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IN-VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACT FROM THE STEM BARK OF *ANNONA SENEGALENSIS* PERS.

Ernest Penn Awa*, S. Ibrahim and D.A. Ameh

Department of Biochemistry, A.B.U., Zaria, Kaduna State- Nigeria

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Correspondence to Author:

Ernest Penn Awa

Department of Biochemistry, A.B.U., Zaria, Kaduna State- Nigeria

ABSTRACT

The antibacterial efficacy of the crude methanolic and aqueous extracts of *Annona senegalensis*, Pers on clinical isolates of *E. coli*, *Salmonella enteriditis* and *Shigella dysenteriae* were determined using Agar Well Diffusion technique on Mueller Hinton Agar (MHA) plates. At the concentration of between 100 and 200mg/ml, the methanolic extract inhibited the growth of all the organisms with an average zone of inhibition diameter ranging from 30±0.2 to 35±0.36mm for *E. coli* and *S. Enteriditis* while for *S. dysenteriae*, it ranged from 21±0.22 to 26±0.36mm. On the other hand, the aqueous extract did not exhibit activity on any of the test organisms. The Minimum Inhibitory Concentration (MIC) of the methanolic extract on the organisms ranged from 0.39mg/ml on *E. coli*, 3.17mg/ml on *S. enteriditis* 25.00mg/ml on *S. dysenteriae*. The result from this study suggests that the crude extract from the stem bark of *A. senegalensis* could be used in the treatment of gastroenteritis caused by these organisms.

INTRODUCTION: In recent times, the rapid development of multi-resistant bacterial strains of clinically important pathogens fetches the interest of scientists to develop newer broad spectrum antimicrobial agents [1]. The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in different traditional literatures [2, 3, 4]. This study is aimed to explore scientifically the antibacterial potential of *Annona senegalensis,* Pers and substantiate the folklore claims.

Annona senegalensis, Pers is a shrub that belongs to the family Annonaceae and is usually found growing in semi-arid to sub-humid regions of Africa. It is native and widely spread from Senegal to Nigeria, also Central African Republic. This specie is little-known outside its natural range [5]. A traditional food plant in Africa, its little-known fruit has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care. Ethno medically, the bark is used by local populations all over Africa in treating guinea worms, diarrhea and especially in northern Nigeria, gastroenteritis, snake bites, toothache and respiratory infections [6]. Gums from the bark are also used in sealing cuts and wounds.

The leaves are used in treating pneumonia, and as a tonic to promote general well-being. A decoction from the roots is used to stop stomachache, venereal diseases, chest colds and dizziness. In South Africa, the roots are also said to cure madness and in Mozambique, they are fed to small children to induce them to forget the breast milk and thus hasten weaning [7].

Various phytochemical compounds such as saponins, tannins, flavonoids, alkaloids, glycosides, phlobatannins, gums, essential oils, anthocyanins etc have been identified as the major contributors to the observed medicinal properties of this plant [8]. Saponins, tannins, alkaloids and glycosides have been shown to have antibacterial activity [9] and hence, the bark extract of this plant is used in treating infectious diarrhea.

Experimental studies have demonstrated the activity of *Annona senegalensis* against helminthes [10] and plasmodium [11]. However, documentation on the antibacterial activity of extracts of this plant against common diarrheal causing bacteria is scarce [12]. Consequently, this study was designed to ascertain the antibacterial effects of *Annona senegalensis* on clinical isolates of *Salmonella enteritidis, Escherichia coli* and *Shigella dysenteriae*.

MATERIALS AND METHODS:

Plant Material: A leaf of *Annona senegalensis*, Pers was initially collected from the plant in the bush around the Samaru District of Zaria, Kaduna state and identified in the herbarium unit of the Department of Biological Science, ABU Zaria, where a voucher specimen number 900167 was deposited. This was followed by the collection of the stem bark to be used in the study.

Test Organisms: The test organisms were clinical isolates of *E. coli, Salmonella enteritidis* and *Shigella dysenteriae.* They were obtained from the Microbiology Laboratory of the Ahmadu Bello University (ABU) Teaching Hospital, Shika, Zaria.

Chemicals and Solvents: All chemicals and solvents used in this study were of chemically analytical grade and in all cases, water used referred to distilled water. Unless otherwise stated, all chemicals used were products of BDH chemicals Ltd, Poole, England.

Preparation of Plant Material: The bark of the stem of the plant was washed with distilled water and air-dried for 21 days. The size was reduced using a knife and coarsely powdered using a mortar and pestle and then further reduced to powder using an electric blender (Philips). The powder was then sieved using sieve number 20 mesh (British Standard) to obtain a fine powder which is referred to hence forth as plant material. The plant material was kept in a closed jar until needed.

