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Research Article

PRELIMINARY QUALITATIVE PHYTOCHEMICAL SCREENINGS AND ANTIMICROBIAL POTENTIALS OF THE LEAF EXTRACTS OF MORINGA OLEIFERA GROWN IN ENUGU STATE, SOUTHEASTERN NIGERIA

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ABSTRACT

Background: Moringa oleifera, being one of the 14 species of family Moringaceae, is an herbal medication that is well-known for its many therapeutic applications. Worldwide, moringa has been used as a traditional herbal medications for a variety of conditions, including anemia, skin infections, blackheads, anxiety, bronchitis, catarrh, chest congestion, asthma, blood impurities, cholera, glandular, swelling, headaches, conjunctivitis, cough, diarrhea, eye and ear infections, fever, hysteria, joint pain, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, intestinal worms, lactation, diabetes, and pregnancy. This study was done to determine qualitatively the phytochemicals and antimicrobial potentials of the leaf extracts of moringa oleifera.
Methods: Cold extraction (maceration) was used using 95 % ethanol as extracting solvent. The phytochemical screening was done using standard procedure/methods (to detect alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, glycosides, and polyphenols), while antimicrobial potentials was done using disc diffusion method. The phytochemical screening showed the presence of alkaloids, terpenoids, flavonoids, tannins, saponins, polyphenols, steroids, and glycosides.
Results: he results also showed that moringa was active against antibacterial (S. aureus and E. coli) and fungal (Rhizopus and A. niger) isolates tested, with petroleum ether extract showing greater activity (larger zones of inhibition), followed by methanol, acetone, and chloroform extracts, respectively
Conclusion: We have concluded that Moringa oleifera can be utilized as a safe and affordable plant antimicrobial agent since it contains active components with antibacterial properties, including flavonoids, tannins, saponins, alkaloids, phenolics, and triterpenoids.

Keywords: Antibacterial, antifungal, Extracts, Moringa oleifera, Phytochemicals.

INTRODUCTION

Moringa oleifera, being one of the 14 species of family Moringaceae, is an herbal medication that is well-known for its many medicinal applications. It originated in northern India about 5000 years ago, and it quickly spread to other regions of the world. It is most often referred to as the horseradish tree, drumstick tree, ben oil tree, miracle tree, and mother's best friend. Its leaves, pods, and flowers are full of nutrients that are vital to both people and animals [1]. Worldwide, moringa has been used as a traditional medicine for a variety of conditions, including anemia, skin infections, blackheads, anxiety, bronchitis, catarrh, chest congestion, asthma, blood impurities, cholera, glandular, swelling, headaches, conjunctivitis, cough, diarrhea, eye and ear infections, fever, hysteria, joint pain, pimples, psoriasis, respiratory disorders, scurvy, semen deficiency, sore throat, sprain, tuberculosis, intestinal worms, lactation, diabetes, and pregnancy [2]. In Nigeria, especially in the northern region where it is known as zogale, moringa leaves are typically eaten as vegetables in soups or prepared into a salad that is served with milled groundnut cake [3].

Though it is more commonly found growing on grazing land or in river basins, Moringa oleifera also thrives on slopes. This tree grows quickly; in regions with less than 400 mm of mean annual rainfall, it has been observed to reach a height of 6 to 7 m in a single year. Despite having low-quality lumber, the perennial softwood tree Moringa has long been used for both traditional industrial and medicinal purposes. In addition to being farmed in West, East, and South Africa, tropical Asia, Latin America, the Caribbean, Florida, and the Pacific Islands, it is already a significant crop in Ethiopia, the Philippines, India, and Sudan [4].

Most people rely on the Moringa tree for their livelihood because every component of it has some medicinal or nutritional applications. Moringa leaves are a great source of minerals, proteins, and vitamins A and C, among other nutrients. One of the least expensive natural antioxidant sources is the moringa tree, which contains about 46 antioxidants. Antioxidants lessen the impact of free radicals and provide the free atoms the body needs. Active substances with antibacterial properties found in moringa include flavonoids, tannins, saponins, alkaloids, phenolics, and triterpenoids. Flavonoids and tannins found in moringa leaves are believed to have anti-inflammatory properties. Because of its high protein and mineral content, Moringa oleifera has been studied for its potential to cure a variety of soft tissue illnesses of the mouth [5].

