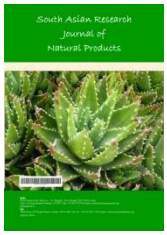

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Preliminary Study of Phytochemical Content and Antimicrobial activities of *Annona senegalensis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Annona senegalensis is one of the widely distributed plants used in folklore for the management of various ailments in Nigeria. The present study examined the phytochemical content and antimicrobial activity of the leaf nethanolic 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 senegalensis* leaf extract. At the highest concentration (100 mg/ml); *Annona senegalensis* leaf ethanolic extract inhibited the growth of *Staphylococcus aureus* (zone of inhibition was 22.00 mm), followed by *Escherichia coli* (zone of inhibition was 20.33 mm) and lastly *Candida albicans* (zone of inhibition was 15.33 mm). Furthermore, the activities of the extract were 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.

Keywords: Antimicrobial; Annona senegalensis; extract; folkore; phytochemicals.

1. INTRODUCTION

A medicinal plants are those plants whose any of its part contain substances particularly secondary metabolites that may be utilized for therapeutic purposes or that can serve as antecedent for the creation of useful drugs. They possess bio-active chemical materials usually refere to secondary metabolites which possess ability to prevent or cure diseases particularly, the ones caused by

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infectious agents. The secondary metabolites are called phytochemicals which are formed by plants to protect them against diseases and predation. However, they have been found to be extremely useful in managing various human diseases [1].

Plants of the tropical rain forests have been used in folklore for medicinal purposes and for the preservation of foods since many of them contain inhibitory properties against microorganisms. In various parts of the world, many plants are known to possess medicinal values and they are employed in tradomedical practices for the treatment of infectious diseases among other uses [2].

The use of plants in traditional medicine is a widely spread practice among the various ethnic groups in Nigeria. It has been reported that majority of the world's population utilize plants and their products for their health-care management. In the past decades, there have been reports of increase in the resistance of bacteria and fungi of medical importance to conventional antimicrobial drugs leading to high cost of managing infectious diseases [3].

The pharmaceutical industry has responded to the constant increase in the incidence of antibiotic-resistant strains of microorganisms by developing more effective drugs which has its own drawback of increased toxicity to human cells [4]. However, the antimicrobial activities of many plants have been documented. Albeit, despite the great quantity of such information on the types of plants, detail, analytical data are available only for a few plants.

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. It commonly grows as a single plant in the understorey of savannah woodlands [5,6]. 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.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Samples

The fresh *Annona senegalensis* 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 Department, 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 mechanical grinder and were stored in clean air-tight containers, and kept in a cool, dry place until required for use.

2.2 Extraction Procedure

One hundred gram (100 g) portion of the powdered samples was soaked in 300ml of solvent (Absolute ethanol) for 48hrs with intermittent stirring using sterile spatula. Thereafter, extracts were filtered through filter paper into sterile containers and then dried using 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].

2.3 Collection of Test Organisms

The test isolates used in this investigation included *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Candida albicans;* and were obtained from the Federal Medical Center, Owo.

2.4 Phytochemical Assays

The tests for screening of phytochemical presence were done using the methods described by Opawale et al. [8] and Sofowora [9].

Tannin Assay: About 1 ml of the extract was brought to boil and then filtered followed by the addition of 0.1% ferric chloride ($FeCl_3$) solution

drops. Green colour appearance shows that tannin is present.

Saponin Assay: A portion of 4 ml of distilled water was added to 1 ml of the extract, then brought to boil which was followed by filtration. About 2 ml of distilled water was added to the filtrate and then agitated vigorously, upon formation of foam, few drops of olive oil added to the mixture with continuous agitation for few minutes. Saponin was confirmed by manifestation of emulsion.

Flavonoid Assay: About 3 ml of 1% aluminum chloride ($AlCl_3$) was added to 5 ml of the plant extract. Then, 5 ml dilute ammonia was added to the mixture followed by 5 ml of concentrated sulphuric acid H_2SO_4. The vanishing of yellow colour reveal positive test of flavonoid.

Terpenoid Assay: To 5 ml of the extract, 2 ml of chloroform was added, then some conc H_2SO_4 solution was added lightly. A development of red-brown colour at the border shows that terpenoid was present.

Glycoside Assay: About 1 ml of glacial acetic acid containing a drop of ferric chloride was added to 3 ml of the extract, then concentrated H_2SO_4 solution was added. Violet-green phase beneath the mixture ring confirms glycoside presence.

Alkaloid Assay: A portion of 1 ml of the extract was mixed with 5 ml of 1 % aqueous HCl, subjected to heating in waterbath and filtered when hot. Distilled water was added to the hot filtrate and few drops of Mayer's solution were added to the mixture. Color change signifies presence of alkaloid.

