



Crude Flavonoids Isolated from the Stem Bark of *Annona senegalensis* have Antimicrobial Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSJ designed the study, wrote the protocol and the first draft of the manuscript. Author WAU managed the analyses of the study. Author AOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at determining the *in vitro* antimicrobial activity of crude flavonoids isolated from the stem bark of *Annona senegalensis*. The study was carried out in the laboratory of Biochemistry department, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria, between July and October, 2013. The antimicrobial activity was carried out using agar well diffusion method. Phytochemical screening of the methanolic stem bark extract of *Annona senegalensis* revealed the presence of steroids, saponins, anthraquinones, flavonoids, tannins and cardiac glycosides. Crude flavonoid of 1.956g was found to be present in 10g of stem-bark extract of the plant. Streptomycin was used as standard which gave the zones of inhibition of 23mm against *Escherichia coli*, 26mm against *Salmonella typhi* and 28mm against *Shigella specie* while the isolated crude flavonoids from the stem bark of *Annona senegalensis* gave the zones of inhibition of 18mm against *Escherichia coli*, 25 mm against *Salmonella typhi* and 26 mm against *Shigella specie*. Crude flavonoids exhibited Minimum Inhibitory Concentration (MIC) against *Shigella specie* and *Escherichia coli* at 100mg/ml while against *Salmonella typhi* at 50mg/ml. In

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conclusion, the crude flavonoids isolated from the stem bark of *Annona senegalensis* have antimicrobial activity against the test organisms.

Keywords: Phytochemical; crude flavonoids; zone of inhibition and test organisms.

1. INTRODUCTION

Herbal medicine is the study and use of medicinal properties of plants. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts [1]. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids.

Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [2].

Annona senegalensis is a shrub or small tree 2-6m tall but may reach 11m under favourable conditions. It has a smooth bark to roughish, silvery grey or grey-brown with leaf scars and roughly circular flakes. Wild fruit trees of this species are found in semi-arid to sub-humid all over regions Africa. It grows on various soil types, it does well on coral rocks dominated by sandy loam soil. Review of documented literature showed that the plant is used for anticancer [3], antimalarial and cytotoxic [4], anticonvulsant [5], analgesic and anti-inflammatory [6].

Flavonoids or bioflavonoids the term is derived from the Latin word *flavus* meaning yellow, their colour in nature, are a class of plant secondary metabolites. Flavonoids were referred to as Vitamin P (probably because of the effect they had on the permeability of vascular capillaries) from the mid-1930s to early 50s, but the term has since fallen out of use [7]. Flavonoids are the most important plant pigments for flower coloration producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen

fixation and floral pigmentation. They may act as a chemical messenger or physiological regulator; they can also act as cell cycle inhibitors [8].

We present here the antimicrobial activity of the crude flavonoids isolated from the stem bark of *Annona senegalensis*.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh stem bark of *Annona senegalensis* was collected within Girei L.G.A., Yola Adamawa State, Nigeria. It was identified and authenticated in the department of forestry of the school of Agriculture and Agricultural Technology, Modibbo Adama University of Technology, Yola, Adamawa state, Nigeria.

2.2 Source of Test Organisms

The microorganisms were obtained from the department of Microbiology, Obafemi Awolowo University Ile-ife (OAU). The organisms are *Escherichia coli*, *Salmonella typhi* and *Shigella specie*. All the microorganisms are clinical isolates identified in the mentioned laboratory.

2.3 Sample Preparation

Fresh stem bark of *Annona senegalensis* was air dried at room temperature. The dried plant stem bark was pounded into fine powder and stored in a well labelled air tight polythene bag for further analysis.

2.4 Extract Preparation

Exactly 80g of the powdered sample was weighed and transferred into conical flask and extracted repeatedly with 825 ml of 80% methanol by maceration then allowed to stand 24 hours at room temperature. The suspension was filtered using a sterile whatman No. 1 filter paper to obtain the methanolic extract. Rotary evaporator was used to concentrate the extract. The concentrated extract obtained was weighed and stored in labelled air tight nylon until when it is required.

2.5 Phytochemical Screening

Phytochemical screening was carried out using the method adopted from [9,10]. This analysis determines the biologically active compounds that contribute to the flavour, colour and other characteristics of the stem bark.

