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A Comparative Study on Phytochemical, Physicochemical and Nutritional Value from Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree)

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Abstract: All parts of *Moringa oleifera* Lamk trees are considered medicinal and used in the treatment of ascites, rheumatism, venomous bites and as cardiac and circulatory stimulant. Scientists have also established that every single part of the *Moringa oleifera Lamk* tree has been of great use not only to the human beings in terms of their health in one form or the other but also for their livestock. From the present research work on "Preliminary Phytochemical investigation of Crude Extracts from Leaf, Bark and Root of *Moringa oleifera* Lamk. (Drumstick tree) have shown the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins, flavonoids, α- amino acids, starch and tannins but reducing sugar was absent in the sample. Qualitative elemental analysis of plant sample by ED-XRF method revealed that the presence of Ca, K and P as minor elements and Fe, Mn, Cu, Si, S, Sr, Zn as trace elements. No toxic elements was detected in the Drumstick tree (leaf, bark and root). Some nutritional values and physicochemical properties such as moisture, ash, fat, fibre, proteins and carbohydrates have also been determined. As a result, it was found that fibre and carbohydrate were present major nutrients in Drumstick tree (leaf, bark and root) but the protein contents of leaf was found as a major nutrient.
Keywords: Phytochemical screening, ED-XRF, *Moringa oleifera*.

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Introduction

Moringa oleifera is a small, graceful, deciduous tree, fast growing, drought resistance, which can reach a maximum height of 7-12 m and a diameter of 20-40 cm at chest height. *Moringa oleifera* Lamk is distributed in many countries of the tropics [1]. It grows in all types of soil, except stiff clays and thrives best under the tropical insular climate of South India. The tree can be propagated by seeds or from cuttings, and cuttings are preferred. Furthermore, cuttings of fairly large size planted in moist soil, strike root readily and grow to sizeable trees within a few months [12]. The leaf of this plant contain betacarotene, riboflavin, nicotinic acid, ascorbic acid, alpha-tocopherol, calcium, iron and phosphorus [5]. Amazingly, it was found that the leaf is an excellent source of protein containing nine essential amino acids [6]. It contains quercetin–3-O-glucoside and quercetin-3-O-(6-malonyl-glucoside) and lower amounts of kaempferol-3-O-glucoside and kaempferol-3-O (6-malonyl-glucoside). It also contained 3-caffeoylquinic and 5-caffeoylquinic acid.

EtOH extract of *Moringa oleifera* leaf found 4-(4´-O-acetyl-alpha-L-rhamnosyloxy) benzylisothiocyanate and niaziminin as new compound [10]. The roots contain pterygospermin, moringine and spirochin [4]. Moreover, roots of *M. oleifera* have high concentration of both 4-(L-rhamnopyranosyloxy)- benzylglucosinolate and benzylglucosinolate. Medicinal plants were and still continue to be an important therapeutic aid and for alleviating aliment of human kind. Thousands of years ago, effective medicinal plants were selected by the process of trial and error, empirical reasoning, and even by experimentation. These efforts are recorded in history by the none discovery of 'medicine' [7] and [8].

The barks contain moringine, moringinine alkaloids and β-sitosterol. The pods contain leucine, threonine, methionine, phenylalanine, tryptophan and lysine [13]. The seeds of *Moringa oleifera* Lamk contain cis-9 octadecenoic (oleic acid), cis-11-eicosenoic acid, cis -11-octadecenoic acid (vaccenic acid [10], palmitic acid, β-sitosterol, stigmasterol, compesterol, alpha–gamma and delta tocopherols [10]. The stems contain 4-hydroxymellein, vanillin, β- sitosterone, octacosanic acid and β- sitosterol.

The flowers and fruits contain amino acid such as alanine, arginine, glycine, serine, threonine, valine, glutamic acid and aspartic acid. Lysine is found in the flower. The flowers contain both sucrose and d- glucose, whereas the fruits show the presence of sucrose only. All parts of trees are considered medicinal and used in the treatment of ascites, rheumatism, venomous bites and as cardiac and circulatory stimulant.

The leaf are rich in vitamins A and C and are considered useful in scurvy and catarrhal affection. A paste of the leaf is used as an external application for wounds. A leaf –juice was dropped into the eyes in fainting fits due to nervous delibity, spasmodic affection of the bowels, hysteria and flatulence [3]. The root bark is use for heart complaints, eye diseases, fevers, inflammation, dyspepsia and enlargement of spleen. The roots are bitter, act as a tonic to the body and lungs, laxative, expectorant, diuretic, emmenagogue, enriches the blood, good for inflammations of throat, chest wound, bronchitis, piles, loss of appetite, cures stomatitis, urinary discharges, abstinate asthama, stimulants in paralytic afflictions, epilepsy and hysteria.The fresh root of the young tree is administrated in case of intermittent fever. Moringa fresh root is vescicant and rubefacient [3].