Anthraquinones Assay: One ml of the extract was mixed with two ml of benzene, then filtered followed by addition of 10 % ammonia (NH_3) to the filtrate. Violet coloration on shaking shows positive result.

2.5 Media Preparation

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

2.6 Test Culture Preparationn

A freshly prepared Nutrient broth was inoculated with a loopful of the organism aseptically and then incubated for 24 h at 37 ^{o}C. About 0.2 ml from the young culture of the test isolate was introduced into 20 ml sterilized Nutrient broth and incubated for about 3 h for standardization of the suspension to 0.5 McFarland standard (10^6 cfu/ml) before use based on the method described by Akinnibosun and Oyetayo [10].

2.7 *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 25 ml aliquot of Nutrient agar (bacteria) and Malt extract agar (yeast) was poured into each dish. After solidification, the test isolates were inoculated on the agar surface (1×10^6 *cfu*/ml) using a sterilized bend glass rod and then allowed to seed for some time. Thereafter, holes were bored into the inoculated plates using cork borer while a volume of 50 µl of the different concentrations of the extract was introduced into different holes. Control pores which contain similar amount of Chloramphenicol and ketoconazole were used as positive controls for bacterial and yeast dishes accordingly and the inoculated dishes were incubated at 37 ^{0}C for 24 h [8]. All the trials were duplicated while the inhibitory zones were measured and recorded in millimeters.

2.8 Data Analysis

Data are shown as mean ± standard error of mean. Significant differences within various groups was assessed by two-way analysis of variance while the comparison of treatment means was done using Duncan's New Multiple Range Test with SPSS package window 7 version 25.0 software. The significant level was taken at 95 % confidence level.

3. RESULTS AND DISCUSSION

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 senegalensis* leaf.

Phytochemicals have been described as natural bioactive substances formed by several plants to protect them against pathogenic attacks. Phytochemicals are the most abundantly circulated materials in plants and many plants cells secrets these bio-active substances. Cheeke [11] submitted that some of these secondary metabolites have great medical functionality which plays key task in prospecting for new drugs and development of various biomedical products.

Table 1. Phytochemical profile of ethanol extract of *Annona senegalensis*

Phytochemical	Status
Alkaloid	Present
Saponin	Present
Tannin	Present
Anthraquinones	Absent
Glycoside	Absent
Flavonoid	Present
Terpenoid	Present

The presence of alkaloids, saponins, tannins, flavonoids and terpenoids in *Annona senegalensis* leaf in this current research is in agreement with those reported by other investigators [12]. The availability of these phytochemicals in the plant material is indicative of possible pharmacological properties.

Generally, alkaloids are known to be among the most potent substance produced by plant which is used for management of diseases [13], evidence from literatures suggests that alkaloids are active and they exact various effects on many organs and systems of the body like the digestive system, circulatory system, excretory system and homeostasis as well as in malaria management [14]. This supports the folklore use of *A. senegalensis* in treating gastrointestinal discomfort. There are reports of pain releif, plasmodial inhibition and antimicrobial activities by alkaloids [15], their effects on the physiological processes in higher animals have been documented as well [16].

The ability of saponins to reduce the blood pressure and protect heart tissues have been reported by Olaleye [17] and they are also reported to have antidiabetic property, cholesterol reducing ability and antimitotic activity. They are also used in managing cough and as emulsifying agent. The presence of saponins in the *Annona senegalensis* leaf extract implies that it may be useful in the management of diabetic condition and cardiac issues. The occurrence of tannins in the plant could be a sign of that the plant extract might be relevant in treating diseases caused by infectious organisms. Tannin is known to posess inhibitory activity against microorganisms [18].

Terpenoids were found in the plant leaf and they have been found to have liver cell protective function thereby serving in preventing hepatic degeneration. Also, they reportedly possess antimicrobial functions [19]. This implies that the plant extract might be of use in handling of liver diseases. The array of phytochemicals present in the *Annona senegalensis* leaf in this current study signify that the plant extract might offer better alternative in the handling of different conditions in man.

The antimicrobial tests showed varied activity of the *Annona senegalensis* 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 senegalensis* plant extract. Furthermore, the activity of the plant extract were dependent on concentration, since 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 inhibitory action of Annona material follow a dose/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

Table 2. Inhibitory activity of *Annona senegalensis* extract on 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]

Legend: values are Mean±S.E, those followed by different superscripts along columns are significantly different at P<0.05

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 senegalensis* may be active against both Gram negative and Gram positive bacteria. There are reports that *Annona senegalensis* 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.

4. CONCLUSIONS

These results exposed the presence of bioactive secondary metabolites in the *Annona senegalensis* leaves and it possesses a promising broad spectrum antimicrobial activity against selected bacteria species which might explain its use in folklore in the management of different disease conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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