Preliminary Study of Phytochemical Content and Antimicrobial activities of *Annona senegalens*

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Abstract

Annona senegalensis is one of the widely distributed plants used in folkloric management of various ailments in Nigeria. The present study examined the phytochemical content and antimicrobial activity of the leaf extract of this important plant. The phytochemical screening was done using conventioal methods whereas, the antimicrobial activity was determined by agar well diffusion technique. The results indicated that anthraquinone and glycosides were absent while alkaloid, saponin, tannin, flavonoids and terpenoid were present in the *Annona senegalens* leaf extract. At the highest concentration used (100 mg/ml), *S. aureus* showed the widest zone of inhibition of 22.00 mm, followed closely by *E. coli* (20.33 mm) while *C. albicans* recorded the least zone of 15.33 mm against ethanol extract of the *Annona senegalens* plant extract. Furthermore, the activities of the extract was concentration dependent, as higher concentrations gave wider zones of inhibition. These results corroborate the folkloric use of the plant as a remedy for various microbial diseases.

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Keywords: antimicrobial, *Annona senegalens*, extract, traditional medicine

Introduction

A medicinal plant has been described as any plant which one or more of its organs contain substances that can be used for therapeutic purposes or serve as precursor for the synthesis of useful drugs. They possess biologically active chemical substances (phytochemicals) such as saponins, tannins, essential oils, flavonoids, alkaloids, and other compounds which have preventive or curative properties. These complex chemical substances generally occur as secondary plant metabolites in these plants and are useful to humanity [1].

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Higher and aromatic plants have traditionally been used in folk medicine as well as in the extension of the shelf life of foods in the case of those with antimicrobial activity. All over the world, hundreds of plants have been identified as good sources of medicinal agents and are used in traditional medicine for different purposes, including the treatment of bacterial and fungal infections [2].

Ethnopharmacological uses of plants feature strongly among Nigerian peoples. It has been pointed out, that plants continue to play a prominent role in primary health-care of about 80% of the world's population. Over the years, there have been alarming reports of multiple drug resistance in medically important strains of bacteria and fungi [3].

The persistent increase in the incidence of antibiotic resistant strains of organisms have led to the development of more potent antibiotic such as the 3rd and 4th generation cephalosporins by pharmaceutical companies [4]. It has long been recognized that some plant materials exhibit antimicrobial properties. In particular, the inhibitory effects of extracts of many kinds of herbs and spices against food-borne bacteria and other pathogens have been reported; among these are cassia, clove, garlic, sage and thyme [5]. However, despite the abundance of such flora detail, analytical data are available only from a few plants (both native and exotic species).

Annona senegalensis is a shrub or small tree with bark smooth to rough, silvery grey or grey-brown, with leaf scars and roughly circular flakes exposing paler patches of under bark. This species are found in semi-arid to subhumid all over regions Africa. They occur along riverbanks, fallow land, swamp forests and at the coast. And it commonly grows as a single plant in the understorey of savannah woodlands [6]. The bark is used for treating guinea worms and other worms, diarrhoea, gastroenteritis, snakebite, toothache and respiratory infections. Gum from the bark is used in sealing cuts and wounds. The leaves are used for treating pneumonia and as a tonic to promote general well being. The roots are used for stomach-ache, venereal diseases, chest colds and dizziness [7]. There is dearth of information on the antimicrobial activity of this plant and this work was designed to assess the phytochemical constituent and antimicrobial activity of the *Annona senegalens* leaf extract.

MATERIALS AND METHODS

Collection and preparation of plant samples

The fresh *Annona senegalens* leaves were sourced from the wild at Iyere village, Owo local government, Ondo state. The plant materials were then authenticated at the Environmental Biology Unit of Science Laboratory Technology Departtment, Rufus Giwa Polytechnic, Owo and voucher specimens (Aas701L) was deposited at the Herbarium Section of the Department. Thereafter, the plant materials were washed thoroughly in distilled water and air dried for three weeks in the laboratory. The dried samples were then ground into powder with the aid of a

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mechanical grinder and stored in clean airtight containers and kept in a cool, dry place until required for use

Extraction procedure

One hundred gram (100g) portion of the powdered samples was soaked in 300ml of solvent (ethanol) for 48hrs with intermittent stirring using a sterile spatula. Thereafter, extracts were filtered through filter paper into sterile containers and then dried using a rotary evaporator at 50^0C. Different concentrations of the extracts were prepared by diluting 0.20 g, 0.50 g and 1.0 g of the extracts in 100ml of 0.01% Tween-20 to obtain concentrations of 20mg/ml, 50mg/ml and 100mg/ml respectively [8].

Collections of test microorganisms

The microorganisms employed in the study were *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Candida albicans;* and were obtained from Federal Medical Center, Owo.

