British Biotechnology Journal
4(11): 1175-1181, 2014
ISSN: 2231–2927

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In vitro Antimicrobial Activity of Crude Tannins Isolated from the Stem Bark of *Annona senegalensis*

Wurochekke Abdullahi Usman[1*], Mahmoud Suleiman Jada[1] and Romanus Moses Jideofor[1]

[1]Department of Biochemistry, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.

Authors' contributions

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

Article Information

DOI: 10.9734/BBJ/2014/11885
Editor(s):
(1) Giuliana Napolitano, Department of Biology (DB), University of Naples Federico II, Naples, Italy.
(2) Kuo-Kau Lee, epartment of Aquaculture, National Taiwan Ocean University, Taiwan.
Reviewers:
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(4) Anonymous, Xiangtan University, China.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=688&id=11&aid=6318

Original Research Article

Received 9th June 2014
Accepted 19th August 2014
Published 4th October 2014

ABSTRACT

The aim of this study is to determine the antimicrobial activity of crude tannins from the stem bark of *Annona senegalensis* on some diarrhoea causing bacteria strains. 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 disc diffusion method. The phytochemical

**Corresponding author: Email: wchekke@yahoo.co.uk;*

analysis revealed the presence of tannins, alkaloids, saponins, cardiac glycosides, steroids, tarpenoids and phenols. Quantitatively, crude tannins of 170.16mg were found to be present in 500mg of the extract which makes up a percentage composition of 34.03%. The extracted crude tannins were used to test for antimicrobial effect against *Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus* and were only effective on *Staphylococcus aureus* with 19mm as the inhibition zone using the concentration of 100mg/ml. The Minimum Inhibitory Concentration (MIC) of the extracted crude tannins was observed at 12.5mg/ml on *Staphylococcus aureus*. This suggests that the isolated crude tannins have an antistaphylococci activity and can be used to treat infections caused by the organism.

Keywords: Antimicrobial activity; Annona senegalensis; phytochemical analysis and Bacteria.

1. INTRODUCTION

Annona senegalensis Pers (*Annonaceae*) commonly known as “Wild Custard Apple” is a shrub or small tree widely distributed in Africa [1]. It has aromatic flowers which are used to flavour food. The ripe fruit is yellow in colour and has a sweet edible jelly with pleasant odour. It is wide spread in the Savannah area and near streams and enjoys great reputation for its immense medicinal value and hence, ethnomedicinal uses [2].

Annona senegalensis decoction is used in the treatment of sleeping sickness in Northern Nigeria [3] and in folkloric treatment of cancer [4], chest pain, coughs, anaemia, urinary tract infections [5], intestinal troubles, stomach ache [6], diarrhoea, bloody stool, dysentery [7], arthritis, rheumatism [8], intestinal and guinea worms [9], venereal diseases [10], head and body ache [11].

Many plants synthesize substances that are useful for the maintenance of health in humans and other animals. These can be classified into two broad groups which are primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines and pyrimidines of nucleic acid, chlorophylls etc., while secondary metabolite include tannins, alkaloids, terpenoids, phenols, steroids, saponins, flavonoids resins glycosides [12]. The quantitative and qualitative distribution of these metabolites differs from plants to plants and parts to parts [12]. About 11% are obtained from plants and a number of synthetic drugs are also obtained from natural precursors. Phytochemicals are known to possess antioxidant, antibacterial, antifungal, anti-diabetic, anti-inflammatory, anti-arthritic, and radio-protective activity, and due to these properties they are largely used for medicinal purpose [13].

Tannins are naturally occurring plant polyphenols and are widely distributed in the plant kingdom. Their main characteristic is that they bind and precipitate protein. Tannins act as a defence mechanism in plants against pathogens, herbivores and hostile environmental condition [14]. They are obtained from different parts of plants which are used industrially for leather processing; it has also been shown to possess potential antiviral, antibacterial and antiparasitic effects. In the past few years tannins have also been studied for their effects against cancer through different mechanisms [15].

