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Phytochemical and Anti-Microbial Analysis of Metabolites in seeds of Moringa oleifera grown in Nigeria

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ABSTRACT

Phytochemical and antimicrobial analysis of *Moringa oleifera* seeds were carried using standard laboratory procedures. The phytochemical content showed the presence of alkaloid, flavonoid, saponin, and steroids respectively as samples contained 5.85g alkaloid, 5.97g flavonoid, and 1.46g saponin. Anti-microbial screening with certain selected human pathogens and the zones of inhibition obtained showed that alkaloids were 3mm, 3mm, 2mm, 4mm, & 5mm, flavonoids were 6mm, 5mm, 4mm, 2mm and 7mm and saponins were 4mm, 3mm, 3mm, 6mm and 5mm for *Escherichia coli, Pseudomonas aureginosa, Klebsiella, Staphylococcus aureus* and *Streptocuccus aureus*. Minimum inhibitory concentration (MIC) were 6.5mg/cm^3, 12.5mg/cm^3, 12.5mg/cm^3, 6.5mg/cm^3 and 12.5mg/cm^3 for *Escherichia coli, Pseudomonas aureginosa, Klebsiella, Staphylococcus aureus* and *Streptocuccus aureus* respectively. This finding shows that the application of the seed extract of *Moringa oleifera* for potential therapeutic use and medicinal drugs in treatment of pneumonia, urinary tract infection and a host of other diseases in humans.

HIGHLIGHTS

- *Moringa oleifera* exist majorly in the tropics
- It has economic use in water treatment and production of perfumes and hair creams
- It has nutritional uses in the formulation of food supplements and animal feeds
- Phytochemical and antimicrobial assessment showed it has therapeutic use in treatment of diseases

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GRAPHICAL ABSTRACT

INTRODUCTION

Medicinal plants have played important roles in the history of humanity from an array of formulation to solve a health-related needs [1]. Plants holds vase number of organic compounds, which are used for nutritional, therapeutic and curative purposes and serves as pharmaceutical development of new drug agents [1,2]. The use of plant as medicines cannot be debated, since human civilization started between 4500 B.C. till date and reported in oldest human repository [3]. The Indians and Chinese isolated different plants by boiling with water or roasting in fire to solve it daily issues and provided leads in development of several life-saving trado-medicinal drugs, which are used today [4,5]. Phytochemicals are biochemical metabolites that occur naturally in plants with no nutritional value to human life. These metabolites include alkaloids, flavonoids, steroids, glycosides, gums, phenol, tannings, terpenes and terpenoids, which are used as chemical precursors for synthetic drug development and manufacturing [5,6]. More than 4000 phytochemicals has been catalogued and classified based on functions as they act to promote health of plants from UV exposure, detoxification, stress alleviation, synthesize and activates hormones, pollution treatment, insects, microbial infection and algae attack, which has shown human potential to fight diseases and illness, acts as antioxidants, hormonal and enzyme stimulation and interference with DNA replication. [5-9].

Moringa oleifera also known as the horseradish tree, is a small or medium-sized shrub that grows to 10m high that grows in the tropics with economic and medicinal uses [10-13], it exist as a perennial softwood tree.

Figure 1.*Moringa oleifera* seed

Over the years, many research articles and reports shows that *Moringa oleifera* have different nutritional, medicinal and economic properties [13,14]. In Nigeria, there has been exponential utilization of herbal medicines, which is currently been assessed for its pharmaceutical active ingredients owing to its natural efficacy and reduced side effects at different concentration matrices. The plant (*Moringa oleifera*) parts such as the leaves, seed, stem, roots, flowers,undeveloped buds and stalk-pods functions as cardiac and circulatory stimulants, possess antitumor, antipyretic, anti-inflammatory [11,12], antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering [13,14], antioxidant, antidiabetic, anticancer[15-18], hepatoprotective [18-20], antibacterial and antifungal activities [21,22]. Economically, *Moringa oleifera* is used as a cheap adsorbent source for water purification purpose as it contains cationic polyelectrolyte that prove efficient as natural coagulant or flocculants [23,24], which is decomposable as it modifies pH, turbidity content, and antiseptic purposes for drinking water [25]. *Moringa oleifera* seeds, when processed into edible oil are sweet, does not become rancid as it is used for manufacturing perfumes and hairdressing purposes [26]. It is used in creation of animal feed [27, 28]. The aim of this study is to determine the phytochemical composition of *Moringa oleifera* and their possible medicinal properties.