In Nigeria, the consumption of the Moringa plant, which is a vital source of nutrients, is significantly lower than that of other traditional vegetables. A significant amount of the nutrients in these vegetables are rendered unavailable to the body due to the anti-nutritive factors present in those plants, despite the fact that many other leafy vegetables are consumed in large quantities throughout Nigeria because starchy staples are frequently regarded as incomplete without accompanying them with generous servings of vegetable soups. These anti-nutrients prevent micronutrients like iron and ascorbic acid (vitamin C) from being absorbed and used. On the other hand, Moringa has a comparatively tiny amount of innocuous anti-nutrients that are readily eliminated by the standard processing procedures that vegetables go through [6].

Although Moringa is underappreciated in many regions of Nigeria especially the Southeast region, this characteristic should make it a more preferred leafy vegetable among Nigerians.

Figure 1. Moringa oleifera

According to nutritional research, Moringa is rich in protein, iron, vitamin C, calcium, vitamin A (β-carotene), and antioxidants like flavonoids, carotenoids, and other polyphenolic substances. It also has significant amounts of other micronutrients like magnesium, phosphorus, zinc, and other minerals and vitamins [5, 7]. In addition to possessing three times the potassium of bananas, the moringa plant has ninety-two (92) minerals and all essential amino acids, according to a pamphlet from the Nigerian Embassy in Hungary [7]. Additionally, it has a lot of phytochemicals and is thought to be quite therapeutic, as many authors have praised and verified [8].

The literature contains shallow information about the therapeutic applications of Moringa oleifera in Nigeria, especially in the Southeast. The use of this species in Nigeria does not appear to have been officially documented. But it seems that current support for the usage of moringa is growing. There is need for much researches and documentations of the medicinal and biological importance of Moringa oleifera in Nigeria [9].

The neglect of moringa in Nigeria, particularly in the south-eastern region, is attributed to both unequal spatial scarcity in its cultivation and the fact that the vast majority of the population is unaware of its significance. According to a research by Popoola and Obembe [6], for example, there is evidence of long-standing knowledge and cultivation of Moringa in many regions of Nigeria. However, the Northern part of Nigeria is where the Moringa plant was initially introduced and domesticated, and it continues to be the place where it is most widely used as a vegetable and for other purposes. However, in the southern part of Nigeria, both private and commercial farmers hardly cultivate Moringa, and there is virtually no documentation of its commercial production or therapeutic applications there. Additionally, its use was still not very widespread in the southern/eastern part of the country [10]. Thus, the purpose of this study was to identify the phytochemicals and antimicrobial properties of moringa leaf extracts grown in the southeast of Nigeria. The findings of this study may stimulate the promotion of Moringa supplementation in diets and raise awareness for the people of the Southeast region of Nigeria about Moringa's numerous therapeutic applications.

METHODS

Using an autoclave and detergents, all of the materials used in this study that are not susceptible to moist heat sterilization were sufficiently sterilized. Before being used, materials like glassware, beakers, conical flasks, etc. were thoroughly cleaned with distilled water and detergent to get rid of impurities and dirt. After that, these materials were sterilized for 15 minutes at 121 degrees Celsius in a portable laboratory autoclave. Every chemical and reagent utilized in this study was of analytical grade [11].

Sample Collection, Identification and Preservation

The leaf sample of Moringa oleifera was collected from Amufie Enugu- Ezike in igbo Eze North Local GovernmentA, Enugu State, Nigeria. The plant was identified by Mr Ohiaba Emmanuel, the department of Science Laboratory Technology, Federal Polytechnic Idah, by Mr. Ohiaba Emmanuel (a botanist). The leaves were then washed with distilled water, dried at ambient temperature and grounded into powder using mortar and pestle and stored in an air-tight container until use.

Sample Extraction and Fractionation

A quantity (300 g) of the powdered leaf sample of Moringa was soaked in 900 ml of 95 % ethanol (1:3w/v) in a reagent bottle, shaken constantly for 20 minutes every day for 7days. The sample was filtered using whatman filter paper and the filtrate was concentrated using rotary evaporator. The crude extract was further fractionated using petroleum ether, chloroform, acetone, and methanol respectively. The fractions were dried and stored in an air-tight container.

