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Phytochemical, Anti-nutritional and Toxicity Assessment of Moringa Oleifera Seeds, Stem Bark and Leaves Using Brine Shrimp (Artemia Salina) Assay

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Abstract: Phytochemical contents of *Moringa oleifere* seeds, stem bark and leaves were determined using various standard methods. The toxicological evaluation was carried out using brine shrimp lethality assay. The phytochemical and anti-nutritional evaluation of the samples revealed that the leaves were highest in alkaloids (6.68±2.35 %), saponins (35.66±0.78%) and tannins (0.64± 0.45 mg/100g), while the stem bark has the lowest contents with values of 0.53±0.25%, 14.3±0.33% and 0.56±0.17mg/100g, respectively. The seeds were however high in flavonoids (14.21±0.66%), oxalates (31.98±0.62mg/100g), phytates (0.85±0.04mg/ 100g) and cyanogenic glycosides (7.4±0.00 mg/100g).
The toxicological study also revealed that extracts from the three parts were not toxic after 24 hours. Mortalities however took place after 48 hours (between 1-5 nauplii death) and 72 hours (5-10 nauplii death) at varying concentrations.
Keywords: *Moringa oleifera*, phytochemicals, anti-nutrients, toxicity, brine shrimps, human health.

Introduction

Moringa oleifera is one of the promising plants which could contribute to increased intake of some essential nutrients and health-promoting phytochemicals. It is recognized as a vibrant and affordable source of phytochemicals, having potential applications in medicines, functional food preparations, water purification and biodiesel production. As world population and affluence continue to grow, the need to feed both man and animal will generate more problems, especially in developing and underdeveloped countries. Even today, the World Health Organization (WHO) estimates that up to 80% of people still rely primarily on traditional remedies, such as herbs, for their medicines[1].

In different parts of the world, *Moringa oleifera* is known by diverse names: among the Igbos of south eastern Nigeria, it is known as "*Okwe Oyibo*", among the Hausas (northern part of Nigeria), it is called '*Zogale*', among the Yorubas (western part of Nigeria), it is called '*Ewe ile'*, among the Fulani, it is called '*gawara*', '*sonjna*' in Marathi, '*Nuggekai'* in Canada, '*Murungai*' in Tamil, '*Mashinga Sanga'* in *Malayalan*. In English language, *Moringa oleifera* is also called Drumstick, Miracle tree, Mother's best friend, never die and Benzolive tree[2]. The leaves are edible and are commonly cooked and eaten like spinach or used to make soups and salads. The composition of the amino acids in the leaf protein is well balanced[3,4]. The leaves and pods are helpful in increasing breast milk in nursing mothers during breastfeeding[5].

However, it is well known that plants generally contain anti-nutrients acquired from fertilizers and pesticides and several naturally-occurring chemicals[6]. Some of these chemicals are known as ''secondary metabolites'' which have been shown to be biologically highly active [7]. They include: saponins, tannins, flavonoids, alkaloids, trypsin (protease) inhibitors, oxalates, phytates, haemagluttinins (lectins), cyanogenic

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glycosides, cardiac glycosides, coumarins and gossypol; the list is inexhaustible. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed at appropriate amounts[8a,b].

Figure 1. Parts of *Moringa* tree (A) *Moringa* tree; (B) *Moringa* pods; (C) *Moringa* leaves and (D) *Moringa* pods with the seeds.

Several investigators have reported the phytochemicals of *Moringa oleifera* seeds, stem bark and leaves but, only limited work has been done on the toxicological assessment of this plant. Taking these precedents into consideration, this study is therefore aimed at investigating the phytochemicals and anti-nutrients present in *Moringa oleifera* seeds, stem bark and leaves and aascertaining the toxicity potential of this plant using the brine shrimp (*Artemia salina*) lethality assay.

Methods and Materials

The leaves, stem bark and seeds of *Moringa oleifera* were collected from a private residence in Ilorin metropolis Kwara State, Nigeria and identified by a taxonomist at the Department of Plant Biology of the University of Ilorin, Nigeria (Figure **1**). The leaves were destalked, rinsed and air-dried with constant turning of the leaves to avert fungal growth. The seeds and stem bark were similarly air-dried before homogenizing using a mortar and pestle. The dried leaves, stem bark and seeds were later milled using an electric blender and stored in well labeled air-tight containers and refrigerated prior to analysis.

Phytochemical Screening

Extraction of the plant parts was carried out by maceration in two different solvents: n-hexane and methanol. The extracts were filtered and concentrated using a rotary evaporator. Qualitative phytochemical screening of extracts was carried out using various standard methods and the phytochemicals screened included: tannins, saponins[9], alkaloids[10], cardiac glycosides[11], anthocyanins[12], flavonoids[13], terpenoids, steroids[10] and coumarins[12].

Quantitative determination of the phytochemicals was also carried out using various standard methods: tannins[14], saponins[15], flavonoids[16], alkaloids[17], phytates[18], oxalates[19,20] and cyanogenic glycosides[21].