According to [9], leaf, flower, root and seed are used for tumours. Medicinal plants and herbs contain substances known to modern and ancient civilization for their healing properties. Seeds crushed to a powder are used to clarify turbid, dirty water. The cleansing take place by a process of electrical charges established between the muddy particles suspended in the water and the pulverized seed and gradually after about an hour, the muddy particles are pulled to the bottom of the water by the force of gravity. Even today, plants are the most exclusive source of drugs for the majority of the world's population.

In industrialized countries, medicinal plants research has had its up and down during the last decades. Traditional medicine is defined as the therapeutic that have been in existence, often for hundreds of year, before the development and spread of modern medicine and are still in use today (WHO, 1991). In 1998, WHO reviewed that medicinal plants are for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drug or as models for pharmacologically active compounds (WHO, TRM, 1998). Hence safety and efficacy data play an important role for the plants, their exacts and active in gradients and the

preparations of them. Thus, assurance of the safety, quality and efficacy of medicinal plants and herbals products has now become a key issue in industrialized and in developing countries [11]. The Government of Myanmar has initiated a national programme for the development of Traditional Medicine System in combating six major types of diseases, namely, malaria, tuberculosis, diarrhea, dysentery, diabetes and hypertension. Scientists have also established that every single part of the Moringa tree has been of great use not only to the human beings in terms of their health in one form or the other but also for their livestock. It makes a great fodder for cattle.

Materials and Methods

Collection and Preparation of Sample

Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree) were collected from Mingalar taung Nyunt Township, Yangon Division, 2019. The collected plant was identified and confirmed as *Moringa oleifera* Lamk at Botany Department, Dagon University. The collected plant samples were washed thoroughly with water. After cleaning, the sample were cut into small pieces and air dried at room temperature for three weeks. The dried samples were powdered by using grinding machine and stored in air- tight container and labeled systematically to prevent moisture changes and other contamination.

Chemicals

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed. All other chemicals and reagents used were of analytical grade.

Preliminary Phytochemical analysis

Qualitative phytochemical analyses were performed in Preliminary Phytochemical analysis were performed in extraction of Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree). Preliminary phytochemical test were carried out according to determine the presence of phytochemicals the alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, α- amino acids, starch and tannins were found to be present but reducing sugar was absent in leaf ,bark and root sample.as described by standard procedure.

Proximate analysis

Drumstick leaf, bark and root samples were evaluated for moisture, crude protein crude fat, crude fiber, ash and nitrogen free extract (NFE) according to their respective methods as mentioned in AOAC (2002). All the tests were carried out in triplicates. Principle of each method is briefly described as follow:

Moisture Content

The moisture content of Drumstick leaf, bark and root samples were determined by AOAC (2002) Method No. 934-01 accordingly. 10 g sample was dried in hot air oven (Model: DO-1-30/02, PCSIR, Pakistan) at a temperature of 105± 5 °C for the duration until weight was constant.

Protein Content

The Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) was used for the determination of nitrogen percent in garlic using AOAC (2002) Method No. 984-13. Accordingly, green tea was digested with concentrated H_2SO_4 by using digestion mixture

(K_2SO_4:$FeSO_4$:$CuSO_4$ i.e. 100:5:10) until the color was light greenish. The digested material was diluted up to 250 mL in volumetric flask. 10 mL of 40% NaOH as well as 10 mL of digested sample was taken in distillation apparatus where liberated ammonia was collected in beaker containing 4% boric acid solution using methyl red as an indicator. This resulted in formation of ammonium borate that was used for nitrogen determination in sample. Thus percentage of nitrogen in sample is assessed by titrating distillate against 0.1N H_2SO_4 solution till color is light golden. Crude protein content was estimated by multiplying nitrogen percent (N %) with factor (6.25).

Crude Fat

The crude fat content in Drumstick leaf, bark and root samples were estimated following guidelines of Method No 920-39 in AOAC (2002). Dried sample (3 g) was refluxed in soxhlet apparatus (Model: H-21045 Extraction Unit, Hoganas, Sweden) using n-hexane as a solvent.

Crude fiber

Drumstick leaf ,bark and root samples were subjected to crude fiber content by elaborating Method No. 978-10 outlined in AOAC (2002). Fat free sample was digested with 1.25% H_2SO_4 followed by 1.25% NaOH solution in Labconco Fibertech apparatus (Labconco Corporation Kansas, USA). After filtration and washing with distilled water reaming residues was weighed and ignited in muffle furnace at temperature of 550-650°C till grey or white ash was obtained. The crude fiber percentage was estimated according to the expression given below.

Total ash

The ash content of peel was estimated according to the procedure mentioned in AOAC (2002) Method No. 942-05. For which, 5 g sample was directly charred on flame in crucible until there was no fumes coming out. Afterwards sample was ignited in muffle furnace (MF-1/02, PCSIR, Pakistan) at 550-600ºC for 5-6 hours or until grayish white residues were obtained.