Herbalists tend to use extracts from parts of plant, such as the roots or leaves but do not know the active ingredient responsible for the efficacy and this created a need to study each

component by its individual traits. This work is meant to identify the phytochemical components present in the Stem bark of *Annona senegalensis*, and the antimicrobial activity of its tannin content.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh stem bark of *Annona senegalensis* was collected within Specialist Hospital quaters Jimeta metropolis 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, Nigeria.

2.2 Microorganisms

The microorganisms used (*Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*) were obtained from the Laboratory of Microbiology Department, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria. All the microorganisms are clinical isolates identified in the mentioned laboratory.

2.3 Preparation of Extract

Exactly 50g of the powdered sample was weighed and transferred into 300ml of distilled water in a conical flask. This suspension was allowed to stand for 24 hours at 30ºC. The suspension was filtered using a sterile Whatman No. 1 filter paper. A rotary evaporator was used to concentrate the extract. The concentrated extract obtained was stored in a refrigerator in a screw-capped bottle until when it is required.

2.4 Phytochemical Analysis

Chemical test was carried out on the aqueous extract using the method described by Sofowora and Adewunmi [16], Trease and Evans [17].

2.5 Quantitative Determination of Crude Tannins

Quantity of tannins was determined using the method of Krishnaiah et al. [18]. The plant sample (0.5 g) was weighed into a 50ml plastic bottle. About 50 ml of distilled water was added and stirred for 1hr. The sample was filtered into a 50 ml volumetric flask and made up to mark. The filtered sample (5 ml) was then pipetted out into test tube and mixed with 2ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008M $K_4Fe(CN)_6.3H_2O$. The absorbance was measured with a spectrophotometer at 395 nm wavelength within 10min.

2.6 Extraction of Crude Tannins

The stem bark was pounded and 100mg of the pounded portion was dissolved with 1ml of concentrated HCl in methanol (1: 100, v/v) and put in a water bath at 30ºC for 30 minutes, which was shaken after every 5 minutes. The sample was centrifuged at 12000 g and the supernatant was collected. Extraction was repeated twice; the combined supernatants were filtered, evaporated to dryness. The extracted tannin was kept for the antimicrobial test [19].

2.7 Antimicrobial Activity Testing

The antimicrobial activity of extracted crude tannins was carried out according to the method used by Emeruwa [20]. Wells were made on the surface of 15ml nutrient agar plates previously streaked with test organisms using 1.0McFarland standard ($3*10^8$ organisms). The extracted crude tannins (0.2ml) were aseptically introduced into the wells made, 100mg of ciprofloxacin was used as positive control which was added into a separate well made. The plates were allowed to stand on the working bench for 30 minutes and were incubated for 24hours at 37ºC. The presence of inhibition zone was regarded as the presence of antimicrobial activity. The antimicrobial activity was expressed as the length of the diameter of the inhibition zone.

2.8 Minimum Inhibitory Concentration (MIC) Determination

MIC was determined by disc diffusion technique. In this method, discs containing different concentrations of the crude tannins extract were placed on the nutrient agar plates were streaked with test organisms and incubated at 37ºC for 24 hours. MIC was recorded as lowest concentration that inhibits the growth of test organisms [21].

3. RESULTS

The phytochemical screening of the stem bark of *Annona senegalensis* revealed the presence of tannins, alkaloids, saponins, cardiac glycosides, steroids, tarpenoids and phenols. Anthraquinones and flavonoids were absent. The result is summarized as shown in Table 1.

Quantitative test of crude tannins revealed that 500mg of the plant extract contains 170.16mg of crude tannins which is 34.03% of the total extract content.

The extracted crude tannins were used to test for antimicrobial effect against *Escherichia coli, Salmonella typhi,* and *Staphylococcus aureus.* The extracted crude tannins were only effective on *Staphylococcus aureus* with 19mm as the inhibition zone using the concentration of 100mg/ml (Table 2).

