

Screening of Antimicrobial Ethanolic Extract of *Peristrophe bicalyculata*

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Abstract

The ethanolic extract from *Peritrophe bicalyculata* leaves was evaluated for the presence of phytochemicals and its antimicrobial activity in vitro against selected bacteria and fungi using the antibiotic gentamycin as control.

The extract showed the presence of secondary metabolites such as alkaloid, saponin, tannin and steroid. It also inhibited the growth of the tested microorganisms at different concentrations. However stronger in-vitro activity was recorded against *Staphylococcus aureus*, *Klebsiella spp*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Asperigillus clavatus* and *Rhizopus stolonifer*. The antimicrobial activity of the plant extract is an evidence of ethnomedicinal potential of the plant.

Key words: Phytochemicals, Antimicrobial, Inhibition, Ethnomedicine, In-vitro, Concentration.

Introduction

Leafy vegetables play a very important role in our diet and nutrition since they are the major sources of not only raw fibers but also essential nutrients, vitamins and minerals. Some of these vegetables are common in Nigeria and West Africa where they are widely used in preparation of various soups. They have been used as medicinal and aromatic plants since ancient times. Some of them are also commonly used for flavouring, local concoction, infusion and spicing.

The importance and awareness of nutrition and medicinal properties of some plant in public health issues has

resulted in the increase demand of knowledge in the nutrient, phytochemical and antimicrobial properties of food (Chinma and Igyor, 2007). Green leafy vegetables are rich source of carotene, ascorbic acid, riboflavin, folic acids and minerals like calcium, iron and phosphorus. (Fasuyi, 2006).

The presence of some phytochemicals determines the antimicrobial properties of various plants. They give plants its colour, flavour, odour and are part of the defense system (disease resistance). According to Liu (2004), phytochemicals are bio actives, non nutrient plants compounds in fruits, vegetables, grains and other plants food that has been linked to reduce the risk of major degenerative disease. Anderson (2004) defined phytochemicals as plant derived chemicals, which are beneficial to human health and disease prevention. It attract beneficial and repel harmful organisms, serves as photoprotectant and respond to environmental changes. For examples, isoflavones, anthocyanins, and flavonoids do function as phytoalexins, a substance that assists a plant to resist pathogens. (Agte *et al*, 2000).

Report shows that the greatest sources of these photochemicals are fruits and vegetables. (Onyeka and Uwambeke, 2007). Most leafy vegetable constitute an indispensable constituent of human diet in Africa generally and Western part of Africa in particular. In addition they are used in the diet of postpartum women during which time it is claimed that they aid contraction of the uterus. Most of these leaves are inexpensive and grow widely in most western part of the country. Medicinal plants have been used for centuries by man in treatment of diseases in early years when there were no clinics or hospitals. In recent years, the potential of the Nigeria flora as a varetals source for pharmaceuticals and other therapeutics have been expressed. Extraction of bioactive compounds from these plants permits the demonstration of their antimicrobial activities and facilitates pharmacological studies leading to synthesis of more potent drug with reduced toxicity (Onyeka, and Uwambeke, 2007).

This research work was therefore, aimed at determining the presence or otherwise of some phytochemicals and also antimicrobial properties of ethanolic extracts of *Peristrophe bicalyculata* leaves on selected microorganisms in order to determine its medicinal use.

Materials and Methods

Source of Materials

Fresh leaves of *Peritrophe bicalyculata* were collected from “Obasoto” in Owo township, Ondo State, Nigeria.

Pure strain of the test microorganisms were obtained from the culture collection of the Department of Science Laboratory Technology (Microbiology Unit) of Rufus Giwa Polytechnic, Owo.

Extraction of Plant Materials

The pulverized fresh leaves (100g) were extracted with 95% ethanol by cold maceration for 24 hours at room temperature (25°C). The extract was filtered and evaporated to dryness over water bath after concentration with rotor vapour to minimal volume.

Determination of alkaloids

A measured weight of the sample was dispensed into 10% acetic acid solution in ethanol to form a ratio of 1:10.

The mixture was allowed to stand for 4 hours at 28°C. It was later filtered with filter paper and the filtrate was treated with drop wise addition of aqueous NH₄OH until the alkaloid was precipitated, this was washed with 10% ammonia solution and dried in the oven at 80°C.

Determination of flavonoids

5g of the sample was boiled in 50ml of 2M HCl solution for 30 minutes under reflux. It was allowed to cool, then filtered through filter paper and the filtrate was treated with equal volume of ethyl acetate.

Determination of tannin

A 5g portion of the sample was dispensed in 50ml of distilled water and mixed properly. This was allowed to stand for 30 minutes at 28°C before it was filtered. 2ml of the plant extract was dispensed into a 50ml volumetric flask. Similarly, 2ml standard solution and 2ml of distilled water were put in separate volumetric flask. The reagent was added to each of the flask, and 2.5ml of saturated Na₂CO₃ solution was also added, the total content of

the flask was made up to 50ml with distilled water and incubated at 28°C for 90 minutes. A spectrophotometer set at 260nm wavelength was used to measure the respective absorbance using the reagent blank to calibrate the instrument.