Preliminary Phytochemical Screenings.

Test for Alkaloids (Mayer's Test).

2 drops of Mayer's reagent were to added 2 ml of extracts. Creamy precipitate confirms the presence of alkaloids [12]

Test for Flavonoids (Salkowski Test).

3 ml of H2SO4 was added to 5 ml of extracts. The formation of orange color indicated the presence of flavonoids]12]

Test for Terpenoids (Salkowski Test).

1 ml of chloroform and 1 ml of concentrated H2SO4 were added to 3 ml of extracts forming a layer. A reddish-brown color confirmed the presence of terpenoids [12]

Test for Tannins (Ferric Chloride Test).

3 drops of 1% ferric chloride solution was added to 1 ml of extract. Green precipitate confirmed the presence of tannins [13].

Test for Saponins (Froth Test).

1ml of distilled water was added to 0.5 ml of the extract, it was shaken vigorously. Formation of frothing confirmed the presence of saponins]12]

Test for Polyphenols (Salkowski Test).

3 drops of 5 % solution if lead acetate was added to 1 ml of extract. Yellow precipitate indicated the presence of polyphenols [12\.

Test for Steroids (Salkowski Test).

2ml of chloroform and 2ml of concentrated H2SO4 was added to 2 ml of extracts. The appearance of yellowish green fluorescence indicated the presence of steroids [12].

Test for Glycoside (Keller-killian Test).

2 ml of chloroform and 2 ml of an ammonia solution were added was added to 2 ml of extract. Pinkish color indicated the presence of glycoside [13].

Antimicrobial Activity

Source of Organisms

Microorganisms used for the antimicrobial activities including Staphylococcus aureus, Escharichia coli, Aspergillus niger, and Rhizopus spp. were obtained from the clinical laboratory of Our Lady's Specialist Hospital Idah Kogi state.

Preparation of Sensitivity Disc.

Whatman No 1 filter papers (of 6 mm diameter) were punched with the aid of paper puncher and placed in 20 bijou bottle and was sterilized, then soaked with the test extract and dried at 40 0C for 30 minutes. The prepared nutrient agar plates were seeded with each of the test bacteria and the filter paper disc were placed on each plate.

The plates were incubated at 370C for 24 hours [14].

Agar Preparation

Nutrient Agar (NA) and Sabouraud dextroxe agar (SDA) were used as medium for culturing bacterial and fungal isolates, respectively. The media was prepared according to manufacturer's instructions14].

Preparation of Stock Solution

A stock solution was prepared by dissolving 0.6g of the extract in 5 mL of DMSO (dimethylsulphoxide). Concentration of 60 mg/ml, 30 mg/ml, and 15 mg/ml were prepared from the stock solution using serial dilution method]14].

Bioassay Procedure

The disc diffusion method was employed, the prepared plates containing the nutrient agar were dried for about 20 minutes to remove excess moisture formed at the surface of the agar medium. Standard inoculum of the isolates was swabbed on to the surface of prepared and solidified agar. The prepared extract disc containing different concentration of the plant and standard antibiotics disc were placed into the surface of the inoculated media at interval in order to prevent any form of over typing of zones. The plates were inoculated at 370C for 24 hours before measurement of zones of inhibition in millimeters. The fungi isolates were similarly cultured on SDA plate, filter paper disc was also placed on each of the plate before incubation at 30 0C for 48hours. Gentamycin (15 mg/ml) and Nystatin (12 mg/ml) were used as controls for bacteria and fungi, respectively [14].

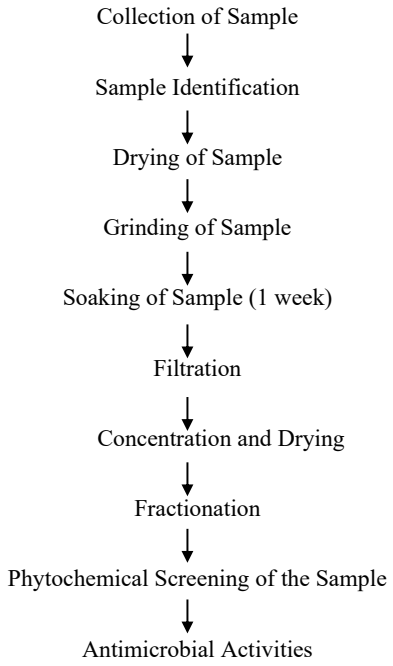

Fig 2: Methodology Flow Chart

RESULTS

Table 1: Weights and Characteristics of the Extracts

	Petroleum ether	Chloroform	Acetone	Methanol
Color of extract	Dark green	Dark green	Dark green	Dark green
Weight of the extract	2.08g	2.66g	1.17g	1.08g
Texture of the extract	Oily	Gummy	Oily	solid

