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PHARMACOGNOSTIC EVALUATION AND ANTIMICROBIAL STUDIES ON *MORINGA OLEIFERA* LAM. (MORINGACEAE)

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ABSTRACT: The pharmacognostic evaluation, anatomy and antimicrobial potentials of the leaf, stem and root of *Moringa oleifera* Lam were evaluated. Powdered extracts obtained from the various plant organs were used for the analytical standardization. The methanolic plant extracts were assayed for antimicrobial activities using the Agar well diffusion method. This was compared with standard antibiotics, Gentamicin and ketoconazole. The Minimum Inhibitory Concentrations (MIC) of the methanolic plant extracts against the sensitive microorganisms was determined via the turbidity method. The results of the analytical standardization of the powdered leaf, stem and root of *Moringa oleifera* were found to be within pharmacopoeial standards. The results of the anatomical studies showed normal tissue distribution with distinctive features and structures, which could be instrumental for delimiting other closely related species. The phytochemical analysis revealed varying concentrations of alkaloids, tannins, flavonoids, saponins, steroids, terpenoids, resins, proteins and carbohydrates. The plants extracts exhibited antibacterial activities against all test bacteria. The stem extract particularly was better effective against *Salmonella typhi* than Standard antibiotic Gentamicin, with an MIC of 10.89 mg/ml and 13.88 mg/ml respectively.

INTRODUCTION: Plants play a significant role in maintaining human health and improving the quality of life and serve as valuable components of medicines, seasonings, beverages, cosmetics and dyes. *Moringa oleifera* is the most widely cultivated species of the family moringaceae and originates from the Himalayas in India¹ and also grows in tropical and subtropical regions². It was introduced to Africa from India at the turn of the twentieth century³.

It grows well in soils with a pH range of 4.5 – 9 and is used for both culinary and medicinal purposes³, which include its use as an antioxidant, anti - carcinogenic, anti - inflammatory, antispasmodic, diuretic, antiulcer, antibacterial, antifungal, anti-nociceptive, as well as its wound healing ability^{4,5,6,7,8}. More so, it has the capacity to boost the immune system⁹. Different parts of this plant are reported to possess these pharmacological actions¹⁰ and therefore, *Moringa oleifera* can be classified as a potential source of lead compounds for drug development¹¹.

This study was aimed at evaluating the pharmacognostic characters of the leaf, stem and root of *M. oleifera* to guard against adulteration and/or substitution; also to verify the claims on antimicrobial effect of the plant.

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MATERIALS AND METHODS:

Collection and Authentication of Plant Material:

The leaf, stem and root of *Moringa oleifera* were collected in the month of November, 2012 at the medicinal plant garden in the Faculty of Pharmaceutical Sciences University of Nigeria, Nsukka. The plant materials were authenticated by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (Inter CEDD) Nsukka. A voucher specimen was deposited with number INTERCEDD1109.

Preparation of Crude Drug Material and

Extraction: A 2 Kg each of the leaf, stem and root of the plant were washed thoroughly under running tap water. These were separately chopped into tiny pieces and dried in an air-circulating oven in the laboratory at 35 – 40°C. The dried plant parts were grinded in an electric grinder. The resulting powders were differently macerated in methanol for 72 hrs. The filtrate was concentrated to dryness using a rotary evaporator attached to a vacuum pump to obtain the crude extracts (130 g, 98 g and 55g respectively). These were stored at a temperature of 4°C.

Phytochemical Screening: Standard methods^{12, 13, 14} were used for phytochemical analysis of the extracts for the presence of alkaloids, tannins, saponins, flavonoids, steroids, glycosides, anthraquinones and terpenoids.

Anatomical Studies: A Transverse Section (TS) of fresh samples of the leaf, stem and root were made. In addition, a Tangential Longitudinal Section (TLS) and Radial Longitudinal Section (RLS) of the stem and root were also made according to the method by Khandelwal¹⁴.

The sections were transferred into a staining jar and stained in safranin for 5 minutes. The safranin was drained off and sections washed three times with distilled water. These were further washed with 97 % and absolute alcohol twice. The sections were counter-stained in 1 % fast green for 5 minutes and washed with absolute alcohol for about 3 – 4 times. The sections were placed in a jar containing 50/50 alcohol/xylene until they became clear. Pure xylene was used to finally clear the sections. The sections were mounted with Canada balsam.