Qualitative phytochemical screening

All the tests described below were carried out on the extracts using the methods described by Opawale [8] and Sofowora [9].

Test for tannins. 1 ml of the extract was boiled in 10 ml of water and then filtered. Observation of green color on the addition of drops of 0.1% ferric chloride ($FeCl_3$) confirms the presence of tannin.

Test for saponins. One portion of the extract was boiled in four-part of water (1:4 v/v) followed by filtration. The filtrate was diluted with a little water and shaken for froth formation; olive oil was added to the mix and shaken continuously for 3 minutes. The appearance of emulsion confirmed the presence of saponins.

Test for flavonoids. 5 ml of the extract was mixed with 3 ml of 1% aluminum chloride ($AlCl_3$). A portion of 5 ml of dilute ammonia and concentrated H_2SO_4 solutions were added sequentially. The disappearance of yellow coloration indicated the presence of flavonoids.

Test for terpenoids (Salkowski test). 2 ml of chloroform were mixed with a 5 ml portion of the extract after which concentrated H_2SO_4 solution was gently added. Presence of terpenoids was confirmed with the appearance of a red-brown color at the interface.

Test for glycosides. A portion of 3 ml of the plant extract was treated with 1 ml of glacial acetic acid containing one drop of ferric chloride followed by addition of concentrated H_2SO_4 solution. The formation of violet-green layer underneath was taken as the positive test for the presence of glycoside.

Test for alkaloids. A 1:5 (v/v) mixture of the extract and 1% aqueous HCl was heated in the water bath and then filtered hot. The filtrate was diluted with water and 4 drops of Mayer's reagent were added. Conclusions were drawn based on color change.

Test for anthraquinone. The extract and benzene were mixed in a ratio of 1:2 (v/v), and then 10% NH_3 was added to the filtrate. The appearance of violet color after shaking the mixture was taken as positive for the presence of anthraquinones.

Media preparation

Malt extract broth, Malt extract agar, Mueller Hinton agar, and Mueller Hinton broth were separately prepared according to the manufacturer's specification in sterile conical flasks. The mixtures were homogenized on a hot plate with a magnetic stirrer (Eka 200P) until a uniform solution was obtained. Thereafter, all the culture media were autoclaved at 121 ^{0}C for 15 minutes and allowed to cool to about 50 ºC before use.

Standardization of microbial culture

A loopful of test organism was aseptically inoculated into Mueller Hinton broth (MHB) and incubated for 24 h at 37 ^{o}C. A portion of 0.2 ml from the 24 h culture of the organisms was dispensed into 20 ml sterile Muller Hinton broth and incubated for 3-5 h to standardize the culture to 0.5 McFarland standards (1.0 x10^6 cfu/ml) before use according to the method of [10].

***In vitro* antimicrobial susceptibility test**

The extracts obtained from the test plants were screened against the test organisms by agar well diffusion method [10]. A 25ml aliquot of Mueller-Hinton agar (bacteria) and Malt extract agar (yeast) was poured into each Petri plate. When the agar solidified, the pathogenic test organisms were inoculated on the surface the plates (1×10^6 *cfu*/ml) using a sterile glass spreader and allowed to sink properly. Subsequently, the surface of the agar was punched with 6mm diameter cork borer into wells and a portion of 50µl of each of the extract concentrations was filled into the wells. Control wells containing the same volume of Chloramphenicol and ketoconazole were used as positive controls for the bacterial and yeast plates respectively and the plates were

incubated at 37 ^{0}C for 24 h [8]. Each experiment was carried out in triplicate and the diameter of the zones of inhibition was then measured in millimeters.

Data Analysis

Data were presented as mean standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version 17.0 software. The significance was determined at the level of $p< 0.05$.

RESULTS AND DISCUSSIONS

The results of the phytochemical screening of the plant presented in Table 1 revealed that anthraquinone and glycosides were absent while alkaloid, saponin, tannin, flavonoids and terpenoid were present in the *Annona senegalens* leaf.

Phytochemicals have been described as natural bioactive substances formed by plants as secondary metabolites that protects against pathogenic attacks. Phytochemicals are the most abundantly distributed substances in the plant kingdom and many plants cells produce these active substances. Cheeke [11] submitted that some of the phytochemicals have great medicinal functions which play major roles in the new drug development process.

Table 1: Phytochemical profile of Ethanol Extract of *Annona senegalens*

Phytochemical	Status
Alkaloids	+
Saponins	+
Tannins	+
Anthraquinones	-
Glycosides	-
Flavonoids	+
Terpenoids	+

Key: + = presence, - = absence

The presence of alkaloids, saponins, tannins, flavonoids and terpenoids in *Annona senegalens* leaf in this present study is in consonance with the reports of earlier researchers [12]. The presence of these secondary metabolites in the plant suggests that the plant may possess pharmacological properties.