Fig 3: Extracted Crude Sample of Moringa

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Table 2: Phytochemical Screenings

S/N	Phytochemical	Petroleum ether	Chloroform	Acetone	Methanol
1	Alkaloids	+	+	+	+
2	Tannins	+	-	+	+
3	Saponins	+	+	-	-
4	Flavonoids	+	-	+	+
5	Steroids	+	-	-	+
6	Polyphenols	+	+	+	+
7	Terpenoids	+	-	-	-
8	Glycosides	+	-	+	+

Table 3: Antibacterial Activity of the Extracts

		Concentration (mg/ml)		
Extracts	Bacteria	15	30	60
Petroleum Ether	S. *auerus*	5	7	10
	E. coli	5	6	8
Chloroform	S. *auerus*	4	5	8
	E. coli	3	4	6
Acetone	S. *auerus*	4	5	7
	E. coli	3	3	5
Methanol	S. *auerus*	3	5	9
	E. coli	2	4	7

Key: Control = 14 mg/ml

Table 4: Antifungal Activity of the Leaves Extracts

		Conc. (mg/ml)		
Extracts	Fungal Isolates	15	30	60
Petroleum Ether	*Rhizopus*	1	3	7
	A. niger	2	4	6
Chloroform	*Rhizopus*	1	3	5
	A. niger	1	3	6
Acetone	*Rhizopus*	1	3	6
	A. niger	1	3	3
Methanol	*Rhizopus*	2	5	8
	A. niger	3	5	7

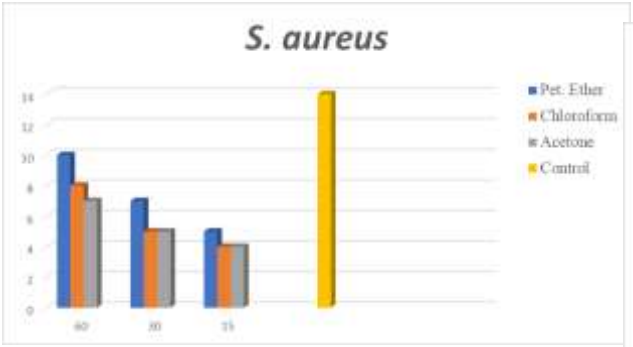

Fig 4: Graph of inhibition against Concentration of S. aureus

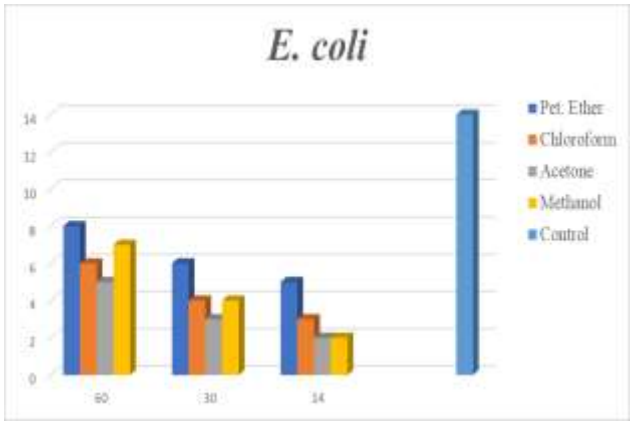

Fig 5: Graph of Inhibition Against Concentration for E. coli

Table 4: Antifungal Activity of the Leaves Extracts

		Conc. (mg/ml)		
Extracts	Fungal Isolates	15	30	60
Petroleum Ether	Rhizopus	1	3	7
	A. niger	2	4	6
Chloroform	Rhizopus	1	3	5
	A. niger	1	3	6
Acetone	Rhizopus	1	3	6
	A. niger	0.5	1	3
Methanol	Rhizopus	2	5	8
	A. niger	3	5	7