The sections were viewed under a microscope and images were captured using a motic image camera attached to the eye piece of the microscope.

Quantitative Microscopy: Fresh leaf samples were washed and cleared in 70 % chloral hydrate and mounted with glycerin. A camera lucida was attached to the eye piece of a microscope and the preparations were examined for the palisade ratio, stomata number, stomatal index, vein-islet number and vein-islet termination number¹⁴.

Analytical Standardization Procedure: Parts of the extracts earlier obtained from the various parts of the plant were evaluated for the following analytical parameters: moisture content, total ash, water soluble ash value, acid insoluble ash value, sulphated ash value, alcohol extractive value and water extractive value¹⁴.

Antimicrobial Sensitivity Test: The plant extracts were reconstituted in normal saline to a concentration of 100 mg/ml. Clinical isolates of *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*, obtained from the Medical Centre of the University of Nigeria Nsukka were diluted to 0.5 McFarland turbidity standard in accordance with the Clinical and Laboratory Standards Institute, CLSI¹⁵. These were inoculated onto solidified Nutrient agar plates (for bacteria) and Sabouraud's dextrose agar plates (for fungi). The agar well diffusion method^{16, 17} was used, using an 8 mm diameter cork borer under sterile conditions. The reconstituted plant extracts were introduced into the agar wells and pre-diffusion was allowed for one hour. Thereafter incubation was at 37° C for 24 hours. Clear zones of inhibition were measured in mm. The Minimum Inhibitory Concentrations of the plant extracts against the sensitive microorganisms were determined using the turbidity method according to the National Committee for Clinical Laboratory Standards, NCCLS¹⁸.

Statistical Analysis: The results of the experiment were analyzed statistically with student's T-test and ANOVA¹⁹.

RESULTS:

