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Phytochemical and Antimicrobial Activity of Moringa Leaf Extracts

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Abstract
The phytochemical and antimicrobial activity of moringa oleifera leaf extracts was carried out on Staphylococcus aureus and Escherichia Coli. The effect of the extracts on the growth of the test organisms was determined using agar diffusion method. The ethanol, n-hexane and aqueous extract were determined at vary concentration of 0.1 mg/mL, 0.2 mg/mL and 0.3 mg/mL respectively. n-hexane and ethanol extract were found to posses more activity than the aqueous extract. They were found to have considerable inhibitory action against the tested organism. The phytochemical contents of the leaf extract revealed the presence of Saponins, alkaloids, tannins, whereas steroids, flavonoids and glycosides were lacking in n-hexane, aqueous and ethanol respectively.

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I. Introduction

Infectious diseases are illnesses spread by an infected person or animal to a vulnerable host [1]. In recent decades, the rise in the occurrence of bacterial infections has become alarming, resulting in a serious health problem [2]. Bacillus aureus, Bacillus subtilis, Bacillus licheniformis, and Escherichia coli have all been identified as pathogenic bacteria for skin infections, septic arthritis, food poisoning, diarrhea, and wounds [3–5]. Antimicrobial drugs play a critical role in lowering the global burden of infectious diseases [6]. One of the most likely ways of treating sickness and ailments in impoverished nations is to employ medicinal plants as a source of drugs [7]. Moringa oleifera, sometimes known as the "horseradish tree," is a tropical and subtropical medicinal plant that has been claimed to have antioxidant, anti-bacterial, anti-inflammatory, and anti-cancerous properties [8-9]. Moringa has long been farmed in the tropics, and its portions have been consumed and used for a number of purposes [10]. This is due to its broad spectrum of nutritional and therapeutic benefits [11]. The leaves, seeds, root, and blossoms of the moringa tree are not only excellent for human use, but also for animal consumption [12]. The leaves, which are high in protein, minerals, B-carotene, and antioxidant compounds, are utilized in traditional medicine as well as for human and animal nutrition [13]. After refinement, the seeds contain a substantial amount of oil (up to 40%) with a high quality fatty acid composition (oleic acid > 70%) and a good resistance to oxidative degradation [14]. It has also been discovered that Moringa leaf extract is 80 percent ethanol and has growth-promoting elements for higher plants [15]. Moringa oliefera leaves have been utilized in ethnomedicine to cure a variety of diseases, including gastrointestinal discomfort, stomach ulcers, diarrhea, dysentery, and skin infection [16].

Ghasi et al. [17] found that combining a high fat diet with a crude leaf extract of Moringa oleifera

reduced high fat diet-induced increases in blood, liver, and kidney cholesterol by 14.4, 6.4, and 11.1 percent, respectively. Antitumor, antipyretic, antiepileptic, antihypertensive, and antioxidant activities have also been discovered in the leaves [18]. Moringa can also be used to control blood sugar levels in some diabetic patients [19].

II. Materials and Methods

Sample and sample,

Moringa oleifera leaf was obtained from a local farm located at Abakpa in Enugu metropolis. It was authenticated by a Botanist from Department of Applied Biology and Biotechnology (ESUT), Enugu state.

Sample preparation

The fresh leaves of moringa oleifera were conveyed into the laboratory using a black nylon bag. The leaves were washed under running tap water, air dried, and was grinded into fine powdery form using mill grinder and stored in an air tight in plastic container until ready for used.

Preparation of plant extract

The preparation were achieved using the aqueous and soxhlet extraction process

Aqueous Extraction

The grinded plant samples were extracted with distilled water using Maceration. 50g of the samples were poured in 600ml of distilled water for 72 hours at room temperature. The extracts were then filtered using filter paper (Whatman no 1). The filtrates were concentrated by boiling over water bath.

Soxhlet extraction

About 70 g of the material was extracted using a soxhlet extraction equipment with a 1-L round bottom flask and a condenser was weighed into two different reflux apparatus set up comprising 800 mL each of n-hexane, and ethanol. The boiling point of each solvent is determined by an electronic hot plate.. The extraction process took 10 hours to get to completion. The extract was made by evaporating the solvent and pouring it into an airtight container using rotary evaporators.

Phytochemical analysis

The ethanol, Aqueous and n-hexane extracts were analysized to test for the presence of tannins, saponins, steroids, alkaloids, flavonoids, and glycosides as described by Kokate(2001).

