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Phytochemical Analysis and Antimicrobial Activity of Moringa Oleifera Leaves Extract

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Abstract:
Phytochemical analysis carried out on Moringa Oleifera leaf extract indicated the presence of alkaloids, saponins, flavonoids, reducing sugar, glycosides, and terpenoids. The antibacterial activity of the leaf extracts of M. oleifera on Staphylococcus Aureus and Escherichia Coli at different concentrations were analyzed, and the maximum zone of inhibition was observed at 15nm and 13nm respectively. Zone of inhibition was found to increase with increasing concentration of the Moringa Oleifera leaves extract.

Key Words*: Phytochemicals, Antimicrobial, Medicinal plants, Antibacterial, Infectious diseases*

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I. Introduction

Recent developments in the scientific field have renewed interest in the medicinal properties of plants because of their low toxicity, pharmacological activities, and economic viability[1] closely linked, interest in plants with antimicrobial properties has rejuvenated due to resistance to routine antibiotics[6] this has resulted in the increased demand for new antibiotics[8], and interest has been developed by the scientific community in the use of herbal medicines with antimicrobial properties. Plants can provide a wide range of complex and structurally diverse compounds, Plant extracts possess antifungal, antibacterial, and antiviral properties and have been screened on a global scale as potential sources of novel antimicrobial compounds, agents promoting food preservation, and alternatives to treat infectious diseases[2].according to the World Health Organization (WHO), above 80% of the world's population depends on indigenous medicines for their basic health care needs[16] The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most essential bioactive compounds of plants are alkaloids, tannins, flavonoids, and phenolic compounds.

Moringa oleifera, a plant with a wide range of medicinal, nutritional, and economic benefits is the most widely cultivated species of the moringaceae family, Native to tropical and subtropical regions of South Asia[3] This fast-growing tree's common names include moringa drumstick tree (from the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which resembles horseradish), and ben oil tree or ben oil tree (from the oil which is derived from the seeds), It is cultivated mainly for its young seed pods and leaves used as vegetables and for traditional herbal medicine.[4] The leaves of moringa oleifera plants are used as a source of vitamins A and C. They are also good sources of vitamin B and are also sources of minerals.[7] The leaf of this plant has diverse biological activities, including antidiabetic, hypocholesterolemic, hypertensive agents [9][10][15], and regulates thyroid hormone,[12] central nervous system, digestive system, nutrition, and metabolism. This plant is also reported to be hepatoprotective against anti-tubercular drugs such as ionized and rifampicin[13] and is being studied for its antimicrobial anti-inflammatory, diuretic, antibiotic, hypertensive, and antimicrobial properties[19] An immune-enhancing polysaccharide having the structural requirement to inhibit tumor promoters[5][11] induced Epstein Barr virus activation has been reported from the leaves[14][17] The alcoholic extract of leaves of *Moringa oleifera* was reported to have analgesic activity[20][25]. Traditionally, the plant is used as an antispasmodic, stimulant, diuretic, and expectorant [21][22][23]. The aim of this study is to carry out phytochemical analysis and also investigate the antimicrobial effect of moringa leaf extracts.

II. Materials and Methods

Materials

Materials used in this research include weighing balance, beaker, conical flask, funnel, filter paper, spatula, temperature-regulated water- bath, Petri-dishes, disinfectants, measuring cylinder, glass slides, autoclave, beaker, wire-loop, Pasteur pipette, microscope, aluminum foil, spatula, distilled water, methanol, oven, spirit lamp, masking tape, Mueller-Hinton agar.

Reagents

Methanol, acetic anhydride, sulphuric acid, hydrochloric acid, diethyl ether, ferric chloride, sodium hydroxide, chloroform, distilled water, Fehling's solution A and B, Dragendoff reagent, nutrient agar, Mayer reagent. The reagents used were of analytical grade and were employed without further purification.

Collection and Identification of Plants

The leaves of Moringa oleifera were collected from different locations in Owerri, Imo State, and were authenticated at the School of Agriculture and Agricultural Sciences, Federal University of Technology, Owerri, Nigeria. The plant leaves were washed with water and dried at room temperature in the Chemistry Laboratory, Federal University of Technology, Owerri. Dried leaves were made to a coarse powder using sterile mortar and pestle and then packed in polythene bags for further analysis.

Sterilization of Media and Glass-wares

All glassware were thoroughly washed with detergent and rinsed with distilled water. They were dried in a hot-air oven and then sterilized at 160^0C for 2 hours. Media were prepared according to the manufacturer's specification and sterilized before carrying out any experiment to avoid contamination.