Elemental Analysis of Plant Sample by ED-XRF Method

In order to determine the heavy toxic metals and macronutrient elements in plant sample, elemental contents in the leaf, bark and root of *Moringa Oleifera* Lamk were determined by ED-XRF method at the Universities Research Center, Yangon. X-ray spectrometry has since long been recognized as a powerful method for multi-elemental analysis.

Results and Discussions

Preliminary phytochemical test were carried out according to determine the presence of phytochemicals the alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, α- amino acids, starch and tannins were found to be present but reducing sugar was absent in Drumstick leaf, bark and root samples. The results obtained from these experiments were summarized in Table 1. For the purpose of quality control, assessment of purity and identification of any sample, standardization is much essential.

The standardization of a crude drug is an integral part for establishing its correct identity. Standardization including physicochemical evaluation is meant for identification, authentication and detection of adulteration and complication of quality control of crude drugs. The physical constant evaluation of the drug is an important parameter in detecting improper handling of drugs.

The medical plants are rich in secondary metabolites which include alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, tannins and α-Amino acid were found to be present. They are of great medicinal value and have been extensively used in drug and pharmaceutical industry.

Qualitative elemental analysis of leaf, bark and root were done by using Energy Dispersive X-Ray Fluorescence (ED-XRF) method, as quality control method. As a result Si, K, Ca, S, Fe, Mn, Sr, Zn, Cu, P were detected in Drumstick leaf, bark and root samples. According to ED-XRF, no toxic elements were found in Drumstick leaf, bark and root samples. These are shown in Figure 5, 6, 7, 8, 9 and 10.

The nutritional values such as fats, protein, carbohydrates and fibre were determined by AOAC method to the Drumstick leaf, bark and root samples. As a result, it was found that fibre and carbohydrate were present as major nutrient in Drumstick leaf, bark and root samples. The determination of ash, fat, moisture and protein content were made according to AOAC methods and the results were shown in Table 2 and Figure 11.

Figure 1. *Moringa oleifera* Lamk. (Drumstick) plant

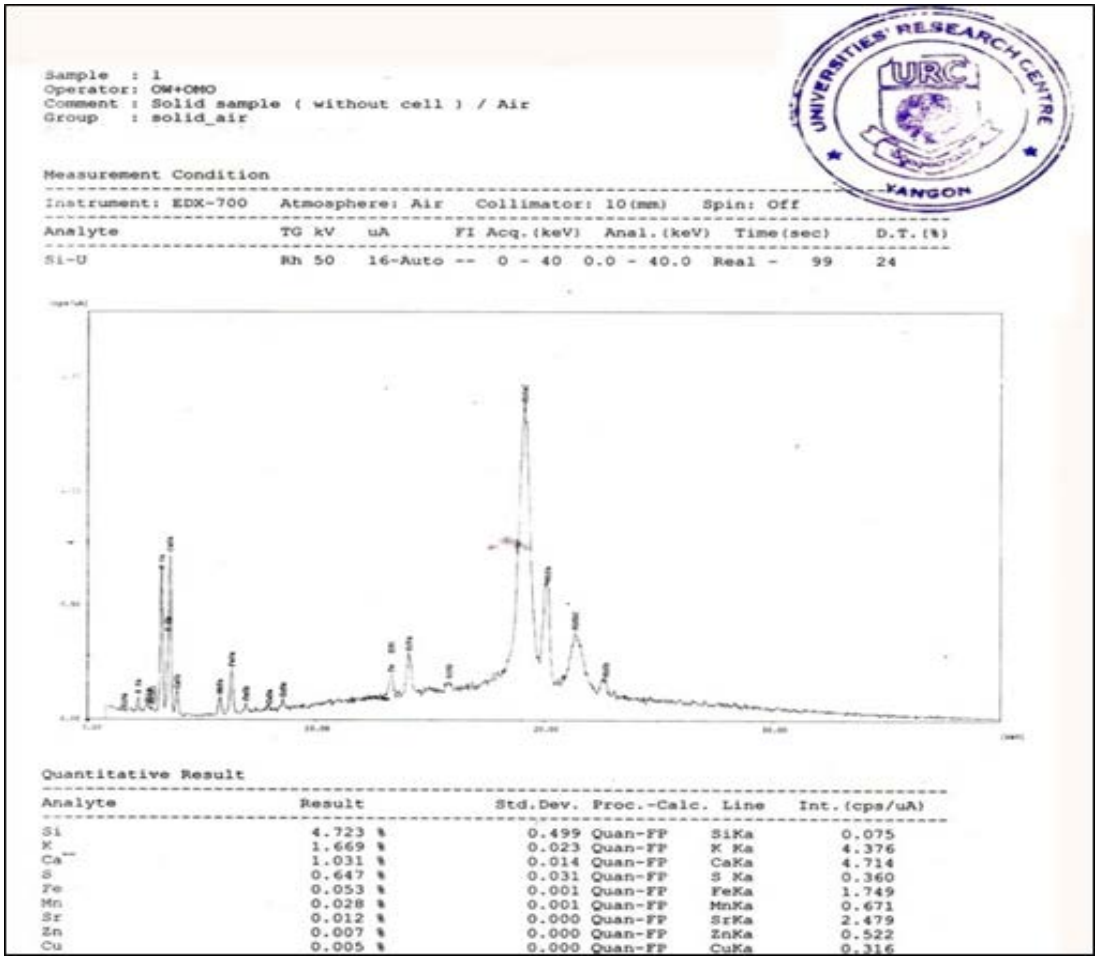
Figure 2. *Moringa oleifera* Lamk. (Drumstick) leaf