Test for Tannins;

Few drops of 1 percent ferric chloride solution were added to 2.5 mL of filtrate. The presence of tannins is indicated by the presence of blue-black, green, or blue green precipitate

Test for Saponins;

0.5mL extract was warmed with 3mL of distilled water and boiled for 2mins, the mixture was filtered while hot and the filtrate was stirred constantly with 2 drops of olive oil. The formation of emulsion indicates the presence of sapponins.

Test for Steriods;

3mL each of acetic anhydride and sulphuric acid was poured 5mL of the extract. The colour changes from violet, which indicates the presence of the steroids.

Test for Alkaloids;

Two drops of dragendoffs reagent was added to 5 mL of the extract with 3mL dilute HCL in water bath and warmed for 7mins, the mixture was cooled and filtered. The formation of a red precipitate indicates the presence of alkaloids.

Test for Flavonoids

A tiny amount of each test extract was dissolved individually in dilute NaOH. The presence of flavonoids is indicated by a yellow solution that turns colourless when strong HCL is added.

Test for Glycosides;

Two point five mL (2.5mL) of dilute sulphuric acid was added to 5mL of the extract in a test tube and boiled for 15mins, then 2mL of sodium hydroxide (NaOH) and 5mL of mixture ferric solution, A and B were added. The formation brick red coloration indicates the presence of glycosides.

Table 1: Phytochemical analysis of moringa leave extracts

S/NO	PHYTO-CONSTITUENTS	Aqueous extract	Ethanol extract	n-Hexane extract
1	Tannins	++	+	+++
2	Saponins	+	+++	+
3	Steriods	++	+	-
4	Akaloids	++	+	+++
5	Flavonoids	-	++	++
6	Glycoside	+	-	+

- = absent, +=detected, ++= moderately detected, +++ = Very detected

Bacterial Test Preparation

Two bacteria strains were employed in this study, one gram negative for E. coli and one gram positive for S. aureus, both collected from stock cultures at the Department of Applied Microbiology in Esut, Nigeria. The bacteria were subcultured on suitable agar and incubated overnight at 37^{o}C. They were then standardized by comparing them to the 0.5 McFarland turbidity standards and producing 1.5x108 colony forming units (cfu) per ml using sterile saline.

Antimicrobial assay

Agar well diffusion was used to test the antibacterial activity of aqueous, n-hexane, ethanol, and methanol extracts of Moringa oleifera leaves. According to Maragathavalliet al. (2012), 0.55 g of each extract was dissolved in DMSO, and then different concentrations of the extracts (0.1mL, 0.2mL, and 0.3mL) were achieved. In duplicates, standardinoculums of 1.5x108 cells were distributed on the surface of sterile Muller Hinton agar plates that matched 0.5 McFarland standards. On the Muller Hinton agar plates, a sterile 6 mm cork borer was used to cut a hole in which 0.1 ml of each of the plant extracts was introduced. The plates were incubated for 24 hours at 37°C. Measurements of inhibition zones in millimeters were used to detect antibacterial activity. Standardized discs were also employed to test bactericidal activity of synthetic antibiotics, and zones of inhibition were discovered.

Determination of the minimum inhibitory concentration (MIC)

Iram Gull et al., (2012) described a method for determining the Minimum Inhibitory Concentrations of extracts with various solvents. The extracts were diluted in a range of concentrations from 100 mg/ml to 0.01 mg/ml and tested against a variety of bacterial species. Sterile discs were dipped in various dilutions of the extracts (aqueous, ethanol, and n-hexane) and placed onto agar plates seeded with uniform concentrations of each bacterial culture separately. The diameter of the clearing zones was used to calculate the zone of inhibition in each.