Preparation of the Crude Extracts: Nine hundred grams of the powdered stem bark of *Annona senegalensis*, Pers were weighed into a conical flask and defatted with 2.5 litres of petroleum ether and then extracted with 2 litres each of methanol and water successively for a period of 72 hours with shaking at 12 hours intervals. The various extracts obtained were filtered using Whartman No.1 filter paper. The filtrates were evaporated to dryness under reduced pressure at 70°C to obtain dried extracts (or residues) that were weighed to give 61.56g of methanolic extract and 38.42g of the aqueous extract. These were then labeled as Methanol Extract (ME) and Aqueous Extracts (AE) respectively. The extracts were stored in a refrigerator or used immediately.

Test for Antibacterial Activity: The antibacterial activity of each extract of the stem bark of *Annona senegalensis*, Pers was evaluated by the Agar Well Diffusion method on Mueller Hinton agar (MHA) plates [13]. In this method, 0.1ml of a 10^3 dilution of a 24 hour old broth culture of each test organism was uniformly spread on the surface of a sterile nutrient agar plate and allowed to dry. Three holes of 9mm diameter each were made on the inoculated plates with sterile cork borers (No.4) and were separately filled aseptically with 0.2ml of 100mg/ml, 150mg/ml and 200mg/ml of ME using a sterile micropipette and appropriately labeled. Holes filled with 0.2ml of equal concentrations of ciprofloxacin were used as controls. The plates were then incubated at 37°C for 24 hours and the diameter of zone of inhibition around each hole was measured and recorded.

Determination of Minimum Inhibitory Concentrations (MICs): Determination of MICs of the methanolic extract on the organisms was carried out using the tube dilution method as described by Kandekai-Olukemi *et al* (2004) with some modifications. Eleven tubes labeled 1-11 were selected. Each tube (1-10) contained 5ml of double strength Mueller-Hinton broth. Five (5) ml of the crude methanol extract was added to tube 1 and serially diluted to tube 10 to give final extract concentration of 50, 25, 12.5, 6.25, 3.17, 1.56, 0.78, 0.39, 0.19, 0.09mg/ml respectively. Tube 11

which contained no crude extract served as the control. After this, 0.05ml of 24 hour old culture containing 10^6 cfu/ml of a test organism previously diluted to 0.5 McFarland standards was each added to tubes 1-10. The tubes were then incubated at 37^0C for 24 hours after which the presence or absence of growth was noted. This was done by observing turbidity. The lowest concentration of extract that inhibited growth of the test organisms was noted as the MIC.

Determination of Minimum Bacteriocidal Concentrations (MBCs): To determine the MBCs of the methanolic extract, the tubes that showed no growth (turbidity) in the MIC determination were selected. One loopful of broth from each of these tubes was sub cultured on sterile Mueller-Hinton agar by streaking on medium. Similarly, Mueller-Hinton agar plates without the extracts were also streaked with the test organisms only to serve as control. They were then incubated at 37^oC for 24 hours. The lowest concentration at which no visible bacterial growth was observed on the petri dish was noted as the MBC of the extract as adopted by Kandakai-Olukemi *et al* (2004).

RESULTS AND DISCUSSION:

TABLE 1: RESULTS OF PRELIMINARY TEST FOR ANTIMICROBIAL ACTIVITIES OF THE STEM BARK METHANOLIC AND AQUEOUS EXTRACTS OF *A. SENEGALENSIS* AGAINST CLINICAL ISOLATES OF SOME GASTROENTERITIS CAUSING BACTERIA

Extract	Methanolic extract			Aqueous extract			Ciprofloxacin		
Concentration (mg/ml)	100	150	200	100	150	200	100	150	200
E. coli zone of inhibition diameter(mm)	30±0.24^a	35±0.36^a	35±0.45^a	GWI	GWI	GWI	50±0.36^c	47±0.51^b	50±0.49^b
S.enteritidis zone of inhibition diameter(mm)	30±0.36^a	32±0.22^c	35±0.36^a	GWI	GWI	GWI	45±0.39^d	50±0.22^b	50±1.96^b
S.dysenteraie zone of inhibition diameter(mm)	21±0.22^b	22±0.22^d	26±0.36^c	GWI	GWI	GWI	TI	TI	TI

Values with different superscripts across the rows and down the columns vary significantly (P < 0.05) with each other for each concentration in relation to the extract and organisms. GWI=Growth without Inhibition, TI=Total Inhibition.