Figure 3. *Moringa oleifera* Lamk. (Drumstick) flower

Figure 4. *Moringa oleifera* Lamk. (Drumstick) bark

Table 1. Results of Phytochemical Investigation of Drumstick (leaf, bark and root) by Test Tube Method

No.	Test	Extract	Test reagents	Observation	Result		
					L	B	R
1	Alkaloids	1% HCl	Wagner's reagent	Brown ppt	+	+	+
			Mayer's reagent	White ppt	+	+	+
2.	Carbohydrates	H_2O	10% α-Naphthol & conc:H_2SO_4	Red ring	+	+	+
3.	Glycosides	H_2O	10% lead acetate	White ppt	+	+	+
4.	Phenolic compounds	H_2O	5% Fe Cl_3 solution	Deep blue colour	+	+	+
5.	Saponin	H_2O	Distilled water Shaken	Frothing	+	+	+
6.	Flavonoids	H_2O	1% NH_3 solution	Yellow	+	+	+
7.	a-amino acid	H_2O	Ninhydrin	Pink spot	+	+	+
8.	Strach	H_2O	I_2 solution	blue colour	+	+	+
9.	Tannin	EtOH	10% $FeCl_3$	Green	+	+	+
10.	Reducing Sugar	5N-H_2SO_4 & NaOH	Benedict's solution	brick red	-	-	-
(+) = presence; (-) = absence							



Sample : 1
Operator: OW+OMO
Comment : Solid sample (without cell) / Air
Group : solid_air

Measurement Condition

Instrument: EDX-700 Atmosphere: Air Collimator: 10(mm) Spin: Off

Analyte	TG kV	uA	FI Acq.(keV)	Anal.(keV)	Time(sec)	D.T.(%)
Si-U	Rh 50	16-Auto	0 - 40	0.0 - 40.0	Real - 99	24

Quantitative Result

Analyte	Result	Std.Dev.	Proc.-Calc.	Line	Int.(cps/uA)
Si	4.723 %	0.499	Quan-FP	SiKa	0.075
K	1.669 %	0.023	Quan-FP	K Ka	4.376
Ca	1.031 %	0.014	Quan-FP	CaKa	4.714
S	0.647 %	0.031	Quan-FP	S Ka	0.360
Fe	0.053 %	0.001	Quan-FP	FeKa	1.749
Mn	0.028 %	0.001	Quan-FP	MnKa	0.671
Sr	0.012 %	0.000	Quan-FP	SrKa	2.479
Zn	0.007 %	0.000	Quan-FP	ZnKa	0.522
Cu	0.005 %	0.000	Quan-FP	CuKa	0.316