Table 2: Antimicrobial activity of ethanol extract of moringa leafs

Test organism	Concentration of extract (mg/ml)			Gentamycin(10µg)
	0.1	0.2	0.3	Standard
E. Coli	10	6	7	13
S.*aureus*	8	9	10	17

Table 3: Antimicrobial activity of n-hexane extract of moringa Leafs

Test organism	Concentration of extract (mg/ml)			Gentamycin(10µg)
	0.1	0.2	0.3	Standard
E. Coli	9	11	7	13
S.*aureus*	8	7	10	17

Table 4: Antimicrobial activity of aqueous extract of moringa leafs

Test organism	Concentration of extract (mg/ml)			Gatamycin(10µg)
	0.1	0.2	0.3	Standard
E. Coli	6	5	7	13
S.*aureus*	5	7	6	17

Table 5: Minimum inhibitory concentration (mg/ml) of moringa oleifera leafs extract of ethanol, n-hexane and aqueous on test organisms

Test organism	Ethanol	n-hexane	Aqueous	Standard
E.coli	0.2	0.3	0.2	0.5
S.aureus	0.2	0.2	0.1	0.6

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III. Results

The phytochemicals indicated in the ethanol, n-hexane and aqueous Moringa oleifera leaf extracts is shown in Table 1. Tannins, saponins, steroids, alkaloids, flavonoids and glycosides were present, except for some phytochemicals such like steroids, flavonoids and glycosides where n-hexane, aqueous and ethanol extracts respectively are not detected. Table 2, 3, 4, and 5 shows the antimicrobial activity of ethanol, n-hexane, aqueous extracts and minimum inhibition concentrations respectively. Table 2 shows the antimicrobial activity of *ethanol* extracts of moringa oleifera leaf on the test organisms. The standard had the highest zone of

inhibition on *E. coli* and *S. aureus* , whereas E.coli is high when compared with S.aureus for 0.1 mg/ml while S.aureus is high against E.coli for 0.3mg/ml. Table 3 shows the antimicrobial activity of *n-hexane*extracts of moringa oleifera leaf on the test organisms. The standard had the greatest zone of inhibition on E. coli and S. Aureus, but E.coli is higher than S.aureus for 0.2mg/ml and S.aureus is higher than E.coli for 0.3mg/ml. Table 4 indicates the antibacterial activity of aqueous extracts of moringa oleifera leaf against the test organisms. The standard exhibited the highest zone of inhibition on E. coli and S. Aureus, while E.coli is greater than S.aureus for 0.3mg/ml and S.aureus for 0.2mg/ml. Table 5 shows the result of the minimum inhibitory concentration (MIC) screening of ethanol, n-hexane and aqueous extract of the leaf of *M. oleifera*. The result revealed that the MIC of the ethanol, n-hexane and aqueous extract on *E. coli* and *S. aureus* ranged from 0.1mg/ml – 0.3mg/ml.

IV. Discussion of the Result

The phytochemical contents of the leaf extract revealed the presence of saponins, alkaloids, and tannins, although steroids, flavonoids and glycosides were absent in n-hexane, aqueous, and ethanol, respectively. Dolara et al [23] reported that tannins are polyphenols with a strong ability to restrict bacterial cell development by inhibiting important microbial metabolism enzymes such as proteolytic macerating enzymes. Furthermore, according to [24], tannin-containing plant extracts are used as astringents, to treat diarrhea, as diuretics, to treat stomach and duodenal tumors, and as anti-inflammatory, antibacterial, antioxidant, and hemostatic medicines. Alkaloids are chemical substances that exist naturally and include basic nitrogen atoms. They are frequently used as pharmaceuticals and recreational drugs due to their pharmacological properties [25]. Flavonoids work as antioxidants and boost the benefits of Vitamin C and they have also been shown to have biological activity against liver toxins, cancers, viruses, and other microorganisms [26].

The antimicrobial potential of moringa leave extracts was evaluated by agar diffusion method and the result obtained from the different extract of the moringa leaf extract unveiled variation of inhibition towards the test organism. The data showed in the table 2 and 3 above illustrated that moringa leafs extracts exhibited higher potential zone on the test organism than that of the data in (table 4), In comparison with the different solvent extract of moringa leaf, the n-hexane extract shows higher inhibition toward the test organism, followed by the ethanol extract. Similarly, the minimum inhibitory concentration was achieved by making dilution of the different extracts of moringaoleifera leafs using method described by maragatharalalli*et al* (2012). It was amazing to note that, both gram +ve microbe's and gram-ve microbes were sensitive to all the extracts of the leafs.

V. Conclusion

Morinna oleifera is a significant breakthrough in the demand for alternative natural medicine for the treatment of disease activities caused by various pathogenic organisms. As a result, the plant could be used to treat diseases such as typhoid fever, diarrhoea, stomach ulcers, tumors, postmenopausal syndrome, arteriosclerosis, blood sugar control, anti-inflammatory drugs, gastrointestinal disorders, anti-oxidants, cancer, diabetes, and other diseases.

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