 °Cfor 4 hours until a white or grey ash residue were obtained.

Calculation;

$$\text{\% of total ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Determination of crude fiber

The crude fiber was determined by acid – base treatment with1.25% H_2SO_4and1.25% Na OH. The residue was allowed to dry to a constant weight at 110°C and then, ignited in muffle furnace at 550°C for 4 hours, cooled and weighed again. The loss of weight due to ignition was crude fiber.

Calculation

$$\text{\% of Crude Fiber} = \frac{\text{Wt. of crucible with dry residue – Wt. of crucible with ash}}{\text{Weight of sample (g)}} \times 100$$

Determination of crude fat (Ether extract)

The crude fat was determined by soxhlet extractor with pet ether (60-80°C)for 8 hours.

Calculation

$$\text{\% of Fat} = \frac{\text{(Weight of flask with ether extract- Weight of flask)}}{\text{Weight of sample (g)}} \times 100$$

Determination of crude protein (Micro Kjeldahl Method)

The crude protein was determined by using the micro-kjeldahal method according to AOAC guidelines.

Calculation

$$\text{\% of Nitrogen} = \frac{(Vs - Vb) \times M \times 14.01}{\text{Weight (g)} \times 10}$$

% of crude protein = % Nitrogen × F
Vs = Volume (mL) of standardized acid used to titrate test
Vb = Volume (mL) of standardized acid used to titrate test
M = Molarity of HCl
14.01 = atomic weight of Nitrogen
W = Sample weight (g)
10 = factor to convert mg/g percent
F = factor to convert N to protein (6.25)

Determination of total carbohydrate

Carbohydrate was calculated by following formula.
% of Carbohydrate =100- (Crude Protein % + Crude Fat % Crude Fiber % + Moisture%+ Total ash %)

Determination of Energy Value

Energy value = [(carbohydrate content x 4) + (protein content x 4) +(fat content x 9)]
(kcal/100g)

Determination of minerals content

Mineral elements were measured by ash digestion method using atomic absorption spectrophotometer[12].

Phytochemical test for types of compounds

Types of compounds present in*M. moringa*leaveswere carried out by Raaman, 2006[13].

Preparation of plant extracts

Fifty grams of cleaned dried sample was extracted with 500 ml of distilled water by using soxhlet at 60°C and 6 hours for 2time [14].

Acute toxicity study of aqueous extract of *M.oleifera* leaves

Acute oral toxicity was done by limit test at the dose of 5000 mg/kg according to OECD 425 guideline (2008). The animals were kept in the cages for at least 5 days prior to testing foracclimatization to the laboratory conditions and maintained with standard food and water.Then, extract was dissolved with distilled water for required concentration to be administered. Animals were fasted food for 3-4 hours prior to testing. The single dose was administered upon fasted weight,orally by using feeding nozzle. After administration, food may be withheld for 1-2 hours[15].

Clinical observations

Animals were observed continuously during the first 30 minutes after dosing and observed periodically (with special attention given during the first 4 hours) for 24 hours and then daily thereafter for 14 days to monitor the systemic toxic effects for each animal[16]. LD_{50} was calculated by AOT 425 Stat program prepared for US Environmental Protection Agency.

Statistical analysis

Each experiment was repeated three times. Data was analyzed by using Microsoft Office Excel v. 2007. Results were presented as mean ± SE.

Results and Discussion

Botanical identification

The collected fresh samples with their inflorescences were identified at Department of Department of Biology (Sagaing University of Education).