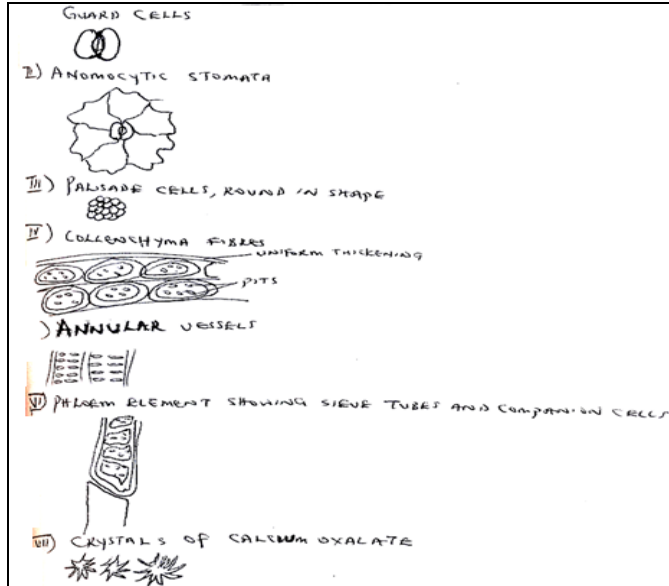


FIG.1: MICROSCOPIC ORGANS OF LEAF OF M. OLEIFERA

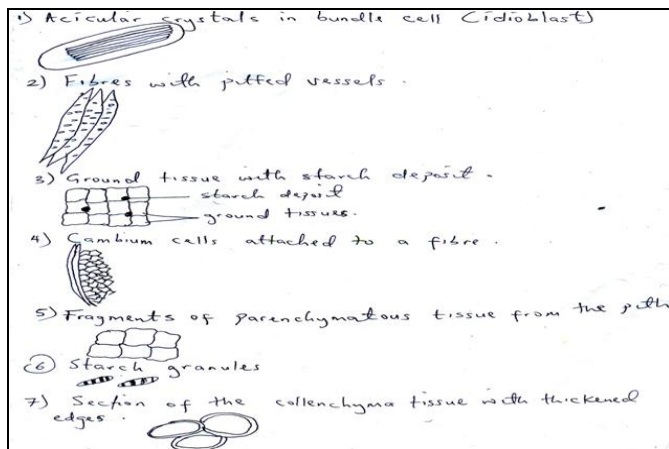


FIG. 2: MICROSCOPIC ORGANS OF STEM OF M. OLEIFERA

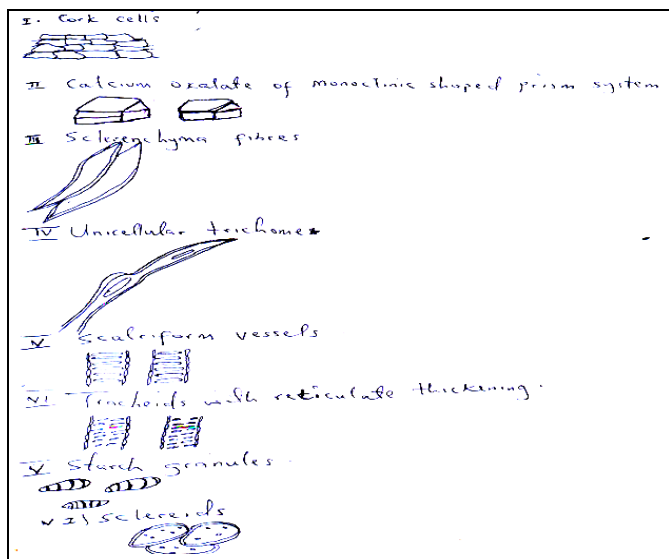


FIG. 3: MICROSCOPY OF THE ROOT OF MORINGA OLEIFERA

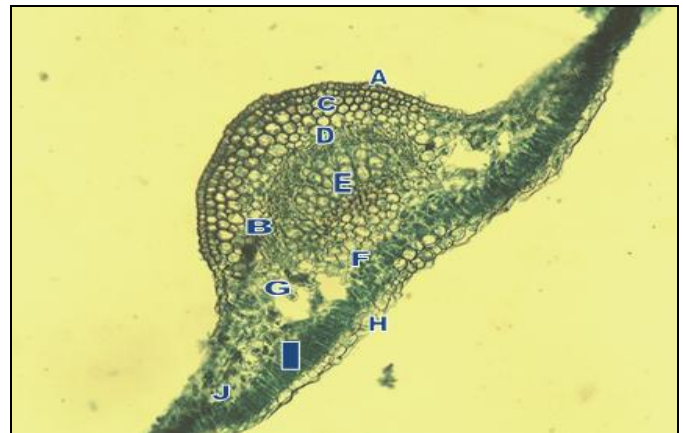


FIG. 4: TRANSVERSE SECTION OF THE LEAF OF MORINGA OLEIFERA

Key: A –Upper epidermis
 B –Starch granules
 C –Collenchyma
 D –Spongy parenchyma
 E –Xylem
 F –Phloem
 G –Respiratory cavity
 H –Lower epidermis
 I –Lower cylindrical palisade cells
 J –Border sclerenchyma

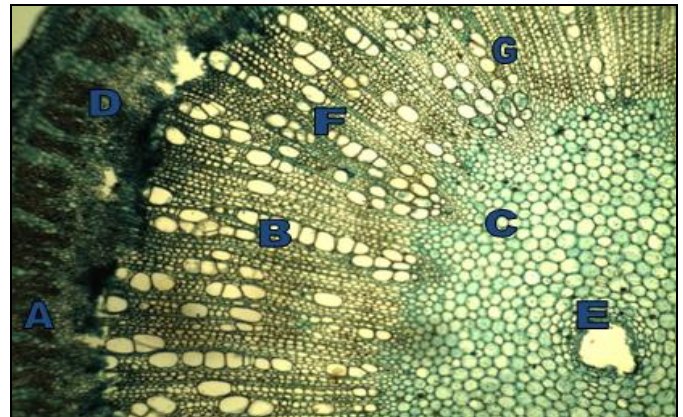


FIG. 5: TRANSVERSE SECTION OF THE STEM OF MORINGA OLEIFERA

Key: A –Bark
 B –Xylem
 C –Pith
 D –Cortex
 E –Cambial ring (showing secondary thickening)
 F –Medullary ray
 G –Parenchyma