EXPERIMENTAL

Sample collection and preparation

The fresh seeds of *Moringa oleifera* were collected at Mpama Egbu, Owerri in Imo State. Nigeria. The samples were dried for about two weeks in the laboratory at ambient temperature (28 – 30ºC) instead of sun or oven drying to prevent loss of phytochemicals during drying. After the period of drying, the seeds were ground to powder. At the end of the drying and grinding process, the sample were immersed with ethanol and water in a separate beaker for soaking and percolation, where the extracts were obtained and tested for various phytochemicals suspected present using respective reagents and equipment [29, 30].

Phytochemical Screening

We identified and proved the presence of pharmacological active constituents present in the sample:

Test for Alkaloids

2g of the sample were weighed into a 200ml flask and 95% ethanol were added and left for four hours. The sample filtered as few drops of Wagner's reagent (iodine crystals and potassium iodide) were added to filtrate. Observation: A yellowish coloration indicates the presence of alkaloids.

Test for Flavonoids

2g of the sample were soaked with 100cm3 of distilled water and allowed to stay for 48hours, filtered thereafter. The filtrate was kept in a conical flask with free drops of magnesium powder and concentrated sulphuric acid (H_2SO_4) were added. Observation: A formation of a reddish precipitate indicates the presence of flavonoids.

Test for Tannins

2g of the sample were weighed into the beaker and 100cm^3 of water added and allowed to soak for two hours thoroughly. The extract were treated with drops of ferric chloride. Observation: The development of a deep bluish-black colour indicates the presence of tannins.

Frothing test for Saponins

2g of the sample were weighed and add to 2cm^3 of water in a test tube. The extract obtained and shaken vigorously accordingly. Observation: The formation of foam (persistent frothing) indicates the presence of saponin.

Salkowki test for Steroids

2g of water extract were obtained and drops of formaldehyde and concentrated sulphuric acid (H_2SO_4) were added. The formation of a reddish-brown colour indicates the presence of steroids.

Test for Glycosides

5cm³ extract were obtained and added with 25cm³ of dilute sulphuric acid in a test tube and boiled, where were allowed for 15 minutes, cooled and neutralized with 10% NaOH. Thereafter, equal volume of Fehling solution A & B added. Observation: Glycosides indicates a brick red precipitate.

Quantitative determination of Phytochemicals

Determination of Alkaloids

85g of the sample were weighed into a 250cm³ beaker as 200cm³ of 20% acetic acid in ethanol added, covered and allowed to stand for 4hours. It was filtered and the extract concentrated to ¼ of its original volume using water bath. Concentrated ammonium hydroxide added to the extract drop by drop until the precipitation of the alkaloid forms. The white solution allowed settling for 24 hours and the precipitate were collected by filtration using a Whatman filter paper (No. 1), dried using an electric oven to a temperature of 110-120ºC for 1½ hours, and weighed. [31, 32].

$$Percentage\ Alkaoid = \frac{Mass\ of\ Precipitate}{Weight\ of\ samples\ used} \times 100\% \qquad (1)$$

Where: Mass of precipitate: $(Weight\ of\ filter\ paper + precipitate) - (Mass\ of\ filter\ paper\ used)$

Determination of Flavonoid

85g of the plant sample were extracted repeatedly with 100cm³ of aqueous methanol at room temperature. The solution obtained filtered using a Whatman filter paper (No. 1). The filtrate transferred into a crucible, evaporated to dryness over a water bath and weighed to a constant mass. [31].

$$Percentage\ Flavonoid = \frac{Mass\ of\ flavoniod\ precipitate}{Weight\ of\ samples\ used} \times 100\% \qquad (2)$$

Where: Mass of flavonoid precipitate $= (Weight\ of\ filter\ paper + precipitate) - (Mass\ of\ filter\ paper\ used)$