Determination of Cytotoxicity

Brine Shrimp Lethality Assay

The brine shrimp eggs were sourced from Ocean Star International, Inc. Company, USA by the Medicinal Plant and Economics Development Unit, Department of Botany, University of Fort Hare, South Africa and the modified method of Ogunnusi and Dosumu[22] was employed in the assay.

The brine shrimps (*Artemia salina*) eggs were hatched in an improvised hatchery made of a plastic container filled with natural sea water collected from Bar Beach, Lagos, Nigeria. *Artemia salina* nauplii (< 48 h old) were exposed to sample solutions for 24 h and frequencies of immobility of the 10 nauplii in 5 ml solutions were scored. The stock solution was prepared by dissolving 0.02g of the n-hexane extract into 2ml of dimethylsulphoxide (DMSO). 0.2ml of this was then dissolved in 1.8ml of brine solution to give a 1000ppm solution[23]. Further dilutions gave 100ppm and 10ppm. 10 nauplii brine shrimps were drawn through glass capillary tubes and placed in test tubes containing 4.0 ml of brine solution and 0.5 ml of plant extract solution. A negative control containing 10 nauplii in 5ml of brine solution mixed with 2 drops of DMSO was set alongside. All assays were carried out in triplicate. The experiments were maintained at room temperature for 24 h in the laboratory.

Results and Discussion

Table 1 presents the results of the phytochemical screening of ethanol and n-hexane extracts of the seeds, stem bark and leaves of *Moringa oleifera*. The sign (+) implies the presence of the phytochemical and (-) indicates the absence of the phytochemical.

The phytochemical screening of the plant parts revealed the presence of bioactive compounds, hence the plant parts could be physiologically active. The phytochemicals present in the different parts of the plant are alkaloids, flavonoids, tannins, saponins, coumarins and glycosides (Table 2). These results are in agreement with those reported by Bamishaiye et al.[24]. The absence of terpenoids in the leaf extract is also in line with the report given by Friedman et al.[25].

Table 2 presents the results of the quantitative determination of the phytochemicals and anti-nutrients. The results showed that the highest concentrations (mg/100g) of flavonoids, oxalates, phytates and cyanogenic glycosides occurred in the seeds. The highest concentrations of alkaloids, tannins and saponin contents were in the leaves. The stem bark has the lowest concentrations of all the phytochemicals investigated except phytates which were lowest in the leaves.

The leaves had the highest content of alkaloids (6.68±2.35) and saponins (35.66± 0.78). This suggests that care should be taken in the consumption of the seeds of *M. oleifere* because of the high content of these anti-nutrients, as continuous consumption may result in an accumulation of these anti-nutrients which may have a negative effect in the end. Studies have shown that the presence of alkaloids even at a low dose of 2mg/kg can be toxic to the body, having the ability to disrupt electrochemical transmissions in the body. Saponins have also been reported to reduce the bioavailability of nutrients decrease enzyme activity and affect protein digestibility by inhibiting various digestive enzymes, such as trypsin and chymotrypsin[27].

The leaves have high content of flavonoids (7.86±0.33), but the content of flavonoids is low in the stem bark (1.55±0.15). Flavonoids was however not detected in the seeds (Table 1). The results suggest that the leaf should be incorporated in meals, especially for pregnant mothers, as it can serve as a source of antioxidants. Flavonoids have been reported to help fend off the onset of cardio-vascular diseases and may help prevent related consequences, such as heart attack or stroke[28]. Flavonoids also have long been recognized to possess anti-allergenic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism[29].

Table 1. Phytochemical analysis of crude n- hexane and methanol extracts from the seeds, stem bark and leaves of *Moringa oleifera.*

Inference	Extracts	Phytochemicals							
		Alkaloids	Saponins	Flavonoids	Tannins	Coumarins	Terpenoids	Glycosides	Phenolics
n- hexane extract	Seeds	+	+	-	+	+	-	-	-
	Stem bark	+	+	+	-	-	-	+	-
	Leaves	+	+	+	-	-	-	+	-
Methanol extract	Seeds	-	+	-	-	-	-	-	-
	Stem bark	+	-	+	+	-	-	-	-
	Leaves	+	-	+	-	-	-	+	-

Table 2. Results of the quantitative evaluation of the phytochemicals in *Moringa oleifera* plant parts.

	Alkaloids %	Flavonoids %	Oxalates mg/100g	Phytates mg/100g	Cyanogenic Glycoside mg/100g	Tannins mg/100g	Saponins %
Seeds	1.51±0.49	ND	31.98±0.62	0.85±0.04	7.4±0.00	0.58±0.52	27.18±0.95
Stem bark	0.53±0.25	1.55±0.15	17.172±0.51	0.62±0.04	6.5±0.00	0.56±0.17	14.3±0.33
Leaves	6.68±2.35	7.86±0.33	21.06±0.52	0.34±0.05	7.2±0.00	0.64±0.45	35.66±0.78

Values are means ± standard deviations of triplicate determinations. ND = Not Detected.

