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Screening for the Phytochemical content and Antimicrobial potential of the n-butanol Root Extract of *Moringa oleifera* Lam (Moringaceae)

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Abstract: *Moringa Oleifera* is a medicinal plant widely used in folkloric medicine in Nigeria for the treatment of wounds, malaria and skin diseases. This research work investigated the alkaloid content, phytochemical content and the antimicrobial potential of the root extract of Moringa oleifera. The phytochemical screening of the extract showed the presence of alkaloid, steroidal glycosides, deoxy-sugar, terpenes, carbohydrates, tannins, proteins, polyphenols and saponins. The extract produced antibacterial activity by inhibiting the growth of *Escherichia Coli, Salmonella typhi staphylococcus aureus, Bacillus Subtilis, and Pseudomonas aeruginosa*.The quantitative determination of the alkaloid content yielded a valueof 55mg (1.1%). The chromatographic separation of the extract produced 91 eluates (G1 - G91). Eluates G42 – G44 with the Retention factor value of 0.54 each were pooled together and preserved for spectroscopic analysis. The phytochemical compounds present in the plant are believed to be responsible for the antimicrobial activities.

I. INTRODUCTION

The World Health Organization (WHO, 2005) has defined medicinal or herbal remedies as finished labeled medicinal products that contains active ingredients of aerial or underground parts of plants or other plant material or combination thereof whether in crude state or as plant preparations. Also ,WHO has defined medicinal plants as plant containing properties or compounds that can be used for treatment or management of diseases.

Statement of Problem

Infectious diseases account for approximately one-half of all deaths in tropical countries due to increasing development of resistance by the pathogenic microorganisms to available drugs. *Moringa Oleifera* is a medicinal plant widely used in folkloric medicine for the treatment of ailments such as malaria, wounds, and skin diseases. Only very little work had been done on the root of the plant probably because the root of *Moringa oleifera*contain some toxic substances that can cause paralysis and death. Other side effects include Heartburn, Diarrhea, Nausea and vomitinga huge antifertility property (Prakash *et al*, 1988). This work investigated the Antimicrobial activity, alkaloid content and the phytochemical content of the root of *Moringa Oleifera*.

Moringa oleifera

Moringa oleifera is the most widely cultivated species of the genus Moringa. It is a fast-growing deciduous, perennial tree. It can reach a height of 10-12m
(Parotta *etal*, 1993).The trunk can reach a diameter of 45cm.The flowers are fragrant and bisexual. Moringa tree is grown mainly in semi-arid, tropical and sub-tropical areas.It tolerates a wide range of soil conditions. Moringa can be propagated from seed or cuttings (Raja *etal*, 2013)
Flowering begins within the first six months after planting.
The fruit is a hanging three-sided brown capsule of 20-45cm size
The seeds have three whitish paperly wings and are dispersed by wind and water (Verzosa *et al*, 2012).

Beneficial Facts about *Moringa Oleifera*

Moringa oleifera contains 92 nutrients, 46 antioxidants, 36 anti-inflammatory agents, 18 amino acids and 9 essential amino acids. It nourishes the immune system, promotes healthy circulation, supports normal glucose levels, contain natural anti-aging benefits, promote healthy digestion, heightens mental clarity, boosts energy, encourages balanced metabolism and supports normal hormonal levels. All plant part of *Moringa oleifera* are traditionally used for different purposes but leaves are generally the most used (Popoola, Obembe,

2013). Roots are soaked in water or alcohol and boiled with other herbs to obtain drinks as remedies for toothache and also used as sex enhancers (Silvasankari*et al*, 2014).

Moringa leaf has been used in the treatment of hypertension, asthma, cancer and diabetes. It also has anti-inflammatory, antihelmintic, antipyretic, analgesic and hypatoprotective activities (Ash fag*et al*, 2012).

Flavonoids are a sub-group of polyphenolic compounds having a benzo-r-pyrone structure and are ubitiquous in plants, (Kumar, Pandy 2013). Phenolic acids have antioxidant, anti-inflammatory, antimutagenic and anticancer properties (Verma *et al*, 2013; El-Seedi*et al*, 2012). Alkaloids are a group of naturally occurring chemical compounds that contain nitrogen atoms in the form of primary amine (RNH_2), secondary amine (R_2NH) and tertiary amine (R_3N). (Cushnier*etal*, 2014). The presence of Alkaloids in *moringa oleifera* leaves have been reported (Kasolo*et al*, 2010).