Determination of Saponins

34g of the sample were digested in 100cm³ of 20% ethanol and stirred using a glass rod. The suspension heated over a hot water bath for 4hours with constant stirring to about 55ºC the mixture filtered and the residue re-extracted with another 100cm³ of 20% ethanol. The combined extract was concentrated or reduced to about 40cm³ over a water bath at about 90ºC. The concentrate transferred into a 250cm³ separating funnel as 20cm³ of diethyl ether added and shaken vigorously. The aqueous layer recovered while the ether layer discarded. The purification process done with another 20cm³ of diethyl ether; 60cm³ of n-butanol added. The combined n-butanol extract was washed twice with 10cm³ of 5% NaCl. The remaining solution wetted in a water bath until evaporation. After evaporation, the sample dried in an oven to a constant mass [31].

$$Percentage\ Saponin = \frac{Mass\ of\ saponin}{Weight\ of\ samples\ used} \times 100\% \qquad (3)$$

Where: Mass of saponin $= (Weight\ of\ saponin\ extract + beaker) - (Mass\ of\ beaker\ used)$

Antimicrobial Screening

The Micro Organisms: *Escherichia coli, Pseudomonas aureginosa, Klebsiella, Staphylococcus aureus and Streptocuccus aureus* were used for the analysis. They were obtained from the stock cultures of Federal Medical Centre Owerri, Imo State, Nigeria brought to the

laboratory and were resuscitated in peptone water and there after sub-cultured into nutrient agar medium and incubated at 37ºC for 24hours.

Anti-Microbial Analysis

The test solution of each extract was prepared by dissolving 0.1g of the plant extract separately in 1.0cm^3 of dimethyl sulphoxide (DMSO) to get a concentration of 100mg/cm^3. The antibacterial activity was performed using filter paper disc diffusion method. Filter paper (Whatman No 1.6mm diameter) were placed in glass petri dishes and sterilized in hot air oven. The media were prepared from 10g nutrient agar in 200cm^3 distilled water, autoclaved at 115ºC for 30 minutes, cooled to 50ºC. The sterile nutrient agar media were poured into the sterile petri dish and allowed to solidify. The bacteria swabbed with a sterile wire loop. Each dis-infused with 0.2cm^3 of plant extract standard; oxacillin was used as a control on a disc with DMSO 100mg/cm3. The disc was used after drying them in an incubator at 40ºC to remove any trace of solvent. The plates were allowed at 37ºC for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each extract and repeated twice for reference; antibiotics to minimize error and the average of these values were recorded [30, 31].

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the *Moringa oleifera* extract were determined by integrating continuous volume of 0.2cm^3 of each extract into the perforated disc on a seeded nutrients agar plate. 0.1g of each of extract dissolved in 1cm^3 of dimethyl sulphoxide to obtain 100mg/cm^3. The concentration of dimethyl sulphoxide were then double to obtain 50mg/ml, then double again to obtain 12.5mg/cm3 and again to obtain 6.25mg/cm3. Each concentration was thereafter used in the method earlier described to obtain zone of inhibition. The least concentration that showed inhibitory zones were taken as the MIC [31].

Results and discussion

Phytochemical Screening

The phytochemical screening of *Moringa oleifera* shows the presence of some metabolites analyzed are presented in **Table 1**.

Results of phytochemical screening of *Moringa oleifera* seeds shows presence of alkaloids, saponins, steroids, flavonoids with glycosides and tannins absent. Alkaloids are significant for protection against microbial and pesticide activities as it is used by ethnomedicinal practioners for analgesic, antispasmodic and antimicrobial treatment. [10, 32, 34]. Saponin are used for antimicrobial activity and inhibit mould as it has haemolytic activities, cholesterols binding usage, also in treatment of yeast and fungal infections [31, 34, 35].

Table 1: Phytochemical screening of *Moringa oleifera*

S/No	Constituents	Phytochemical Test (*Moringa oleifera*)
1	Alkaloids	+
2	Saponins	+
3	Steroids	+
4	Flavonoids	+
5	Glycosides	–
6	Tannins	–

+ = Present, - = Absent

Steroids are fat-soluble chemicals used for performance enhancing drugs. Flavonoids are water-soluble polyphenolic molecules used for anti-inflammatory activity, enzyme inhibition, antimicrobial activity, estrogenic activity, anti-allergic activity, antioxidant activity, anti-ulcerogenic activity, vascular activity and cytotoxic antitumor activity [10,30, 31].

Quantitative determination of Phytochemicals

Alkaloids

The seed extract of *Moringa oleifera* contained 5.85% alkaloids. Alkaloids rank among the most efficient therapeutically significant plant substance. Pure form of isolated alkaloids with its synthetic derivatives are used as anodyne (painkilling), asthma, convulsion, and bactericidal treatments [31].