The results also revealed that the seeds have an appreciable amount of oxalates and phytates when compared to the other parts. This suggests that the intake of the *Moringa* seeds should be minimal as it they have the ability to form complexes with proteins at both low and high pH values. These complex formations alter the protein structure, which may result in decreased protein solubility, enzymatic activity and proteolytic digestibility[30].

Oxalates are anti-nutrients and studies have shown that they cause gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi and hypocalcaemia[31].Golden et al.[32] reported that oxalates cause nephrotic lesions in the kidney. Phytates have a similar effect as oxalates since they bind with minerals and interfere with their metabolism. On average, daily intake of phytates was estimated to be 2000–2600 mg/kg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries and 150–1400 mg/kg for mixed diet[33].

The cyanogenic glycosides in the three parts are appreciable but not negligible (Table 2) and are higher than the tolerable intake as stated by the FAO/WHO to be (0.009mg/100g). Alikwe *et al.*(2013) also reported that the cyanogenic content obtained in *Moringa* leaves was (0.25%) which is also higher than the tolerable intake stated above. Care should be taken in the consumption of the seeds of *M. oleifera* as they contained the highest cyanide equivalents. Studies have also shown that cyanogenic glycosides are toxic and on hydrolysis release hydrogen cyanide (HCN) which has been reported to have the ability to cause marked weight change[34]. It acts by inhibiting cytochrome oxidase, the final step in electron transport, thus blocking ATP synthesis. Symptoms include faster and deeper respiration, a faster, irregular and weaker pulse, salivation and frothing at the mouth, muscular spasms, dilation of the pupils and bright red mucous membranes[35].

The results above also showed a low concentration of tannins in the three parts, but not negligible. It has been reported that tannins have astringent properties; they hasten the healing of wounds, parasitic skin diseases and inflamed mucous membranes[28,10]. The anti-nutritional properties of tannins depend upon their chemical structure and dosage and the total acceptable tannin daily intake for a man is 560 mg/kg which is quite higher than the concentration in the three parts. This result suggests that more of the leaves can be consumed or formulated in our beverages, tea or coffee. Other studies have also shown that *Moringa* leaves are rich in tannins and this is one of the reasons why they are used in the treatment of urinary tract infection and diarrhea, as well as healing of wounds and dysentery[36,29].

Table 3 shows the LC_{50} values of the methanolic extracts of the seeds, stem bark and leaves on brine shrimps. The three extracts of *Moringa oleifera* did not result in any mortality after 24 hours. Mortalities however took place after 48 hours (between 1-5 nauplii deaths) and 72 hours (5-10 nauplii deaths) at varying concentrations. This is an indication that the degree of lethality is dependent on concentration and time, since mortality increased as the number of days increased.

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity, which in most cases correlates reasonably well with cytotoxic and anti-tumor properties[37].

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. In the course of this study, the brine shrimp lethality assay has proven to be a convenient system for monitoring biological activities of the *Moringa* plant species.

Therefore, since the three plant parts screened for toxicity against the brine shrimp gave negative results in terms of mortality after 24 hours, even at different concentrations (10 ppm, 100 ppm and 1000 ppm), this is an indication that the plant is not toxic at its early stage, but if consistently consumed and accumulated in the body for a number of days, it could result in toxic effects.

Brine Shrimp Lethality Assay

Table 3. Data of *in-vitro* brine shrimp lethality assay of the n-hexane extract of *Moringa oleifera* seeds, stem bark and leaves.

Conc. (ppm)	LogC	No. of nauplii deaths at 24hrs	No. of nauplii deaths at 48hrs	No. of nauplii deaths at 72 hrs	% mortality at 24hrs	LC_{50} (ppm)
Seeds						
10	1	0	1	4	0	0
100	2	0	3	7	0	0
100	3	0	5	8	0	0
Leaves						
10	1	0	2	6	0	0
100	2	0	3	9	0	0
1000	3	0	5	9	0	0
Stem bark						
10	1	0	1	7	0	0
100	2	0	3	8	0	0
1000	3	0	4	10	0	0
Control						
(1000)	3	0	0	1	0	0

Conclusions

The results obtained from the analysis carried out on the three parts (seeds, stem bark and leaves) of *Moringa oleifera* have shown that all the parts studied contain phytochemicals and anti-nutrients. The stem bark contains the lowest quantities of the phytochemicals and anti-nutrients evaluated except for phytates the lowest content of which was found in the leaves. Although the seed is a good deposit of flavonoids which are desirable phytochemicals, it is also high in anti-nutrients, especially oxalates, and so should be consumed with caution. The leaves on the other hand are rich in alkaloids and saponins. All parts of the plant contain phytochemicals and anti-nutrients, but the levels of the anti-nutrients are quite very high, especially oxalates and cyanogenic glycosides; hence caution should be taken in terms of the level of consumption by humans.

Brine shrimp lethality bioassay also revealed that the toxicity of the three parts increased as the number of days increased. This may suggest that accumulation of *Moringa* in the system may be toxic over time. It could be concluded that while *Moringa* plant is rich in nutrients and desirable phytochemicals and acclaimed to be physiologically active, caution must be exercised in its level of consumption because of the high levels of anti-nutritional factors and the possibility of toxicity on accumulation.

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