Extract Preparation

Soxhlet extraction method following [22] was used for the extraction of Moringa oleifera leaves. 250g weighed amount of Moringa oleifera leaves powder was packed in an extraction thimble and placed in an extraction chamber which was suspended above the flask containing the solvent and below a condenser. The flask was heated and the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the extract was removed and the solvent was evaporated by using a rotary evaporator. The crude extract was stored in a refrigerator ready for phytochemical analysis.

Phytochemical Analysis Procedures

Test for alkaloids

About 0.2g of the extract was warmed with 2% H_2S0_4 for a few minutes. It was filtered and a few drops of Dragonorff reagent were added. An orange-red precipitate indicated the presence of alkaloids.

Test for tannins

A small quantity of the extract was mixed with water and heated in a water bath and filtered. A few drops of ferric chloride were added to the filtrate. A dark-green solution indicates the presence of tannins.

Test for saponins

About 0.2g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of a creamy mist of small bubbles) indicates the presence of saponins.

Test for glycosides

The extract was hydrolyzed with HCI and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. A red precipitate indicated the presence of glycosides.

Test for reducing sugar

The extract was shaken with distilled water and filtered. Then, boiled with few drops of Fehling solution A and B for a few minutes. An orange-red precipitate indicates the presence of reducing sugar.

Test for flavonoids

About 0.2g of the extract was dissolved in dilute NaOH and HCI was added. A yellow solution that turns colorless indicates the presence of flavonoids.

Test for steroids

2ml of acetic anhydride was added to 0.5g of the extract of each with 2ml of H_2S0_4. A colour change from violet to blue or green in some samples indicates the presence of steroids.

Test for terpenoids

0.2g of each extract was mixed with 2ml of chloroform and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish-brown colouration of the interface was formed that indicates positive results for the presence of terpenoids.

Test Organisms

The pure cultures of test bacterial strains used in the study were *Staphylococcus Aureus* gram-positive (respiratory pathogen) and *Escherichia Coli* gram-negative (food-borne pathogen) the strains were obtained from the culture collection of the Microbiology laboratory at the Federal University of Technology, Owerri. The strains were inoculated in the freshly prepared nutrient broth liquid medium and incubated at 37^0C. Aseptically some colonies from the pure culture were mixed (emulsify) in nutrient broth (7µl/ml broth) and incubated at 37^oc overnight prior to antibacterial sensitivity. At the time of screening agar plates were inoculated with bacterial cell culture (10^8 CFU/ML) by using 0.5 McFarland turbidity standards using sterile culture moistened cotton swab.

Media for the test organism

26.6g of Mueller Hinton agar was dissolved in 700ml of sterile distilled water and autoclaved at 121^0c for 15 minutes. After cooling it was poured into a sterile petri dish of approximately 4mm and allowed to set at ambient temperature. Mueller Hinton agar is the most commonly used media due to its ability to support the growth of most pathogens and its lack of inhibitors towards common antibiotics.

Antimicrobial Activity Measurement

For the antimicrobial assay, 0.02g of leaves extract was dissolved in 10ml of distilled water to give a solution concentration of 2000µg/ml, and further concentrations of (1000µg/ml, 500µg/ml, 250µg/ml, and 125µg/ml) were prepared by two-fold serial dilution of extracts. The modified agar well diffusion method of [26] was used, where 0.2ml of the bacteria isolates of each test organism was mixed with 20ml of molten Mueller Hinton agar at 40^0C. The agar was poured aseptically into sterile Petri dishes and allowed to solidify. The solidified agar was punched with a 6mm diameter sterile cork borer to create wells on the agar. The bacterial lawn was spread on nutrient agar using a sterile glass rod. The wells were filled with 1ml of the prepared extracts concentration. Sterile distilled water was used to fill one of the wells which served as the solvent control, while Ciprofloxacin (lµg/ml) was used as standard antibiotics (positive control). These were then kept at a low temperature (4^0C) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37^0C) for 12-18 hours to allow the growth of the microorganism. If the test material has antimicrobial activity, it will inhibit the growth of the microorganism, giving a clear, distinct zone called the "Zone of inhibition". The antimicrobial activity of the test agent was determined in terms of millimeters by measuring the diameter of the zone of inhibition. The greater the zone of inhibition, the greater the activity of the test material against the test organism [5].