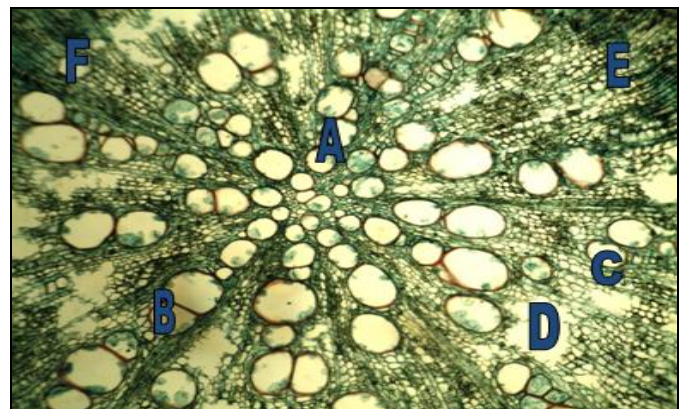


FIG. 6: TRANSVERSE SECTION OF THE ROOT OF MORINGA OLEIFERA

Key: A –Primary xylem
 B –Secondary xylem
 C –Medullary ray
 D –Intercellular air space
 E –Cambium
 F –Bark

TABLE 1: QUANTITATIVE LEAF MICROSCOPY OF *MORINGA OLEIFERA*

Quantitative Standards	Composition (square per cm)
Palisade ratio	11.75 ±0.75
Stomatal number	6.75±0.48
Stomatal index	0.54±0.43
Vein-islet number	7.00±1.08
Veinlet termination	4.00±0.41

n = 3 ± SEM

TABLE 2: PHYSICO-CHEMICAL PROPERTIES OF *MORINGA OLEIFERA*

Evaluation Parameters	% Composition		
	Leaf	Stem	Root
Moisture content	5.45	5.65	9.60
Total ash	1.35	2.35	2.50
Acid insoluble ash values	1.60	1.10	1.40
Sulphated ash values	1.85	1.85	1.30
Water soluble ash values	1.80	12.70	0.85
Alcohol soluble extractive values	15.0	6.25	11.25
Water extractive values	13.75	6.25	10.00

TABLE 3: PHYTOCHEMICAL SCREENING OF *MORINGA OLEIFERA*

Phytochemicals	Leaf	Stem	Root
Tannins	+	-	-
Saponins	++	++	+
Alkaloids	+++	-	+
Resins	+	-	-
Steroids	-	++	+
Terpenoids	-	+	+
Flavonoids	+	-	-
Carbohydrates	++	++	+++
Proteins	+++	++	+

Key: + = Slightly Present, ++ = Present, +++ = Highly Present, - = Absent

TABLE 4: ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACTS OF *MORINGA OLEIFERA*

Microorganisms	Methanol Crude Extracts (100mg/ml)			Standard Antibiotics (100µg/ml)	
	Leaf	Stem	Root	Gentamicin	Ketoconazole
<i>Bacillus subtilis</i>	+	+	+	+	-
<i>Staphylococcus aureus</i>	-	-	-	+	-
<i>Pseudomonas aeruginosa</i>	+	-	+	+	-
<i>Escherichia coli</i>	+	+	-	+	-
<i>Salmonella typhi</i>	+	+	+	+	-
<i>Candida albicans</i>	+	-	+	-	+
<i>Aspergillus niger</i>	-	-	-	-	+

Key: - = Insensitive, + = Sensitive

TABLE 5: MINIMUM INHIBITORY CONCENTRATION (MIC)

Microorganisms	Methanol Crude Extracts			Standard Antibiotics	
	Leaf	Stem	Root	Gentamicin	ketoconazole
<i>Bacillus subtilis</i>	7.24±0.33	9.27±0.24	7.56±0.28	3.81±0.58	-
<i>Pseudomonas aeruginosa</i>	-	25.20±0.03	26.26±0.28	16.40±0.00	-
<i>Escherichia coli</i>	15.03±0.00	24.85±0.33	-	12.84±0.21	-
<i>Salmonella typhi</i>	22.49±0.30	10.89±0.21	24.99±0.27	13.88±0.88	-
<i>Candida albicans</i>	-	23.53±0.18	24.13±0.31	-	10.438±0.32
<i>Aspergillus niger</i>	-	-	-	-	11.264±0.66

Key: - = No reaction

DISCUSSION: The macroscopical and organoleptic standards which include the visual and sensory characters provided the simplest and

quickest indication of the identity and quality of *Moringa oleifera*. The leaf, stem and root showed marked variations in their organoleptic properties.

The leaves have a similar taste to spinach, the stem and roots have an acrid taste. The leaf, stem and roots have a peculiar odour.