The Minimum Inhibitory Concentration (MIC) of the extracted crude tannins was observed at 12.5mg/ml on *Staphylococcus aureus* (Table 3).

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

Phytochemicals	Result
Tannins	+
Alkaloids	+
Saponins	+
Flavonoids	-
Cardiac Glycoside	+
Anthraquinones	-
Steroids	+
Tarpenoids	+
Phenols	+

Key: + = Present, - = Absent

Table 2. Antimicrobial activity of the crude tannins from the stem bark of *Annona senegalensis*

Microoganism	Inhibition Zone (mm)
Escherichia coli	NI
Salmonella typhi	NI
Staphylococcus aureus	19.00

Key: NI = No inhibition

Table 3. MIC of crude tannins from the stem bark of *Annona senegalensis* on *Staphylococcus aureus*

Organism	Concentrations (mg/ml)					
	50	25	12.5	6.25	3.0	1.5
Staphylococcus aureus	-	-	-	+	+	+

Keys: + = indicates growth and - = indicates no growth

4. DISCUSSION

Phytochemical analysis of the aqueous extract of the stem bark of *Annona senegalensis* revealed the presence of chemical constituents namely tannins, alkaloids, saponins, cardiac glycosides, steroids, tarpenoids and phenols. This result agrees with the findings of Cletus [22] but variations exist due to the presence of anthraquinones, and the absence of steroids and cardiac glycoside in his result. This may be as a result environmental factors which affect metabolic processes in plants. The presence of some of these phytochemical components like saponins, tannins and phenolic compounds have been attributed to the antibacterial activity of the crude drugs being used by traditional practioners [23]. The presence of these bioactive components in the crude drugs have been linked to their activities against disease causing microorganisms [24] and also offering the plants themselves protection against infection by pathogenic microorganisms [25].

This study showed that the isolated crude tannins from the stem bark of *Annona senegalensis* were effective on *Staphylococcus aureus* (Table 2). This observation may be attributed to the fact that *Staphylococcus aureus* was reported to absorb more tannin into their cell wall and aggregate its presence [26-27]. The ability of this absorption is due to the strong repulsive negative charge of lipopolysaccharide on the surfaces of gram-negative bacteria. Yoda et al. [28] reported that the binding of tannin to the peptidoglycan of a cell results in disruption of cell's function in osmotic protection of cell division and cell wall biosynthesis. However, it is possible that the crude tannins from the stem bark of *Annona senegalensis* acted via the same mechanism mentioned.

Tannin has been reported to have a high binding to iron and may work like a siderophore to chelate iron from the medium thereby making iron unavailable to the microorganism. Microorganisms growing under aerobic conditions need iron for a variety of functions including reduction of the ribonucleotide precursor of DNA and other essential purposes [29]. Tannin was also found to change membrane fluidity and cell morphology and to cause leakage of cytoplasmic material [26].

The minimum inhibitory concentration conducted on *Staphylococcus aureus* revealed 12.5mg/ml as the lowest concentration of tannin that prevents visible growth of the microorganism after overnight incubation with media. The MIC value is used clinically to

determine the concentration of antimicrobial agent needed by a patient and also the type of antibiotic that will be used to disrupt microbial growth, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

5. CONCLUSION

In conclusion, the crude tannins from the stem bark of *A. senenegalensis* have antimicrobial effect on *Staphylococcus aureus*. This study support the use of the plant by traditional medicine practitioners in the treatment of infectious diseases caused by the test organism such as diarrhoea.

ACKNOWLEDGEMENTS

The authors wish to extend their sincere appreciation to the department of Biochemistry of Modibbo Adama University of Technology for giving them the privilege to use their laboratory during the study. The authors are also indebted to the Chief Laboratory Technologist of the department for his assistance and valuable contributions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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