Determination of minimum inhibition concentration

There are three main reagents necessary for this assay; the media, an antimicrobial agent, and the microbe being tested. The minimum inhibitory concentration is generally determined by turbidity. Sterile Nutrient broth was used to prepare the extract concentration. 4ml of the concentration of the extract was introduced into a sterile test tube. 1ml of standardized bacterial broth culture of test bacterial isolates was added to the concentration of the extract. The control test tube was inoculated with sterile distilled water. All the test tubes were cotton plugged and incubated at 37^0c for 24 hours. The minimum inhibitory concentration was taken as the lowest inoculated extracts concentration that did not permit any visible growth when compared with the turbidity of the test tube containing Mueller Hinton agar and the test tube inoculated with distilled water as control [28].

III. Results and Discussion

TABLE 1: Results of Phytochemical Analysis

Test	Parameters	Results
1	Alkaloids	+
2	Saponins	+
3	Tannins	_
4	Flavonoids	+
5	Reducing Sugar	+
6	Glycosides	+
7	Terpenoids	+
8	Steroids	_

KEY

- ABSENT

+ PRESENT

Table 2: Shows the Antimicrobial Activity of the Methanol Leaves Extract of the Moringa Oleifera on test organisms.

	ZONE OF INHIBITION (mm)					
	1000µg/l	500µg/l	250µg/l	125µg/l	+ve control	-ve control
Staphylococcus Aureus	15	12	10	6	15	-
Escherichia Coli	13	10	8	4	14	-

KEY:
+ PRESENT
- ABSENT

Table 3: Shows the Minimum Inhibitory Concentration of Methanol Extract of Moringa Oleifera Leaves

	CONCENTRATION OF EXTRACTS (%)				
	1000µg/l	500µg/l	250µg/l	125µg/l	MIC
Staphylococcus Aureus	15	12	10	6	15
Escherichia Coli	13	10	8	4	14

IV. Discussion

Preliminary phytochemical analysis revealed that the leaf possessed the phytoconstituents, alkaloids, saponins, flavonoids, terpenoids, reducing sugar and glycosides (Table 1) and also revealed the absence of tannins and steroids. In this research, methanol leaf extracts of Moringa oleifera showed antimicrobial activity on the test organisms. A similar result was observed in the study of [20] who investigated the antibacterial activity of methanolic extract of moringa oleifera by using the agar well diffusion method and reported the most significant activity of this plant was seen against *Staphylococcus aureus*. While working on the same plant species [6] investigated the antibacterial activity of the methanolic extract of leaves by agar well diffusion against *Bacillus spp*. and *Staphylococcus Aureus*. The results are also in agreement with [4] who reported methanolic leaf extract of *C australis* had the highest activity against *Staphylococcus Aureus* at 200mg/ml concentration with 10.5mm zone of inhibition. The methanol extract of leaves has shown strong antibacterial activity against the test organisms [10] reported that most of the plant extracts show activity against gram-positive than gram-negative bacteria. This was seen in this present research, methanol extract possesses better inhibitory activity against gram-positive than gram-negative bacteria. This revealed the medicinal potential against gram-positive bacteria.

Minimum inhibitory concentration

Moringa oleifera methanol leaves extract at 125µg/ml concentration shows a minimum inhibitory concentration against gram-positive *Staphylococcus Aureus* while at 500 µg/ml extract concentration shows minimum inhibitory concentration against gram-negative *Escherichia coli*. A previous study reported that microbes exhibit little or no antimicrobial activity in water [3]

References

[1]. Afolayan, A-J 2003. Ex-zac--s "-Yom the shoots of Arctictis actinoids inhibiting the growth of bacteria and fungi. Pharmacological Reviews., 41
[2]. Ahmad, S.M., Muhammed, S.K.S., and Abdulkarim, J.P. (2012). Physiochemical properties of Moringa oleifera seed oil extracted using solvent and enzymatic methods. Food Chemistry. 51(8) 21442155.
[3]. Aiyegoro F, Latif S, AshrafM, Gilani AH. Moringa oleifera: Evaluation of the antioxidant activity of plant extracts and their heat, pH, and storage stability. Food chemistry. 2008; 27: 17-25.
[4]. Aliero, M. and Afolayan A.J. (2006) Antioxidant and total phenolic content of moringa oleifera leaves in two stages of maturity. Plant food Human Nutrition 2009; 64:3-11.
[5]. Barry, S.I (1976). Some Physico-chemical properties of Moringa oleifera seed oil extracted using solvent and aqueous enzymatic methods. Food Chemistry. 93, 2, 253-263
[6]. Ben Salem and Makkar I.E (2009). Frying quality and stability of high oleic Moringa oleifera seed oil in comparison with other vegetable oils. Food Chemistry. 2009, 105, 1382-1389
[7]. Bhattacharya R. G.; Barba, C. V. C. Utilization of Malunggay (M. oleifera Lam.) leaves in rice (Orya sativa L.) flat noodle production. Philippine Journal of Science. 1982, 128, 79-84.
[8]. Chande L. S, and Dave S.-M. Alpha-Tocopherol content in 62 edible tropical plants. Joo, .culture and Food Chemistry 2009, 49, 3101-3105
[9]. Devis D Obnubiyi, J. A.; Umaru, H. A. Phytochemical screening and antiulcerogenic effect of Moringa. African Journal of Traditional, Complimentary and Alternatives Medicines. 2011, 3, 3, 70-75
[10]. Fahey J. W. Moringa oleifera: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Trees for Life Journal 2005, 1