They exhibit physiological activity when administered to animals; high alkaloid content is part of reasons in its utilization in the treatment of malaria and fever. [36]. Alkaloid have many pharmacological functions such as antimalarial, antihypertensive, anticancer, antifungal and antibacterial abilities in treatment of diseases or illnesses [31,34].

Flavonoid

The flavonoid percent content of the seed extract of *Moringa oleifera* were 5.97%. Flavonoids act as antioxidants in many biological activities such as allergic, antiviral, anti-carcinogenic, and anti-inflammatory actions [18]. Flavonoids in the duodenal tract lowers the risk of heart diseases [37-39]. In addition, flavonoids protect ulcer development by initiating a gastric mucosa cover, increasing capillary resistance, and improve microcirculation, which renders the cells less injurious to precipitating factors [19].

Saponins

Saponins were found to be available at 1.46% in the seed extract of *Moringa oleifera*. Some of the general characteristics of saponins include formation of forms in aqueous solution, hemolytic activity, and cholesterol binding properties. Saponin content of the sample may be the reason for its usage as natural antibiotic and aids in the fight of infection and microbial invasion [38-40]. Saponins also prevents cancer cell multiplication, thus inhibiting unwanted cancerous cell generation in the body [7, 18, 19].

Anti-Microbial Analysis

Antimicrobial analysis conducted for *Moringa oleifera* seed extract in comparison with Oxacillin for alkaloids, saponins and flavonoids with different microorganisms as shown in **Table 2 – 4.**

Table 2. Antimicrobial results of Alkaloids.

Micro Organisms	Zone of Inhibition (mm)	MIC (mg/cm^3)	1mg Oxacillin
Escherichia coli	6	6.5	12
Pseudomonas aureginosa	5	12.5	21
Klebsiella	4	12.5	14
Staphylococcus aureus	2	6.5	13
Streptococcus aureus	7	12.5	20

Table 3. Antimicrobial results of Saponins

Micro Organisms	Zone of Inhibition (mm)	MIC (mg/cm^3)	1mg Oxacillin
Escherichia coli	3	6.5	12
Pseudomonas aureginosa	3	12.5	21
Klebsiella	2	12.5	14
Staphylococcus aureus	4	6.5	13
Streptococcus aureus	5	12.5	20

Table 4. Antimicrobial results of Flavonoid

Micro Organisms	Zone of Inhibition (mm)	MIC (mg/cm^3)	1mg Oxacillin
Escherichia coli	4	6.5	12
Pseudomonas aureginosa	3	12.5	21
Klebsiella	3	12.5	14
Staphylococcus aureus	6	6.5	13
Streptococcus aureus	5	12.5	20

The role of *Moringa oleifera* seed extract in inhibiting the activities of *Staphylococcus aureus, Escherichia coli, Klebsiella, Streptococcus aureus,* and *Pseudomonas aureginosa* have been documented above. The extracts showed marked inhibition when compared with the standard antibiotic (1mg oxacillin). The inhibition therefore supports the use of the seed extract in treatment of pneumonia, respiratory tract infection, bacteremia, urinary tract infection and much more. In view of this, the concentration of the phytochemicals present in the seed of *Moringa oleifera* can be increased when used for pharmacological activities and antimicrobials utilization [32, 35, 40]. Having assessed alkaloids, saponins and flavonoids in the inhibition of different microbes, we can state that the concentration of the phytochemicals is dependent in its potency to cure or treat microbial illness in humans.

CONCLUSION

The results obtained from the preliminary phytochemical screening and investigations into the antibacterial potentials of *Moringa oleifera* seed extract revealed the presence of an array of bioactive principles called phytochemicals, which includes alkaloids, saponins, steroid's, flavonoids, whose antibacterial potentials were comparable with those of the standard antibiotics - Oxacillin showed marked inhibition. *Moringa oleifera* seed extract is a promising and capable naturally occurring antibacterial agent with potential applications in the pharmaceutical drug development for controlling the pathogenic bacteriological infections such as: respiratory and urinary tract infections, skin infections and other diseases such as pneumonia, kidney failure, fever, bacteraemia and others caused by the test microbial strains used in this research.

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