Scientific Name - *Moringa oleifera*Lam.,Enc.1: 398.1785.
Family - Moringaceae
English Name - Horse radish, Drum stick
MyanmarName - Dan-tha-lon
Part Used - Leaves, Fruits, Seeds
Flowering Period - March - May

Plant Description

Perennial, large tree, up to 12m high; stems and branches are terete, pubescent while young. Leaves are tripinnately, compound, alternate; leaflets 3 to 9 paired, opposite, ovate, obovate to oblong. Inflorescences are panicles, erect; peduncles 8 to 30 cm long. Flowers are white, or yellowishwhite, greenish at the base, about 2 cm in diameter at anthesis, bisexual, fragrant. Sepals 5,ovate or oblong. Petals 5, obovate hairy at the base. Stamens 5, free, hairy at the base; filaments filiform; stminodes 5, alternating with stamens. Gynophore 2-3 mm long. Ovary superior, linear, hairy unilocular with numerous ovules on the parietal placentae; style glabrous, with hollow stigma. Capsules 18 to 45 cm long, pendulous, 3 valves with blunt ribs. Seeds are compressed sub orbicular, 3 winged.

Proximate Investigation

The proximate investigation of dried *M. oleifera* leaves are shown in (Table 1).

Table 1. Proximate compositions of *M. oleifera*leaves

Name	Moisture (%)	Ash (%)	Crude Fibre (%)	Crude Fat (%)	Crude Protein (%)	Carbo-hydrate (%)	Energy Value (kcal/100g)	Reference
M. oleifera	6.69	11.974	10.13	4.84	23.88	42.49	309.04	
Nigeria, 2019	4.42	8.85	9.26	2.38	15.23	59.86		9
Algeria, 2018	-	14.1	-	6.5	22.8	56.6	376	17
Nigeria, 2015	7.88	9.82	12.57	3.88	28.00	37.87	2625.25	18

The low moisture content in *M. oleifera* prevents the growth of microorganisms and prolongs storage life. Ash is inorganic residue and it gives us an idea of the mineral matter contained in a plant. Crude fibre has small nutritional value.Fibre can lowerthe risk of coronary heart disease, diabetes, constipation, hypertension, colon and breast cancer and reduced serum cholesterol level, aids absorption of trace elements in the gut, help in bowel movement. Dietary fat promotes the increase of palatability of food by absorbing and retaining flavors. Crude protein are essential for natural synthesis and maintenance of body tissues, enzymes, hormones and other substances required for healthy functioning. Carbohydrate provides energy to cells such as brain, muscles and blood contribute to fat metabolism and acts as mild laxative tohuman beings. The contribution of energy from plants is sufficient, supply of energy in the diet is required for protein to be fully utilized[5,18].

Determination of mineral content

The minerals contentfromdried *M. oleifera* leaves are shown in (Table2).

Table 2. Minerals content (ppm) from*M. oleifera* leaves and other countries

Present/ Other Study	Macrominerals				Microminerals					Toxic mineral		Ref
	Ca	Mg	K	Na	Cr	Cu	Fe	Mn	Zn	Cd	Pb	
M. oleifera	1603.89 ± 10.42	66.99 ± 0.25	205.63 ± 3.15	51.78 ± 0.31	2.05 ± 0.28	13.71 ± 0.76	57.38 ± 0.53	17.71 ± 0.05	13.83 ± 1.26	ND	4.19 ± 1.00	
Nigeria,2019	1.93%	0.39%	-	-	-	7.05	105.2		59.37	-	-	9
Algeria, 2018	15550	2366	23831	3190	-	8.1	390	52.1	33.7	-	-	17
Nigeria, 2015	825	6433.3	4300.00	-	-	-	5.8		641.7	-	-	18
Ref Value	3600*	2000***	6380 - 36600***	400-500*	2****	73**	425**	500**	100**	**0.**3****	10****	

* = Ref 19, **= Ref 20, *** = Ref 21, **** = Ref 22, ND=Not Detected

According to table 2, *M. oleifera* leaves contained some essential macrominerals, microminerals. All minerals content were within maximum permissible limits(WHO 1996 , FAO/WHO 2001, Ajasa 2004and WHO, 2005)[19,20,21,22].

Preparation of plant extracts

Yield percent of aqueous extract *M. oleifera* leaves was11.29%.

