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Phytochemical, Proximate Analysis and Antimicrobial Activities of Methanolic Crude Extract of *Tylophora glauca* (Bullock)

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ABSTRACT: The proximate analysis of *Tylophora glauca* showed moisture (7.11%), ash (6.66%), crude protein (20.03%), Fat (14.01%), fiber (7.19%) and carbohydrate (45.00%). The phytochemicals detected were tannin, alkaloid and saponin while steroid, phlobatannin, terpenoid, flavonoids and cardiac glycoside were not found. The mineral analysis in mg/100g indicated that the leaves contained calcium (42-90), sodium (39.86), potassium (37.35), magnesium (31.49), zinc (40.49), iron (6.70), copper (0.31), manganese (1.57) and phosphorus (38.71). The antibacterial activity revealed that *Staph aureus* was sensitive to the extract with inhibition zone ranging from 2mm (3.12mg/ml) to 14mm (50mg/ml). *Pseudomonas aeruginosa* with inhibition zone ranging from 4mm (3.12mg/ml) to 16mm (50mg/ml) while *Escherichia coli* zone of inhibition ranging from 2mm (6.25mg/ml) to 12mm (50mg/ml) while *Enterococcus sp*. had zone of inhibition ranging from 4mm (3.12mg/ml) to 16mm (50mg/ml). The effect of the extract on the radial mycelial growth of *Botrydiopodia theobromae* varied from 71.0mm (3.13mg/ml) to 62.0mm (50mg/ml) while *Rhizopus sp* had ranged from 71.0mm (3.10mg/ml) to 61.0mm (50mg/ml).

Key words: Phytochemical, Antimicrobial activity, Methanolic extract, *Tylophora glauca*

INTRODUCTION

Plants have been considered since immemorial time, among the common sources of medicaments. Most of plant-derived medicines have been developed on the basis of traditional knowledge in health care and in many cases; there is a correlation between the indications of pure substances and those of respective crude extracts used in traditional medicine (Osho *et al.*, 2007). To provide scientific basis for use of some of these plants in the treatment of many diseases of microbial origin, many studies have been screened for antimicrobial activities and have been found promising. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity (Holetz *et al*.,2002).

The health care delivery of the larger proportion of the rural communities in Nigeria today hinged to a large extent on medicinal plants based traditional health care delivery system. Even today, according to the World Health Organization (WHO, 2003), as many as 80% of the world's people depend on traditional medicines for the primary health care delivery needs. The greater parts of traditional medicines and therapy involve the use of plant extract or their active ingredients (George and Roger 2002). The active ingredients of plants that can provide effective therapeutic potential can occur in all plant structures but concentration is often higher in one part, such part is preferred. Examples include roots, flower, fruit, leaves, bark of the stem and seeds (Akinleye, *et al.,* 1996).

Biologically, active compounds from natural sources have always been of great interest to scientists working on infectious diseases. In recent years there has been growing interest to evaluate plants possessing antibacterial activity for various diseases (Dhir *et al*., 2002). A

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number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Dhir *et al*., 2002; Adodo, 2004; Idu, 2007).

The healthcare delivery of the larger proportion of the rural communities in Nigeria and most part of Africa today hinged to a large extent on medicinal plants based on traditional health care delivery system. Even today, according to the World Health Organization (WHO), as many as 80% of the world's population depend on traditional medicines for their primary health care delivery and needs (WHO, 2008). In Nigeria today, 70-80% of the rural population still rely on traditional medicine for primary health care, most of which involve the use of extract from plants. For this purpose, various parts of plant are extracted, from the roots, leaves stem-bark, flowers, fruits and seeds that have always done the magic.

Today, it is estimated that plant materials are present in, or have provided the model for 50% western drugs (Osho *et al.*, 2007). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity.

Tylophora glauca, also known as 'ipecacuanha' belongs to the family Asclepiadaceae. In Yoruba language it is called '*Albaro'* while in Igbo language it is called '*Okashi Nwanzu'* (George and Roger, 2002). This plant has been used in folklore medicine in the form of decoctions for patients with smallpox, skin infections, measles and gastroenteritis (Gills, 1992).The dried leaves are emetic, expectorant, diaphoretic, blood purifier and stimulant. The decoction of the leaves is given for dysentery and diarrhea while the infusion of the leaves and the root is given in chronic bronchitis, as an appetizer, for skin diseases and syphilis (Gills, 1992; George and Roger, 2002). This study tends to determine the chemical constituents, proximate, phytochemical analysis and antimicrobial activities of the crude methanolic extract of the leaves of *T. glauca*.

MATERIALS AND METHODS

Plant Collection

The fresh leaves of *Tylophora glauca* was collected from Ikere -Ekiti in Ekiti State of Southwestern Nigeria. The plant sample was identified at the Department of Plant Science, University of Ado Ekiti, Nigeria. Voucher specimen (No 06/242) was deposited at the herbarium of the Department of Science Technology, Federal Polytechnic, Ado-Ekiti. The sample used for the analysis was air-dried at room temperature of $\pm 27^0$C and pulverized**.**

Proximate, Phytochemical and Mineral Analysis

These were carried out using the methods of Association of official Analytical chemist, AOAC (2005). The parameters determined for proximate analysis include ash, moisture, crude protein, fat, fiber and carbohydrate, For phytochemical analysis; Tannin, alkaloid, saponin, steroid, phlobatannin, terpenoid, flavonoids and cardiac glycoside were determined qualitatively while for mineral analysis Na, K, Ca, Mg, Zn, Fe, Cu, Mn and P were the parameters determined.