Determination of Steroid

A measured weight of the sample was dispensed in 100ml freshly distilled water and homogenized in laboratory blender. This was filtered and was eluted with normal ammonium hydroxide solution (PH 9). 2ml of the eluate was put into the test tube and mixed with 2ml of chloroform. 3ml of ice-cold acetic anhydride were added to the mixture in the flask and 2 drops of concentrated H₂SO₄ were added to cool. Standard sterol solution was prepared and spectrophotometer at 420nm was used to measure the absorbance.

Antimicrobial Screening

The crude ethanolic extract of the fresh leaves was screened for antimicrobial activities against clinically isolates using Agar diffusion steak methods. The test organisms were prepared by sub culturing them overnight in culture media and incubated in a freshly prepared nutrient broth at 37°C for 3 hours. 100mg of the test extract was dissolved into 1ml to give a concentration of 100mg/ml of the extract. Clinically isolated bacteria (*E. coli*, *Klebsiella spp*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) were inoculated using steak method. 1ml of prepared extract was then introduced into 5mm hole in a solidified inoculated agar, bored with a cork borer and was placed at 54⁰C. Gentamycin, a broad-spectrum antibiotic was used as standard.

Result and Discussion

The presence of alkaloids, tannin, steroid and flavonoid in the plant as shown in Table1 may be collectively or individually responsible for the observed antimicrobial activities. This result also corresponds with the results of phytochemicals of plants and fruit common in the region (Onyeka, and Uwambeke, 2007).

Table 2 shows the result of the antimicrobial potency of crude ethanolic extract of *Peritrophe bicalyculata* against some selected microorganisms. The diameters of the zone of inhibition of this extract were compared

with Gentamycin. The effect of the ethanolic extract of the *Peritrophe bicalyculata* was strongly effective against the test bacteria: *Staphylococcus aureus*, *Klebsiella spp.*, *E. coli*, and *Pseudomonas aeruginosa*, to be 2.1cm, 1.9cm, 2.0cm, 2.1cm zones of inhibition at concentration of 100mg/ml respectively. The fungi: *Aspergillus niger*, *Aspergillus clavatus*, *Rhizopus stolonifer* also recorded 1.8cm, 1.5cm and 2.2cm (zone of inhibition) respectively. The results obtained compared favourably with gentamycin used as control antibiotics (Table 2).

The MIC of the extract from the *Peritrophe bicalyculata* were 90g/ml, 90g/ml, 60g/ml and 60g/ml for *Staphylococcus aureus*, *Klebsiella spp.*, *E. coli*, and *Pseudomonas aeruginosa*, respectively and 60g/ml, 90g/ml, 60g/ml for the fungi isolates; *Aspergillus niger*, *Aspergillus clavatu* and *Rhizopus stolonifer* respectively. The varied minimum inhibitory concentration revealed that the extract is highly effective against *E. coli*, *Pseudomonas aeruginosa*, *Asperillus niger* and *Rhizopus stolonifer* at the concentration of 60g/ml while an increase in concentration to 90g/ml is required to produce noticeable effect on *Staphylococcus aureus*, *Klebsiella spp* and *Aspergillus clavatus*.

This suggest that the species can be gainfully employed in the production of antibiotics as the low MICs means that only a small quantity of extract will be required to inhibit the organisms. The antimicrobial activities shown by *Peritrophe bicalyculata* is in line with the previous antimicrobial works on different plants analyzed in the region (Onyeka, and Uwambeke, 2007).

There is a need for further study to ascertain if the yield in this species will be increased by using stronger fractionating solvent such as ethyl acetone or methyl acetone. These solvents have been reported to be more vigorous than other solvents used in crude extraction of plants (Ajayeioba and Fadare, 2006). Efforts should also be made to quantify the identified phytochemicals.

In conclusion, the results from this study have shown the potency of the plant crude extract on the tested microorganisms, which is indication of the medicinal value of the plant extract. The extract

compared favourably well with gentamycin that was used as control.

TABLE 1. Phytochemicals of crude extract of *Peritrophe bicalyculata*.

Secondary metabolite	<i>Peritrophe bicalyculata</i>
Alkaloid	+
Saponin	+
Tannin	+
Steroid	+
Flavonoid	+

+ = Presence of secondary metabolite - = Absence of secondary metabolite

TABLE 2. Minimum inhibitory concentration (MIC) of ethanolic extracts *peritrophe bicalyculata* on selected test isolates in (mg/ml) and the zone of inhibition measure in centimeter.

Extract (mg/ml) of <i>Peritrophe bicalyculata</i>	Zones of inhibition (cm)						
	<i>Staphylococcus aureus</i>	Klebsiella Spp	E.coli	<i>P.aeruginosa</i>	<i>A. niger</i>	<i>A. clavatus</i>	<i>R. stolonifer</i>
100	2.1	1.9	2.0	2.1	1.8	1.5	2.2
90	1.4	1.1	1.4	1.5	1.1	1.1	2.0
60	0.0	0.0	0.6	0.4	0.3	0.0	0.3
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gentamycin (positive control)	1.8	1.9	1.3	1.1	1.5	2.0	2.0

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