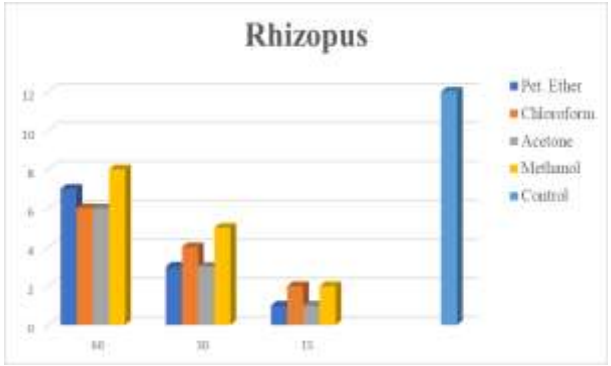

Fig 6: Graph of zone of inhibition against concentration for Rihizopus

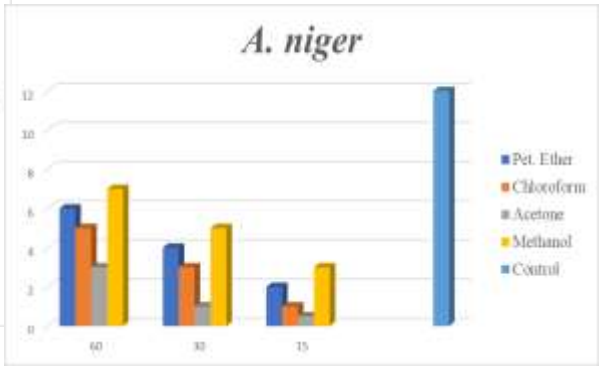

Fig 7: Graph of zone of inhibition against concentration for Aspergillus niger

DISCUSION

The results for phytochemical screening was reported in table 2. The results revealed the presence of terpenoids, flavonoids, tannins, saponins, glycosides, polyphenols, steroids, and alkaloids. The majority of the phytochemicals were found in petroleum ether extract. The phytochemicals found in this study correspond with the finding which reveals the presence of flavonoids, phenols, saponins, and amino acids [16]. Many antibiotics used to treat common pathogenic strains also contain tannins, alkaloids, saponins, phenols, glycosides, and flavonoids, all of which have therapeutic value. Those recuperating from stroke and other cardiac conditions may utilize the vegetable in their meals since saponins have antihypertensive qualities. Because moringa leaves contain saponins, they are utilized to make a diet for people with hypertension. In a similar vein, patients are treated for congestive heart failure and cardiac arrhythmias with herbal remedies that include glycosides [15].

The antimicrobial properties of the petroleum ether, chloroform, acetone, and methanol extracts on two enteric bacterial and two fungal isolates are presented in Table 3 and 4 and figures 4, 5, 6, and 7. The results from the antibacterial properties of Moringa oleifera showed that the petroleum ether and methanol leaf extracts inhibited the growth of Staphylococcus aureus and E. coli much more than chloroform and acetone extracts. The results for antifungal (table 4) also showed methanol extract inhibiting the growth of Rhizopus and A. niger, followed by petroleum ether extract, chloroform and acetone respectively. It is observed that the extracts showed lager zones of inhibition against bacterial isolates than in fungal isolates. The antibiotic controls used were gentamycin and nystatin, which exhibited high levels of inhibition to the growth of these microbes with zones of inhibition between 12-15 mm. These findings correspond to the findings of research studies some of which have revealed that nearly all types of moringa oleifera tissues exhibit antimicrobial activity including antibacterial, antifungal, antiviral, and anti-parasitic property [17].

CONCLUTION

Moringa oleifera can be utilized as a safe and affordable plant antimicrobial agent since it contains active components with antibacterial properties, including flavonoids, tannins, saponins, alkaloids, phenolics, and triterpenoids. Moringa oleifera leaf extract has a high protein and mineral content. It may be used in traditional medicine to treat a variety of soft tissue disorders of the mouth.

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CONFLICT OF INTERESTS

The authors have declared that there is not conflict of interests in the course of this research work.

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