Figure 5. ED-XRF spectrum of *Moringa oleifera* Lamk (Drumstick) leaf

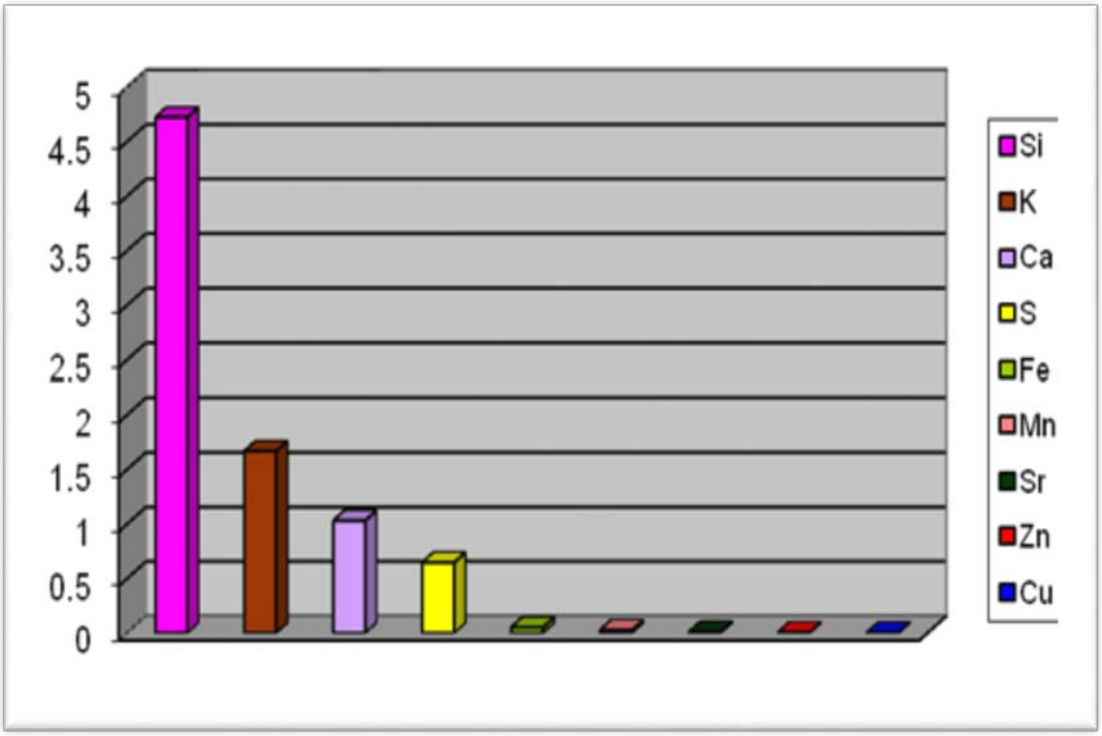
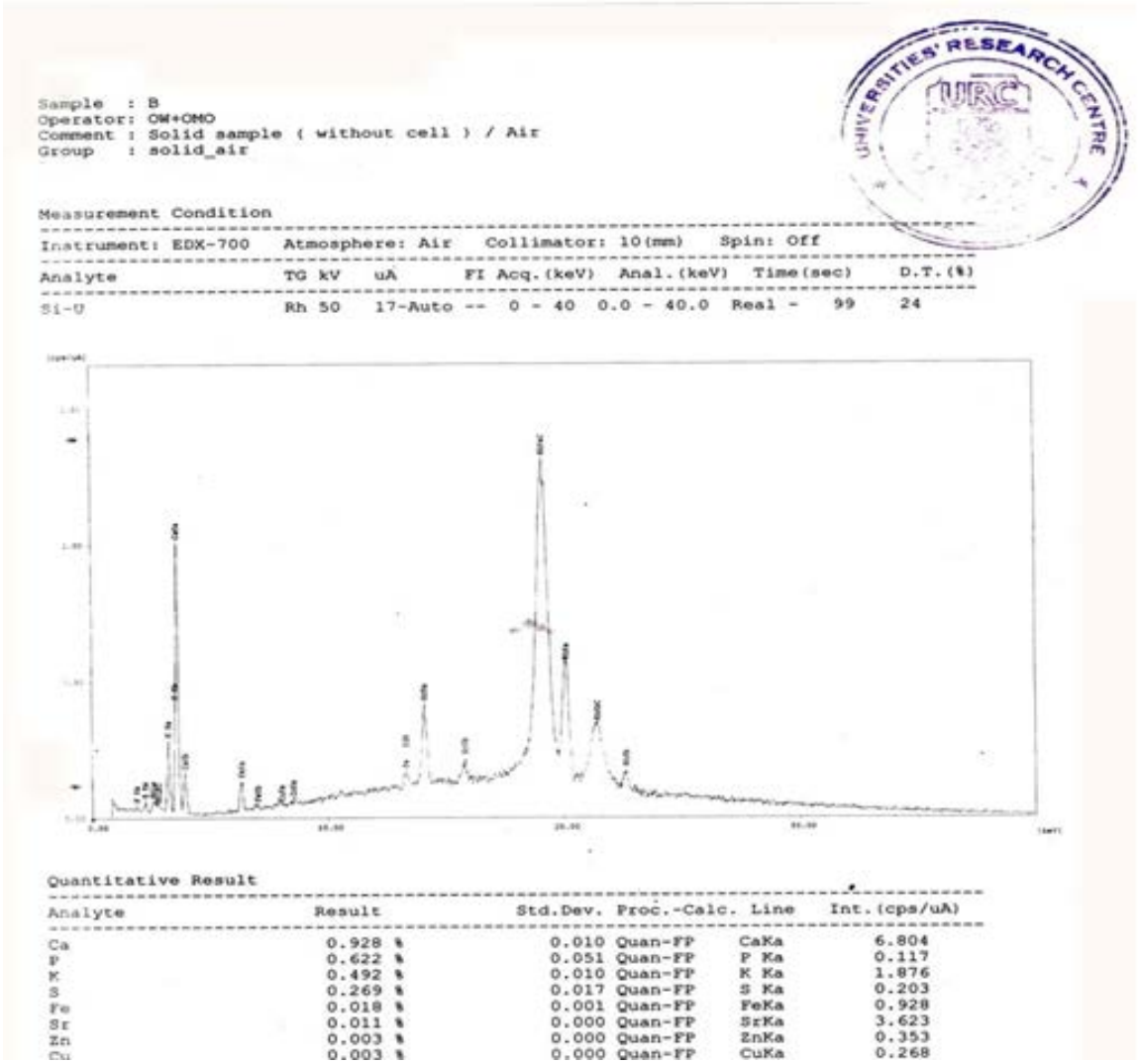