Alkaloids are believed to be one of the most effective and therapeutically significant plant substances [13], they are reported to be pharmacologically active and their actions are felt in different parts of the body system such as the nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases and malaria [14]. This may explain the use of the plant in treating gastrointestinal discomfort. Alkaloids have analgesic, antiplasmodial and bactericidal effects [15] as well as marked physiological effects on animals [16].

Saponins have hypotensive and cardiac depressant properties according to Olaleye [17] and have been shown to possess beneficial properties by lowering the cholesterol level, have anti-diabetic and anticarcinogenic properties as well as being used as an expectorant and emulsifying agent. The presence of saponins in the leaf of *Annona senegalens* implies that it may be useful in the treatment of diabetes and management of heart conditions. The presence of tannins in the plant could be a sign of that the plant may be useful in the management of infectious diseases. Tannins are known to posess antimicrobial activity [18].

Terpenoids were found in the plant leaf and they reportedly possess anti- hepatoxic properties, thus helping to prevent liver damage (cirrhosis); they equally have anti-microbial or anti-septic properties [19]. This suggests that the plant may be useful in the management of liver problems. The array of phytochemicals present in the *Annona senegalens* leaf in this current study indicate that the plant may offer useful alternative in the management of different conditions in man.

Table 2: Antimicrobial Activity of Ethanol Extract of *Annona senegalens ascalonicum* against Selected Pathogens

Sample	*S. aureus*	*Bacillus spp*	*E. coli*	*K. pneumoniae*	*C. albicans*
20 mg/ml	11.33 ± 0.15^{a}	12.67 ± 0.03^{a}	10.33 ± 0.04^{a}	13.33 ± 0.58^{a}	10.33 ± 0.01^{a}
50 mg/ml	15.67 ± 0.01^{b}	15.33 ± 0.05^{b}	17.33 ± 0.00^{b}	16.33 ± 0.07^{b}	12.67 ± 0.01^{b}

100 mg/ml	22.00±0.07[c]	18.67±0.20[c]	20.33±0.02[c]	19.33±0.02[c]	15.33±0.06[c]
Control	34.33±0.58[d]	25.33±0.25[d]	32.33±1.00[d]	29.50±0.58[d]	21.00±0.07[d]

The antimicrobial tests showed varied activity of the *Annona senegalens* plant as presented in Table 2. At the highest concentration used (100 mg/ml), *S. aureus* showed the widest zone of inhibition of 22.00 mm, followed closely by *E. coli* (20.33 mm) while *C. albicans* recorded the least zone of 15.33 mm against ethanol extract of the *Annona senegalens* plant extract. Furthermore, the activities of the extract was concentration dependent, as higher concentrations gave wider zones of inhibition.

The plant recorded a wide spectrum of activity against the both Gram positive and Gram negative organisms. Both *E. coli* and *K. pneumoniae* (Gram negative bacteria) as well as *S. aureus* and *Bacillus* spp (Gram positives) showed similar range of zone of inhibition to the extracts at all the concentrations used. This is in line with the previous reports on the some plants used as remedies in African traditional medicine [20, 21]. Further, all the bacterial test isolates were susceptible with comparable zones of inhibition suggesting a possible wide spectrum of activity against different bacteria species while also having a promising activity against the test yeast. This may be connected to the types of the phytochemicals found in the plant extract. Antimicrobial activities of plant materials have been linked with the quality and quantity of the phytochemicals that are present in plant material.

Moreover, the antimicrobial activity of the plant material follow a concentration dependent pattern as wider zones of inhibition were observed at higher concentration of the extracts. Compared with the control, the extracts had significant activity against the test organisms. The high activity of the plant extract against the test organisms suggest that the plant

materials may contain precious antimicrobial agents which may be utilized for development of new antimicrobial agents.

The susceptibility of *S. aureus, Bacillus* spp., *E. coli and K. pneumoniae* were comparable at all the concentration suggesting that *Annona senegalens* may be active against both Gram negative and Gram positive bacteria. There are reports that *Annona senegalens* species possess phytochemicals such as alkaloid, tannin, saponin, steroids, terpenes and glycosides, some of these are known to exhibit inhibitory activity on microorganisms [22]. The difference in the antifungal activity recorded and those reported for other spices may be due to the differences in phytochemical constituents, time of harvest and the difference in the geographical location of the plants.

Conclusion

The results of this study revealed the presence of bioactive secondary metabolites in the *Annona senegalens* leaves and it possess a promising broad spectrum antimicrobial activity against selected bacteria species which might explain its use in folklore in the management of different disease conditions.

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