Phytochemical Test

In phytochemical analysis, *M. oleifera*leaves contained alkaloids, α amino acid,carbohydrate, flavonoids, glycosides, phenols, protein,reducing sugar, saponins and tannins. Alkaloids possess analgesic, antispasmodic, antibacterial, anti-inflammatory, anticancer and antioxidant activities. Flavonoids have anti-allergic, anti-inflammatory, antibacterial, anticancer, antidiabetic, antidiarrhea, antioxidant properties and lower the risk of heart disease. Glycosides used as antibiotic, anticancer, antidiabetic, purgative, and treatment of congestive heart failure, cardiac arrhythmia and skin diseases. Saponins show antifungal, antibacterial, anticancer, antidiabetic, antiprotozoal, hypolipidemic, hypocholesterolemic and responsible for central nerveous system.Tanninshave antidiarrheal,antioxidant, peptic ulcers and wound healingactivities.Phenolic compounds like alkaloids, flavonoids and tannins have important biological activities and essential for human diet, found in plants[23].

Acute toxicity test of the aqueous extract papaya leaves

Acute toxicity test was performed using OECD 425 guideline for minimizing the number of animals. Rathi B.S.*et al*. (2006), India study, reported that, LD_{50} of aqueous extract of *M. oleifera* leaves was found to be 5000 mg/kg by OECD 425 [24].Chivapat S.*et al*. (2011), from Thailand revealed that aqueous extract of *M. oleifera* leaves was non-toxic at the dose of 20 g/kg[25].Therefore, limit test, at 5000 mg/kgwas used. The results showed that no toxic signs and lethality for all mice up to 14 days observation period, at the dose of 5000 mg/kg. Skin and fur changes, eyes, mucous membrane, respiratory rate, motor activity and behavioral pattern were found to be normal. Salivation, convulsion, cyanosis, tremors, and diarrhea did not occur in all animals. There was no significant change in body weight before and after administration of test extract. Therefore, median lethal dose (LD_{50}) was greater than 5000 mg/kg and acute safe for consumption.

The differences in proximate compositions and minerals content of *M. oleifera* leaves have been reported by various investigators might be due todifferent geography, climatatic conditions, times of harvests, habitat and part used of plant samples.

Conclusions

Theresults of proximate compositions and minerals content of *M. oleifera* leaves indicated the presence of considerable amount of protein, carbohydrate, fats, and minerals. Therefore,*M. oleifera*leavesprovide good source ofmany nutrients for human health andused as food supplement for community.