Figure 6. Qualitative elemental analysis of (Drumstick leaf) by ED-XRF



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Sample   : B
Operator: OW+OMO
Comment : Solid sample ( without cell ) / Air
Group    : solid_air

Measurement Condition
Instrument: EDX-700   Atmosphere: Air   Collimator: 10(mm)   Spin: Off
Analyte          TG kV   uA       FI Acq.(keV)  Anal.(keV)  Time(sec)   D.T.(%)
Si-U             Rh 50   17-Auto --  0 - 40  0.0 - 40.0  Real -   99     24
```

Quantitative Result

Analyte	Result	Std.Dev.	Proc.-Calc.	Line	Int.(cps/uA)
Ca	0.928 %	0.010	Quan-FP	CaKa	6.804
P	0.622 %	0.051	Quan-FP	P Ka	0.117
K	0.492 %	0.010	Quan-FP	K Ka	1.876
S	0.269 %	0.017	Quan-FP	S Ka	0.203
Fe	0.018 %	0.001	Quan-FP	FeKa	0.928
Sr	0.011 %	0.000	Quan-FP	SrKa	3.623
Zn	0.003 %	0.000	Quan-FP	ZnKa	0.353
Cu	0.003 %	0.000	Quan-FP	CuKa	0.268

Figure 7. ED-XRF spectrum of *Moringa oleifera* Lamk (Drumstick bark)

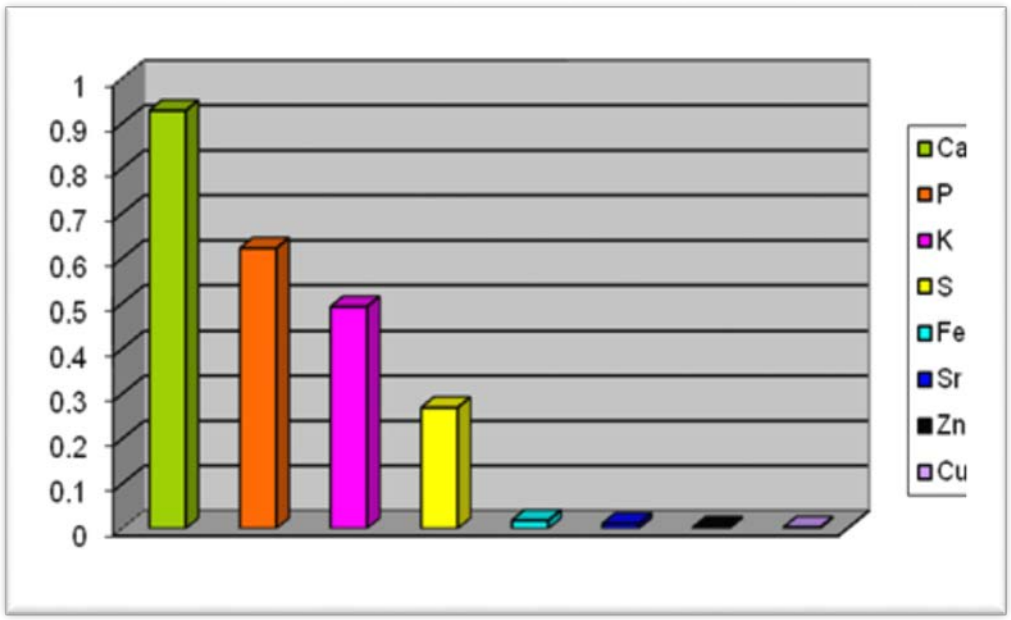
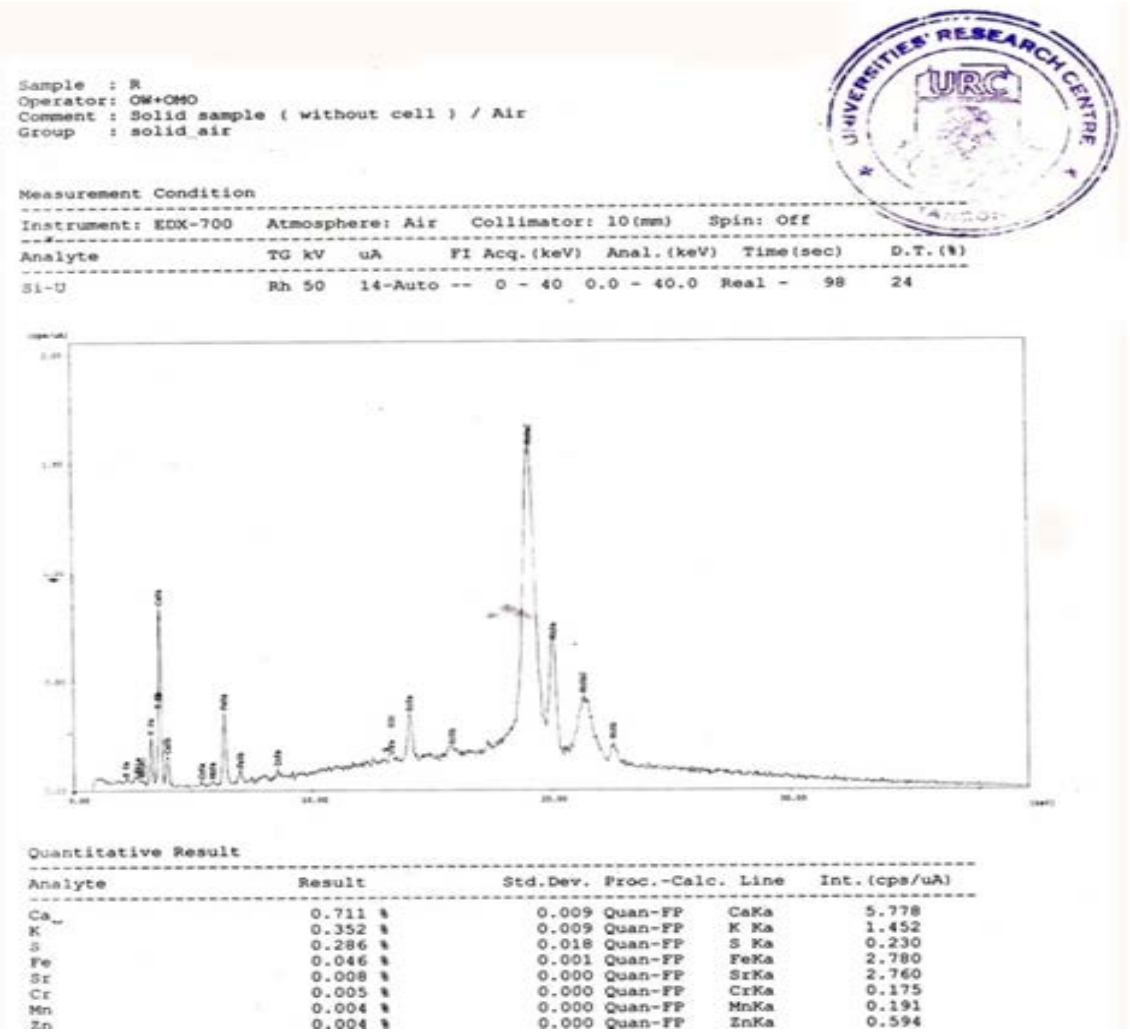


Figure 8. Qualitative elemental analysis of (Drumstick bark) by ED-XRF



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Sample   : R
Operator: OW+OMO
Comment : Solid sample ( without cell ) / Air
Group    : solid_air

Measurement Condition
Instrument: EDX-700   Atmosphere: Air   Collimator: 10(mm)   Spin: Off
Analyte          TG kV   uA       FI Acq.(keV)  Anal.(keV)  Time(sec)   D.T.(%)
Si-U             Rh 50   14-Auto --  0 - 40  0.0 - 40.0  Real -   98    24
```