The analytical parameters of the leaf, stem and root of *Moringa oleifera* were within pharmacopoeial standards and this could be used as a reference guide for identification and assessment for quality and purity (**Tables 1 and 2**).

A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug²⁰. The total ash values for the leaf, stem and root were 1.35 %, 2.35 %, and 2.5 % respectively (**Table 2**). The powdered roots have the highest value (2.5%), which may indicate higher concentration of inorganic salts occurring naturally in it. The total ash value usually consists of carbonates, phosphates, and silicates that include both plant derived ash as well as those residues that may adhere to the plant surface such as soil contaminants²⁰.

The acid insoluble ash value on the other hand indicates contamination with siliceous materials e.g. earth and sand. The result showed that the leaves stem and root gave acid insoluble ash values of 1.6 %, 1.1 %, and 1.4 % respectively (**Table 2**). The water soluble ash is part of the total ash content which is soluble in water; this is a good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation²⁰. It is an important indication of the presence of exhausted materials substituted for the genuine article²⁰. The sulphated ash value also produces a more consistent ash than the total ash because all oxides and carbonates are converted to sulphates at high temperatures used. The sulphated ash values for the leaves stem and roots were 1.85 %, 1.85 %, and 1.3 % respectively (**Table 2**). The alcohol and water soluble extractive values for the leaves stem and roots were 15.00 %, 6.25 %, 11.25 % and 1.8 %, 12.70 %, 0.85 % respectively (**Table 2**). The extractive yields are used as means of evaluating drugs, the constituents which are not readily estimated by other means. The results showed that the leaves are very soluble in alcohol than in water, while the stem is more soluble in water than in alcohol and the roots are more soluble in alcohol than in water.

The results of the moisture content (**Table 2**) also showed that the leaves contained the least amount of moisture (5.45 %), while the roots contained the highest amount of moisture (9.60 %), the stem contains a moderate amount of moisture (5.65 %). This could discourage microbial decomposition. These also were in the prescribed range (≤ 14 %) for moisture content in crude drug²¹. The results of the quantitative microscopy gave accurate cellular micrometry of all the tissues and also determined the proportions of the substances present by means of the microscope, using the Lycopodium method²².

The results of the anatomical sections of the leaf, stem and root of *Moringa oleifera* gave distinct features and structures which will help in the distinguishing of this plant from other closely related species, such features include simple boarded pits in the tracheids and vessels, calcium oxalate of monoclinic shaped prism system, reticulate vessels, idioblasts, cork cells, xylem vessels, starch granules, anomocytic stomata, collenchyma cells and phloem elements showing sieve tubes and companion cells (**Fig. 1 – 6**).

The preliminary phytochemical analysis of the plant (**Table 3**) showed the presence of carbohydrates, saponins and proteins in the leaves stem and roots. Alkaloids seemed to be more present in the leaf, while moderately present in the root and were not found in the stem. Steroids were present in only the stem and root. Flavonoids were present only in the leaves, terpenoids were found in the stem and root. Resins and tannins were present only in the leaves. These phytochemicals are known to exhibit antioxidant, anti-inflammatory, antibacterial, immunomodulatory and antisickling activities among others²³. Thus the presence of these phytochemicals in *M. oleifera* might have contributed to various claims on numerous pharmacological activities of the plant.

All three parts of *Moringa oleifera* exhibited potency against *Bacillus subtilis* and *Salmonella typhi* (**Tables 4 and 5**) but was ineffective against *Staphylococcus aureus* and *Aspergillus niger*. Also shown by the result, the leaf was relatively most effective. This is particularly interesting as the leaf is largely eaten as a vegetable across the African continent.

The Minimum Inhibitory Concentration (MIC) against *Salmonella typhi* was 10.89 mg/ml by the stem extract and 13.88 mg/ml by Gentamicin (Table 5). This indicated that the methanolic stem extract exhibited better efficacy than standard antibiotic, Gentamicin against *Salmonella typhi*.

CONCLUSION: This study evaluated the pharmacognostic parameters of *Moringa oleifera*, which is essential for its standardization in order to supplement monographs of the plant for optimum therapeutic benefits as reported by several researchers. More so, its antimicrobial activity in this study supports its use against infectious diseases.

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CONFLICT OF INTEREST: There was no conflict of interest whatsoever among the authors of this manuscript. Every data and its analysis as presented were unanimous.

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