2.6 Quantitative Determination of Crude Flavonoids

Quantitative determination of flavonoids from the stem bark of *Annona senegalensis* was carried out using the method adopted by Boham and Kocipai [11]. Fine powder (10g) of the stem bark of *A. senegalensis* was extracted repeatedly with 80% aqueous methanol (100ml) at room temperature. The whole solution was filtered through Whatman filter paper no.1. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed until a constant weight was obtained.

2.7 Extraction of Crude Flavonoids Using Partition Method

The crude flavonoids from the stem bark of *A. senegalensis* were extracted according to the procedure reported by Oyedapo and Amos [12] and Oyedapo et al. [13]. Exactly 10g of crude methanol extract was weighed and dissolved in 250mls of warm distilled water and filtered through wool. Exactly 250mls of hexane was added to the filtrate and shaken together then poured into the separating funnel forming two layers of lower hexane fraction and upper aqueous fraction. The hexane layer was collected into a dry clean container and more hexane (150mls) was added to the aqueous layer until the hexane layer becomes colourless. Exactly 145mls of ethyl acetate was added to the aqueous layer (exhaustively) until the ethyl acetate layer becomes colourless. The crude flavonoids obtained was concentrated using rotary evaporator and air dried to complete dryness at room temperature. The yield of the crude flavonoids was weighed and recorded.

2.8 Determination of Antimicrobial Activity of the Crude Flavonoids

The crude flavonoids were screened for antibacterial activity using the agar well diffusion method as described by Akinpelu [14]. With the aid of a sterile 1ml pipette, 0.2ml of the broth cultures of the test organism was added to 18mls of sterile molten sensitivity agar which had

already cooled down to 45°C. This was well mixed and poured into already labelled sterile Petri dish according to the test organism. The medium was then allowed to set with the aid of sterile cork borer; the required numbers of holes was bored into the medium. The wells were then filled up aseptically with the solution of the crude flavonoids using sterile Pasteur pipettes. Streptomycin was used as the standard antibacterial agent at a concentration of 1mg/ml. The plates were allowed to stand for about one hour on the bench to allow proper diffusion of the antibacterial agent into the medium and then incubated upright at 30°C for 24hrs and care was taken not to stockpile the plates. Clear zone of inhibition indicates the relative susceptibility of the bacteria to the extract. This was measured in millimetres (mm).

2.9 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the crude flavonoids was determined using the method of Russel and Furr [15]. Different concentrations of crude flavonoids were used which include 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.5625mg/ml. Exactly 2mls of the extract from each dilution was pipetted into sterile plate with the aid of sterile pipette and then mixed with 18mls molten Muller Hinton agar plate and allowed to set. The surface of the Muller Hinton agar plate was allowed to dry before streaking with overnight broth cultures of the bacterial isolates. The plates were then labelled accordingly and incubated at 37°C for 48hrs. They were later examined for the presence or absence of bacterial growth. The lowest concentration preventing growth was taken as the Minimum Inhibitory Concentration (MIC) of the extract.

3. RESULTS

Phytochemical screening of the methanolic stem bark extract of *Annona senegalensis* revealed the presence of steroids, saponins, anthraquinones, flavonoids, tannins and cardiac glycosides while alkaloids were absent. This is presented in Table 1.

Crude flavonoid of 1.956g was found to be present in 10g of stem bark extract of the plant.

Streptomycin was used as standard which gave the zones of inhibition of 23mm against *Escherichia coli*, 26mm against *Salmonella typhi*

and 28mm against *Shigella specie* while the isolated flavonoids from the stem-bark of *Annona senegalensis* gave the zones of inhibition of 18mm against *Escherichia coli*, 25 mm against *Salmonella typhi* and 26 mm against *Shigella specie*. This is shown in Table 2.