Quantitative Result

Analyte	Result	Std.Dev.	Proc.-Calc.	Line	Int.(cps/uA)
Ca	0.711 %	0.009	Quan-FP	CaKa	5.778
K	0.352 %	0.009	Quan-FP	K Ka	1.452
S	0.286 %	0.018	Quan-FP	S Ka	0.230
Fe	0.046 %	0.001	Quan-FP	FeKa	2.780
Sr	0.008 %	0.000	Quan-FP	SrKa	2.760
Cr	0.005 %	0.000	Quan-FP	CrKa	0.175
Mn	0.004 %	0.000	Quan-FP	MnKa	0.191
Zn	0.004 %	0.000	Quan-FP	ZnKa	0.594

Figure 9. ED-XRF spectrum of *Moringa oleifera* Lamk (Drumstick tree root)

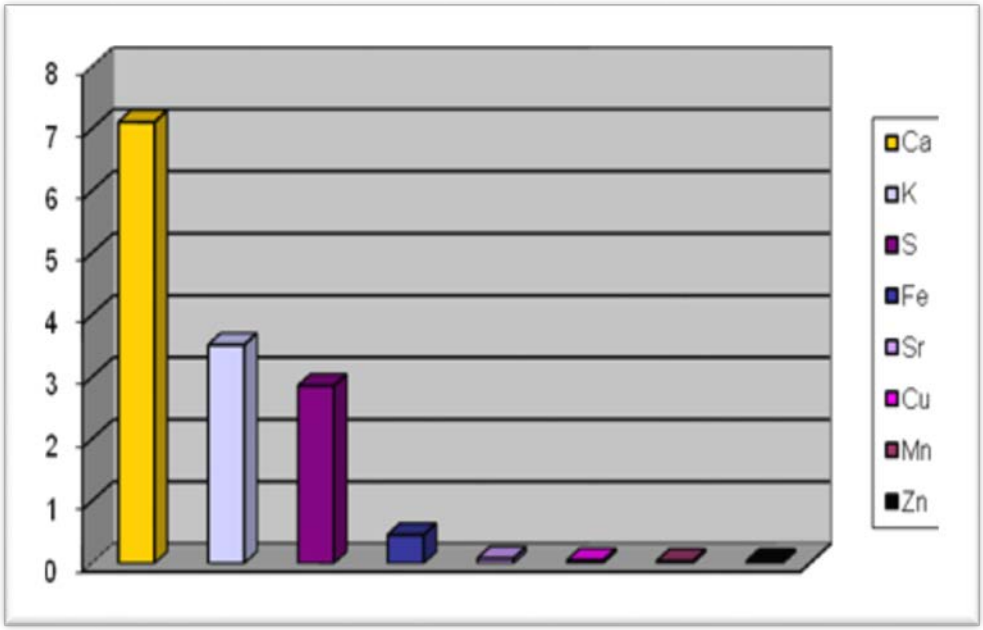

Figure 10. Qualitative elemental analysis of (Drumstick root) by ED-XRF

Table 2. Results of Nutritional Values and Physicochemical Characterization of Drumstick Leaf, Bark and Root sample

No	Analyte	Content (%)		
		Leaf	Bark	Root
1	Moisture content	10.6	9.9	10.4
2	Protein content	31.2	5.9	3.7
3	Crude Fibre content	19.31	46.35	54.32
4	Ash content	9.23	15.84	16.12
5	Carbohydrate content	27.35	21.18	10.54
6	Fat content	2.31	0.83	0.92

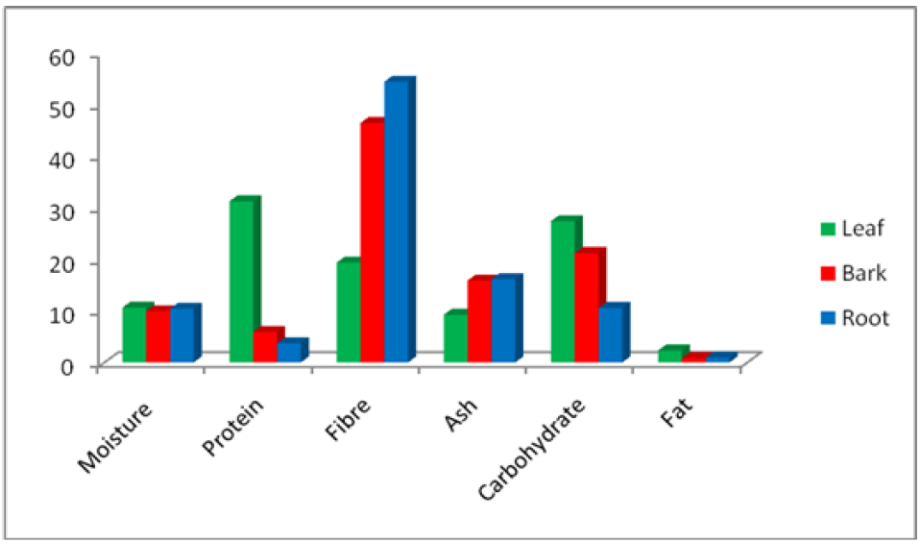

Figure 11. Nutritional Values of Drumstick leaf, bark and root sample

Conclusion

From the present research work on "Preliminary Phytochemical Investigation and Antibacterial Activity of Crude Extracts from Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree), the following inferences may be deduced. The preliminary phytochemical screening test has shown the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins, flavonoids, α- amino acids, starch and tannins but reducing sugar was absent in Leaf, Bark and Root of *Moringa oleifera* Lamk sample. Some nutritional values and physicochemical properties such as moisture, ash, fat, fibre, proteins and carbohydrates have also been determined. As a result, it was found that fibre and carbohydrate were present major nutrients in Drumstick leaf, bark and root but the protein contents of leaf was found as a major nutrient. Qualitative elemental analysis of plant sample by ED-XRF method revealed that the presence of Ca, K and P as minor elements and Fe, Mn, Cu, Si, S, Sr, Zn as trace elements. No toxic elements was detected in the Drumstick leaf, bark and root. Scientists have also established that every single part of the Moringa tree has been of great use not only to the human beings in terms of their health in one form or the other but also for their livestock.

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