[11]. Faizi, S.; Siddiqui, B. S.; Saleem, R.; Siddiqui S.; Aftab K.; and Giliani A.H. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from Moringa oleifera. Journal of Phytochemistry. 1994, 38, 957-963.
[12]. Gassenschmidt, U, R.D. Tauscher, B. and Niebergall, H. (1995). Isolation and characterization of a flocculating protein from Moringa oleifera Lam. Biomedicine and Pharmacotherapy., 1243:477-481
[13]. Gheldof M. Vargas, Sakurai, H.; Fujiwara, Y.; Hashimoto, K.; Maoka, Kozuka, Ito, Y.; Tokuda, H.; Nishino, H. An antitumor promoter from Moringa oleifera Lam. Mutation Research. 2002, 440, 181-188
[14]. Halliwell, J. L. Plants used against cancer. A survey. Lloyodia 1999, 3034.
[15]. Iqbinosa, S.; Bhanger, M. I. Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan. Journal of food Composition and Analysis, 2009, 19, 544-551.
[16]. Karatas, R.; Fonseca, Y.; Simon, J. E. Assessment of the nutritional value of Moringa oleifera species from Zambia, 2009, Unpublished.
[17]. Lambole, L.S., Kumar, V.A., Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruits, vegetables, and other readily available foods. Food chemistry. (2000) 101, 4. 1 .41
[18]. Locket, E., Dongmeza. H.. Francis, P., Becker, K., Effects of dehydrated methanol extracted Moringa (moringa oleifera lam) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (Oreochromis Niloticus) Aquaculture. 2000, 261, 1, 407-422
[19]. Mahomed, H. P. S, and Ojewole, K. Nutritional value and whole and ethanol anti-nutritional components of extracted Moringa oleifera leaves. Animal Feed Science Technology. 2006, 63, 211-228.
[20]. Mbikay M. Therapeutic potential of moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review front pharmacology 2012; 3:24. doi:10.3389/fpharm.2012.0024
[21]. Nwosu, M. O., Okafor, J. 1., (1995) Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolous and some other pathogenic fungi. 38(5-6): 191-195
[22]. Okeke, M, Ukwuoma, C., Eze, N., Iroegbu, O. (2001) Evaluation of extracts of root of Landonphin Owerrience for antibacterial activity. Journal of ethnopharmacology, 7(2): 216-219
[23]. Olsen, A. (1987). Low technology water purification by bentonite clay and Moringa oleifera seed flocculation as performed in Sudanese villages. Effects on Schistosoma Mansoni Cercariae. Water Research, 21(5): 517-522
[24]. Olson, C., (2010) Practical Manuals on the Industrial Utilization of Medicinal and Aromatic plants, Tamilnadu, India. Middle East Journal of science, 4:144-146.
[25]. Parotta, J., 1993 Stability during frying of Moringa oleifera seed oil variety 'Periyakulam I" Journal of Food Composition and Analysis 15, 79-101
[26]. Perez, H.U., Clifford, M.N., Kuhnert, N., Pharmacolognostic studies on the leaves of Moringa oleifera. Nigerian Journal of Natural Product and Medicine. 1990, 5, 45-49. 105
[27]. Rastogi, R.P. and Mehrotra, B.N. (2002). Compendium of Indian Medicinal plants, vol. 1-6. Central Drug Research Institute/National Institute Science Communication, Lucknow/New Delhi.
[28]. Rojas J.J., Ochoa, V.J., Ocampo, S.A and Munoz, J.F. (2006). Screening of antimicrobial activity of ten traditional plants used. Colombian folkloric medicine. A possible alternative in the treatment of non- nosocomial infections. BMC complementary Alternative Medicine, 6:2

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