Tannins are water soluble phenolic compounds that binds to and precipitate alkaloids, gelatin and other proteins. They exhibit various biological properties such as anticancer, antiatherosclerotic, anti-inflammatory, antihepatotoxic, antibacterial and anti-HIV replication activity (Kancheva, Kasaikina, 2013). *Moringa oleifera* leaves are an appreciable source of tannins (Bhatta *et al*, 2012)

Saponins are a group of natural compounds that consist of an isoprenoidal-derived aglycone, designated genin or sapogenin, covalently linked to one or more sugar moiety (Augustin et al, 20100). Even though some saponins have haemotytic side effects, they are studied for their anti-cancer properties. Moringa leaves are good source of saponins (Tiam*et al*, 2013).

Oxalates and phytates are anti-nutritional compounds as they bind minerals inhibiting the intestinal absorption. *Moringa oleifera* leaves present high contents of these compounds (Teixeira *et al*, 2014).

II. METHODS

Root of *Moringa oleifera*

Plant of study: *Moringa oleifera*

Collection and Identification of the plant

The fresh root of the plantwere collected in September, 2018 from a farmland in ObotAkaraLocal Government Are of Akwa Ibom State. The plant was identified and authenticated as *Moringa oleifera* (Moringaceae) by Prof. Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The root were cut into tiny pieces ,shade dried and pulverized using a mortar and pestle.

PROCEDURES

Extraction Procedure

The pulverized plant (200g) was macerated using n-hexane and chloroform
n-butanol successively in a maceration tank each for 72 hours at room temperature ($27^0C\pm 2^0C$).. The tank was agitated three times daily to enhance the extraction process. The extract was then collected through filtration using filterpaper into beakers. The extractwas evaporated in a water bath at 40^0C until the extract is concentrated . Upon complete drying, the n-butanol, n-hexane andchloroform extracts were weighed. The n-butanol extract gave the highest yield hence it was stored in a refrigerator at -4^0C for subsequent use.

Phytochemical screening

The n-butanol extract was screened phytochemically to identify the bioactive constituents. These tests were carried out using the standard methods of analysis by Evans 1996.

Determination of alkaloid

5.0g of sample of root powder was taken into 250ml beaker and 200ml of 20% Acetic acid in ethanol was added to it. Magnetic stirrer was used to mix the solution for 10h at room temperature. The solution was filtered using Watman filter paper Number 1. The filtrate was placed on a hot water bath (60^0c) until the extract volume reduced to one-quarter of the initial volume. Conc. NH_4OH was added dropwise until a thick precipitate was formed. The whole solution was allowed to settle down . The ppt was collected by filtration, dried in an oven and weighed.

Moringa Oleifera root (5.0g)

↓ Extracted with a solution (200ml)
containing 20% acetic acid + Ethanol

Filtrate

↓ Conc. NH40H Solution added to the filtrate dropwise
until Precipitate is formed

Precipitate

↓

Precipitate was dried, weighed and recorded
Dried Precipitate

ANTIMICROBIAL ACTIVITY STUDY

The experiments were carried out by adopting the Agar-well diffusion, method (Gramer, 1976, Murray, *et al*, 1995) using the following media: Mueller-Hinton agar medium (for antibacterial analysis) at a pH of 7.4 Sabouraud dextrose agar (for antifungal analysis), Nutrient agar medium for storing and preserving bacterial organisms at pH of 7.5 and Nutrient both for inoculation of the test organisms to obtain broth culture. The Mueller-Hinton agar powder and the sabouraud dextrose agar powder were products of the International diagnostic group England.

COLUMN CHROMATOGRAPHY

An open glass column (gravity) was used for this chromatographic analysis. The column was packed with silica gel of 60-120 mesh. The silica gel was made into slurry with enough quantity of petroleum ether. The slurry was poured into the glass column with gentle tapping. A little quantity of cotton wool was inserted to cover the gel in the column. The n-butanol extract (0.525g) was crushed with 10g of silica gel until a powder was obtained. The powder was poured into the column and more of the solvent (petroleum ether gradually added. The column was allowed to equilibrate. Collection of the eluate commenced the following day. The column was eluted into labeled tubes. 91 eluates were obtained from different solvent systems

THIN LAYER CHROMATOGRAPHY

A pre-coated aluminum TLC plate of dimensions (20cm x 20cm) was used for the analysis. The solvent-system was a mixture of benzene and 2-propanol (3:2). The solvent system was mixed in a TLC tank and gently swirled and allowed to equilibrate for 10 minutes. The TLC plate was then inserted into the already saturated tank for development.