5. References

1. Hussein H.J., Hadi M. Y.*et al*,. Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophot ometer and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy.* 2016; 8(3): 60-89.
2. Revathi P., Jeyaseelansenthinath T. and Thirumalaikolundhusubramaian P. Preliminary Phytochemical Screening and GC-MS Analysis of Ethanolic Extract of Mangrove Plant-Bruguiera Cylindrica (Rhizho) L. *International Journal of Pharmacognosy and Phytochemical Research*. 2014-15; 6(4): 729- 740.
3. Ullah I., Gul S.*et al.*. Analysis of nutrients and minerals of some wild edible plants. *International Journal of Fauna and Biological Studies.* 2017; 4(6): 35-39.
4. Khan S. A., KhanL., *et al.*. Profile of Heavy Metals in Selected Medicinal Plant. *Pak. J. Weed Sci. Res*. 2008; 14(1-2): 101-110.
5.Samia M.Y., Elbadri O. E*et al.*. Proximate Composition of *Moringa oleifera* Lam. from different Regions in Sudan. *CPQ Microbiolog.* 2018; 1 (4), 01-13.
6. Gajalakshmi S., Iswarya V., *et al.* Evaluation of heavy metals in medicinal plants growing in Vellore District. *European Journal of Experimental Biology,* 2012; 2 (5):1457-1461.
7. Debebe M. and Eyobel M. Determination of proximate and mineralcompositionsof *Moringa oleifera* and *Moringa stenopetala* leaves cultivated in Arbaminch Zuria andKonso, Ethiopia. *African Journal of Biotechnology*, 2017; 16 (15): 808-818.
8. Kumar A., Singh R. P and Singh N.P. Analysis of macro and micro nutrients in some Indian medicinal herbs grown in Jaunpur (u.p.) soil. *Natural Science*. 2011; l3(7): 551-555.
9. Ali A. M. *et al*. Phytochemical Screening, Proximate and Mineral Analysis of Moringa Oleifera Leaf in Kano, Northern Nigeria.*Journal of Allied Pharmaceutical Sciences.* 2019; 1(1): 1-5.
10.Nwe Nwe Yi, 2007. Taxonomic Study on Magnoliophyta (Angiospermae) of Loikaw Township. PhD Thesis, University of Mandalay.
11. AOAC, 1990. Association of Official Analytical Chemist. USA. 15th Edn: 69-90.
12.Niaz A, Ullah N *et al.* Pollution Based Study of Heavy Metals in Some Selected Medicinal Plants by Dry Digestion Method. *International Journal of Pharma Sciences and Research (IJPSR).* Feb 2013; 4 (2):17-24.
13. Raaman N. 2006. 'Qualitative phytochemical screening', in: Phytochemical Techniques, *New India Publishing agency*, New Delhi: Pitampura; 19-24.
14. Handa S.S., Khanuja S.P.S., Longo G. and Rakesh D.D. (2008). Extraction technologies for medicinal and aromatic plants. *International center for science and high technology Trieste*. 21-25.
15. Organisation for Economic Co-operation and Development (OECD) (2008). Acute oral toxicity- up and down procedure. In: *OECD Guideline for Testing of Chemicals 425.* 1-27.
16. Loomis T.A. and Hayes A.W. (1996). Toxicologic testing methods. In: *Loomis'Essentials of Toxicology*. 4th Edition, *Academic press*, USA.212-213.
17. Ziani B.E.C. *et al*.Detailed chemical composition and functional properties of Ammodaucus leucotrichus Cross. & Dur. and Moringa oleifera Lamarck.*Journal of Functional Foods.* 2019;53: 237–247
18. Okiki P.A., Osibote I.A. *et al*. Evaluation of Proximate, Minerals, Vitamins and Phytochemical Composition of *Moringa oleifera* Lam. Cultivated in Ado Ekiti, Nigeria. *Advances in Biological Research.* 2015; 9 (6): 436-443.
19.Tadzabia K., Maina H. M., Maitera O. N. and Osunlaja A. A. Elemental and PhytochemicalScreening of *Vitex Doniana* Leaves and Stem Bark in Hong Local Government Area of Adamawa State, Nigeria. *International Journal of Chemical Studies.* 2013; 1(3):150-156
20.FAO/WHO (Codex Alimentarius Commission) (2001). Food additives and contaminants. Joint FAO/WHO Food Standards Program; ALINORM 01/12 A:1-289.
21. Ajasa OM, Bello MO, Ibrahim AO, *et al.* Heavy trace metals and macro nutrients status in Herbal plants of Nigeria. *Food Chemistry.* 2004; 85: 67-71.

22. WHO (2005). Quality control methods for medicinal plant materials. Revised, Geneva.
23.Lei Lei Win *et al.* Elemental Composition, Phytochemical Screening and Antibacterial Activity of *Aegl marmelos* (L.) Correa(□□□□□□). 8th Traditional Medicine ResearchCongress.
24.Rathi B.S*et al*.Evaluation of aqueous extract of *Moringa oleifera* Linn for wound healing in albino rats. *Indian Journal of Experimental Biology*.2006; 44: 898-901.
25.Chivapat S.,Sincharoenpokai P. *et al*.Acute and Chronic Toxicity of*Moringaoleifera* Linn Leaves Extracts. *Thai Journal of Veterinary Medicine*. 2011;41(4): 417-424.