Antimicrobial assays

Bacterial and fungal strains

Tests were performed against the following microorganisms: *Staphylococcus aureus* (ATCC 6538P), *Pseudomonas aeruginosa* (ATCC 12228), *Klebsiella spp* (ATCC 9341), *Enterococcus spp* (ATCC 6633), *Escherichia coli* (ATCC 25922) *Rhizopus spp* and *Botrydiopodia theobromae* (ATCC 10231) were acquired from The American Type Culture Collection (ATCC). The strain of *Rhizopus spp* was obtained from the Federal Polytechnic, Ado-Ekiti Stock Culture Collection. Microorganisms cultures are maintained on Nutrient agar medium (Merck) for bacteria, and Sabouraud dextrose (Merck) for fungi.

Antimicrobial screening

The agar-diffusion method adopted by the Brazilian Pharmacopeia (George and Roger, 2002), described in detail by Schapoval *et al.* (1988), was used in this study.

The microorganisms were maintained on agar slants, and subcultures were freshly prepared before use. Bacterial inocula were made in 5ml of Nutrient broth (Merck), and grown for 24 h at 37 ° C. The fungi were inoculated in Sabouraud broth (Merck), and grown for 48 h at 25 ° C. The final inocula were prepared with Nutrient agar medium (Merck) or Sabouraud agar (Merck) (5 ml, 48°C), seeded with the test microorganism (0.5% for bacteria and 1.0% for fungi).Plates were prepared by pouring freshly prepared Nutrient agar and adjusted to 45 ◦C (Merck) (20 ml) or Sabouraud agar (Merck) (20 ml) into 20mm × 100mm Petri plates.
The Inoculum (5 ml) was poured directly over the surface of prepared plates, allowed to solidify for 5 min; stainless steel cylinders (7 per plate) were applied to the surface of the inoculated plates with sterile forceps. 200 -l of crude extracts (50 mg/ml) were inoculated through each cylinder, and plates incubated overnight at 37°C and 25°C for bacteria and fungi, respectively. After 24h incubation, inhibition zones were recorded as the diameter of the growth-free zones. Two control (200-l of methanol and 200-l of water) cylinders were used in all plates and extracts analyzed in quintuplicate. Chloramphenicol (40 g/ml, 200-l) was used as the positive control for bacteria, and nystatin (30 mg/ml, 200 -l) as the positive control for fungi.

RASULTS AND DISCUSSION

The results of proximate analysis are shown in table 1. The ash content of the plant was 6.66%, moisture 7.11%, crude protein 20.03%, fat 41.01%, fiber 7.19%, while the carbohydrate content was 45.00%.

Table 1: Proximate Analysis

Parameters	Results (%)
Ash	6.66
Moisture	7.11
Crude Protein	20.03
Fat	14.01
Carbohydrate	45.00

Table 2: Phytochemical analysis

Constituents	Results
Tannin	+ ve
Saponin	+ ve
Alkaloid	+ ve
Flavonoids	- ve
Steroid	- ve
Phlobatannin	- ve
Terpenoid	- ve
Cardiac glycoside	- ve

+ve Positive.
-ve Negative

The results of phytochemical screening are shown in table 2. The plant contained Tannin, alkaloid and saponin. However, steroid, phlobatannin, terpenoid, flavonoids and cardiac glycoside were absent. The results of mineral analysis are shown in table 3. The plant contained Sodium 39.86mg/100g, Potassium 37.35mg/100g, Calcium 42.49mg/100g, Magnesium 31.49mg/100g, Zinc 40.49mg/100g, Iron 6.70mg/100g, Copper 0.341mg/100g, Manganese 1.57mg/100g, while the Phosphorus content was 38.71mg/100g. The results of antibacterial and antifungal activities are shown in tables 4 and 5 respectively.

Table 3: Mineral analysis

Constituents	Results (mg/100g)
Sodium (Na)	39.86
Potassium (K)	37.35
Calcium (Ca)	42.90
Magnesium (Mg)	31.49
Zinc (Zn)	40.49
Iron (Fe)	6.70
Copper (Cu)	0.31
Manganese (Mn)	1.57
Phosphorus (P)	-

The results obtained in the proximate analysis of the plant showed that the dry matter of the plant is as high as 6.66%. This has always been the findings of most researchers and it is complemented by the reports of Harbinger (1994) and Pamplona-Roger (2000). The carbohydrate and crude protein composition of a plant are of importance because of their nutritive values. Both exist as 45.00% and 20.3% respectively in the plant.