The plate was removed from the tank as the solvent reached the solvent front on the plate and air-dried. The spots on the TLC plate were detected using UV-light (wavelength 254nm). The visible spots were enriched faintly with pencil. The distance from the centre of the spot to the origin and distance of the solvent from the origin to the solvent front were measured and recorded. The retention factor (Rf) was calculated for each spot using the formula:

$$Rf = \frac{\text{Distance of spot from origin}}{\text{Distance of solvent front from origin}}$$

III. RESULTS

Table1 Phytochemical screening of the n-butanol extract of *Moringaoleifera* root

Constituents	n-butanol extract
Alkaloid	+++
Steroidal glycosides	++
Deoxy sugar	++
Terpenes	++
Carbohydrate	++
Tannins	+
Balsams	-
Protein	++
Quinine	-
Polyphenols	+
Resins	-
Saponins	+

+++ = Present in abundance

++ = Moderately present

\+ = Present

\- = Absent

Table 2 Zones of Inhibition(mm) by n-*Butanol* Extract of *Moringa oleifera* Root and Standard Drug Excluding the Diameter (4mm) of the Borer

Organism	n-*butanol* extract3.5mg/ml	Chloramphenicol 1 mg/ml	Nystatin 50,000 IU/ml
Escherichia coli (NCTC 10418)	7	24	-
Pseudomonas aeruginosa ATCC 15442	8	23	-
Staphylococcus aureus (NCTC 6571)	9	22	-
Bacillus subtilis (NCTC 8853)	6	25	-
Salmonella typhii NCTC(8571)	4	28	-
Candida albicans	-	-	20

IV. DISCUSSION

Microbes are tiny living things not visible to the naked eye for example bacteria, some fungi, viruses.

Antimicrobial drugs are used in the treatment of infections caused by bacterial, fungi, viruses, protozoa and some parasites.

Antimicrobial resistance occurs when the drug that normally would kill or stop the growth of the microbe causing infection is no longer effective against it. The microbe that changes in this way is said to resist the action of the drug.

When this happens infections become difficult to treat as they no longer respond to drugs that were formerly used in their treatment. The patient remains ill for a longer period and the infection is more likely to spread to other people.

This study tried to find out if the n-*butanol* Root Extract of *Moringaoleifera*has antimicrobial activity.

The phytochemical screening showed that the extract contained alkaloid, steroidal glycoside, deoxysugar, terpenes, carbohydrate, tannins, protein, polyphenols and resins.

Most alkaloids preparations have long been used as psychoactive substance and pain killers (Arnold, 1989). Some cardiac glycosides are used in the modern treatment of congestive heart failure and atrial fibrillation.This is a result of the ability of these compounds to increase cardiac output by increasing the force of contraction. Terpenes are class of compounds that comprises of essential oil, lavoursand fragrance. These are bases for perfumery industry (Choudhary, 1997).

Tannins have been shown to constrict blood vessels, thus providing protective covering for wounds (Evans, 1996). Saponins are glycosides with foaming characteristics and haemolytic properties. They have beneficial effects on blood cholesterol and emetic action (Evans, 1996).

The antimicrobial assay showed that the test organisms, *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 6571), *Bascillussuntilis* (NCTC 8853), *Salmonella typhi* (NCTC 8571) and *Pseudomonas aeruginosa* (ATC 15442) were all susceptible to the extract and the standard chloramphenicol by showing different zones of inhibition. The extract also demonstrated antifungal activity by recording a zone of inhibition of 3mm.

The chromatographic separation of the n-*butanol* extract gave 91 eluates (G1 – G91). Three eluates (G42-G44) having the same R_f (0.54)value
were pooled together because they had the same Rf value of 0.54 and preserved for spectroscopic analysis.

V. CONCLUSION

It can be concluded as follows:

1. That the percentage alkaloid content of *Moringaoleifera* root was determined to be 55mg (1.1%)
2. That the *Moringaoleifera* (n-butanol root extract) contained alkaloid, steroidal glycoside, deoxy sugar, terpenes, carbohydrates, tannins, protein, polyphenols and saponins.
3. That the extract inhibited the growth *E. coli, P. aeruginosa, S. aureus, B. subtilis,*and *S. typhi.*

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