Table 1. Phytochemical constituents of stem bark of *Annona senegalensis*

Phytochemicals	Results
Flavonoids	+
Saponins	+
Antraquinones	+
Cardiac glycosides	+
Tannins	+
Steroids	+
Alkaloids	-

Key: + = Present- = Absent

Table 2. Antimicrobial activity of crude flavonoids from the stem bark of *Annona senegalensis* on test organisms

Test organisms	Streptomycin	Crude flavonoids
<i>Shigella specie</i>	28 mm	26 mm
<i>Salmonella typhi</i>	26 mm	25 mm
<i>Escherichia coli</i>	23 mm	18 mm

Crude flavonoids exhibited Minimum Inhibitory Concentration (MIC) against *Shigella specie* and *Escherichia coli* at 100mg/ml and against *Salmonella typhi* at 50mg/ml. This is presented in Table 3.

Table 3. MIC of crude flavonoids from the stem bark of *Annona senegalensis* on test organisms

Test organism	Concentrations			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
<i>Shigella specie</i>	I	NI	NI	NI
<i>Salmonella typhi</i>	I	I	NI	NI
<i>Escherichia coli</i>	I	NI	NI	NI

KEY: I = Inhibition NI = No Inhibition

4. DISCUSSION

The phytochemical screening of the stem bark extract of *Annona senegalensis* showed that it contains flavonoids, saponins, anthraquinones, Cardiac glycosides, Tannins and steroids. Bako et al. [16] reported that alkaloids were present in his study. The absent of alkaloids in this present study may be attributed to the method used to determine it, the area where the plant material

was obtained from or the solvent used. The plant decoction which contains these phytochemicals are responsible for the treatment of different ailment like sleeping sickness in Northern Nigeria [17] and chest pain, coughs, anaemia, urinary tract infections [18], diarrhoea, bloody stool, dysentery [19], arthritis, rheumatism [20], intestinal and guineaworms [21], venereal diseases [22] and leishmaniasis [23]. The presence of these phytochemicals in the stem bark of *Annona senegalensis* suggested that it could be a potential source of medicine for the treatment of the mentioned ailments.

Most phytochemicals have antioxidant activity and protect the body cells against oxidative damage and reduce the risk of developing certain type of cancer. Other phytochemicals which interfere with enzymes are protease inhibitors, terpenes (citrus fruits and cherries). Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls [24]. Saponins were reported to interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells [25].

Modern authorised physicians are increasing their use of pure flavonoids to treat many important common diseases, due to their proven ability to inhibit specific enzymes, to stimulate some hormones and neurotransmitters and to scavenge free radicals [26].

In vitro studies showed that flavonoids from other plants have anti-allergic, anti-inflammatory, antimicrobial, anti-cancer and anti-diarrhoea activities [26]. However, the crude flavonoids from the stem bark of *Annona senegalensis* could also have these effects mentioned. Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens; they have been proposed for use against fungal pathogens of man [27].

The antimicrobial activity of isolated flavonoids from the stem bark of *Annona senegalensis* on *Shigella specie*, *Salmonella typhi* and *Escherichia coli* showed a significant zone of inhibition as presented in Table 2. The crude flavonoids showed high degree of inhibition zone in *Shigella specie* followed by *Salmonella typhi* while *Escherichia coli* showed the lowest zone of inhibition. *Escherichia coli* was least inhibited because it has the ability to develop resistance against antibiotics than other microorganisms used in this study despite the fact that all the

microorganisms are gram negative. According to Havsteen [26], flavonoids have the ability to inhibit or kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease and destroy some pathogenic protozoans. Therefore, it is possible that the crude flavonoids from the stem bark of *Annona senegalensis* also exerted the same effect on the test organisms as mentioned.

The results obtained from the minimum inhibitory concentration as presented in Table 3, showed that flavonoids inhibited *Shigella specie* and *Escherichia coli* at a higher concentration than *Salmonella typhi*. The result showed that the higher the concentration of the flavonoids, the more effective it becomes. Clinically, the minimum inhibitory concentrations are not only used to determine the amount of antibiotics that the patient will receive but also the type of antibiotics to be used which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents [28].

5. CONCLUSION

In conclusion, the results obtained have shown that crude flavonoids from the stem bark of *Annona senegalensis* have antimicrobial effect on test organisms which are causative agents of diarrhoea. Hence, the crude flavonoids from the stem bark could be a source of synthetic drugs for diarrhoea and other diseases caused by these species of microorganism.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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