Table 4: Antibacterial activity of methanolic extract on test organisms

Test organisms	Diameter of zone of inhibition (mm)				
	Concentration (mg/ml)				
	3.12	6.25	12.5	25	50
Klebsiella spp	0.0	0.0	0.0	2.0	4.0
Enterococcus spp	4.0	6.0	12.0	12.0	16.0
Pseudomonas aeruginosa	4.0	4.0	8.0	12.0	16.0
Staphylococcus aureus	2.0	4.0	4.0	10.0	14.0
Escherichia coli	0.0	2.0	2.0	6.0	12.0
Chloramphenicol (40 g/ml)	0.0	0.0	0.0	0.0	0.0
Methanol	0.0	0.0	0.0	0.0	0.0
Water	27.0	27.0	27.0	27.0	27.0

Table 5: Antifungal activity of methanolic extract using radial mycelia growth assay

Test organisms	Radial mycelial growth (mm)				
	Concentration (mg/ml)				
	3.12	6.25	12.5	25	50
Rhizopus spp	73.0	69.0	69.0	65.0	61.0
Botryodiopodia theobromae	71.0	69.0	68.0	65.0	62.0
Nystatin (30 mg/ml)	61.0	61.0	61.0	61.0	61.0

The value of carbohydrate is high because most plants store glucose as starch which is a source of energy. The elemental analysis revealed the presence of phosphorus, magnesium, calcium and sodium in appreciable quantities. This precludes that the plant could be a good source of nutrition for body building and a booster to immune system. Phosphorus has been reported to be good for bones and teeth formation. It contributes to energy production by participating in the breakdown of carbohydrates, protein and fats. It is needed for growth, maintenance and repair of tissues and cells and for the production of DNA and RNA (Harbinger, 1994). Phosphorus is also needed to balance, and metabolize vitamins and minerals such as vitamin D, calcium, iodine, magnesium and zinc. Magnesium is an essential mineral involved in various metabolic reactions (Nishiura *et al.*, 2005). It is necessary for major biological processes, including the production of cellular energy and the synthesis of nucleic acids and proteins. It is also important for the electrical stability of cells and maintenance of membrane integrity and plays a key role in many physiological functions especially protein synthesis of chlorophyll (de Oliveira *et al.*, 2001). The presence of calcium explains why the plant is important in blood clotting, muscle contraction and in the metabolic processes of certain enzymes. The concentration of iron is also of significance in the plant. Most studies indicated that iron deficiency leads not only to behavioral changes but also to biochemical changes in the brain (de Oliveira *et al.*, 2001). Iron plays a pivotal role in erythropoiesis and in many intracellular reactions of oxygen transport. It facilitates the oxidation of carbohydrate, proteins and fats. Calcium in conjunction with phosphorus and magnesium are activator of many enzyme systems and maintains the electrical potential in the nerves (de Oliveira *et al.*, 2001; Freitas *et al.*, 2002). Phosphorus assists calcium in many body reactions although it also has independent functions. Sodium and potassium are required to maintain osmotic balance of the body fluids, pH of the body, regulate muscle and nerve irritability and control of glucose absorption. (Nwanjo, 2007). The presence of active components especially, tannins and flavonoids provide the bitter taste experienced from the

plant. These findings correlate with that of Akinleye *et al*. (1996), Dhir *et al*,(2002) and Idu, (2007). Active principles like saponin and alkaloids have been discovered to be very potent against clinical pathogens such as *Escherichia coli*, *Salmonella typhi* and *Staph aureus* (Ajibade and Falegan, 2007). The susceptibility of different concentrations of the extract on test microorganism is shown in Table 4. *Pseudomonas aeruginosa* and *Enterococcus spp* were more susceptible to the extract at the various concentrations (50–3.12mg/ml). *Pseudomonas aeruginosa, Enterococcus spp Staphylococcus aureus, Escherichia coli,* were susceptible to zones of inhibition of 16.0mm, 16.0mm 14.0mm and 12.0mm respectively at a concentration of 50mg/ml. There is a considerable susceptibility observed with the fungi as shown in table 5. *Rhizopus spp* was more susceptible to the extract since at 50mg/ml, the radial mycelial growth did not exceed 61.0mm which is the initial core diameter. The positive control with nystatin showed radial growth of 61.0mm. However, *B. theobromae* only increased by 1.0mm after 8days of incubation. The data obtained in this study have led to the conclusion that the presence of saponin and alkaloid is responsible for the significant antibacterial effect of *T. glauca* on a wide range of organisms. Evaluation of the *T. glauca* in rural areas of Nigeria is more urgent than ever. At the same time, ethnographic, ethnobiological, and ethnopharmacological surveys dealing with traditional Nigerian uses of plants and other aspects of folk pharmacopoeias could represent the basis for this research implementation focusing on eco-sustainable interdisciplinary projects involving biological conservation, and most importantly, the conservation of local cultural heritage. Last, but not least, a lot of unknown uses of medicinal plants, do exist even in Africa and Europe, and ethnobotanical studies such as the present one could provide inputs for new phytochemical and phytopharmacological studies. These, on their turn, could lead to integrated projects for the sustainable cultivation of local plant resources for the small-scale production of raw phytotherapeuticals.

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