TABLE 2: THE MINIMUM INHIBITORY CONCENTRATIONS OF STEM BARK METHANOLIC EXTRACT OF *A. SENEGALENSIS*, PERS ON CLINICAL ISOLATES OF SOME ORGANISMS CAUSING GASTROENTERITIS

Concentration (mg/ml)	Ciprofloxacin	50	25	12.5	6.25	3.17	1.56	0.78	0.39	0.19	0.09	Normal saline
E. coli	-	-	-	-	-	-	-	-	-	+	+	+
S. enteritidis	-	-	-	+	+	+	+	+	+	+	+	+
S. dysenteriae	-	-	+	+	+	+	+	+	+	+	+	+

Key: +=Turbidity; - =No Turbidity

TABLE III: MINIMUM BACTERIOCIDAL CONCENTRATIONS OF STEM BARK METHANOLIC EXTRACT OF *A. SENEGALENSIS*, PERS AGAINST CLINICAL ISOLATES OF SOME BACTERIA CAUSING GASTROENTERITIS

Concentration (mg/ml)	50	25	12.5	6.25	3.17	1.56	0.78	0.39	0.19	0.09
E. coli	-	-	-	-	-	-	-	-	-	+
S. enteriditis	-	-	-	-	-	+	+	+	+	+
S. dysenteriae	-	-	+	+	+	+	+	+	+	+

Key: += Growth; - = No Growth

Traditionally, *Annona senengalensis* stem bark had been used in the treatment of diarrhea, dysentery, respiratory diseases, snakebite, feminine barrenness, convulsions and fever in some parts of Africa. The use of the decoctions of the parts of this plant as anti-gastroenteritis drugs by some communities in Northern Nigeria provides the theoretical basis for carrying out this research project.

There fore the null hypothesis for the work is that "there is no scientific basis for the use of this plant extract to treat gastroenteritis". To test this hypothesis therefore, crude and partially purified extracts of the stem bark of this plant were tested on three gram negative bacteria mostly responsible for gastroenteritis in Northern Nigeria: viz *E. coli*, *Salmonella enteritidis* and *Shigella dysenteriae.*

From the results obtained, the amount of extracted materials was found to increase with polarity of the solvents except with diethyl ether and water. These findings may be due to the fact that the non-polar components might have been extracted by petroleum ether during the defatening of the powdered plant material. And this could be responsible for highest percentage recovery of the most polar and highest components by methanol.

The crude methanolic extract of the stem bark of this plant showed an appreciable anti bacterial activity against the test organisms compared to the standard drug ciprofloxacin but the aqueous extract aqueous extract showed no activity against all of the test organisms. The extract is however less active against *S. dysenteriae* compared to the other two test organisms. This is in line with many workers who had shown that plant methanol extracts inhibited the growth of tested bacteria than plant aqueous extracts [13]. This could also be attributed to the fact that different solvents have different polarities which could be responsible for the variation in the degrees of solubility of the various phytoconstituents [14].

The study shows that the highest activity was demonstrated by the methanolic extract of the plant against the gram negative bacteria tested. However, this is contrary to the findings of Abu-Shanab *et al* (2004) and Basri *et al* (2005) who had reported that plant extracts are usually more active against gram positive than gram negative bacteria. This may be due to the fact that the plant extracts might have provided antimicrobial components that overcame the permeability barrier provided by the cell wall or cell membrane of gram negative bacteria as asserted by Wei *et al* (2008). This demonstrates that *A. senegalensis* stem bark can be used to source antibiotic substances for the development of antibiotics with broad spectrum of activity.

The minimum inhibitory concentration (MIC) of the methanolic extract against the susceptible organisms was 0.39mg/ml for *E. coli*, 50mg/ml for *S. dysenteriae* and 25mg/ml for *S. enteritidis*. Although there is no agreement on the acceptable inhibition levels for plant materials, a classification based MIC results: strong, below 0.50mg/ml; moderate, between 0.60 to 1.50mg/ml and weak inhibitors above 1.60mg/ml was proposed by Aligianis *et al* (2001). Thus, the present study has established that the MIC of the extract was strong against *E. coli* (0.39mg/ml) and weak against *S. enteritidis* and *S. dysenteriae* with MICs of 25.00mg/ml and 50.00mg/ml respectively. It can therefore be concluded that the *A. senegalensis* extract could be considered as a potential antibacterial agent particularly on *E. coli*. This is further supported by Harami *et al* (2005), who reported that extracts of *A. senegalensis* exhibited good antibacterial activity against *E. coli*.

Like wise, the MBC of the extract also varied from 0.19mg/ml (*E. coli*) to 25mg/ml (*S. dysenteriae*) confirming the trend that *E. coli* isolate is more susceptible to the extract than the other clinical isolates. Therefore, the susceptibity of these enteric clinical isolates at these concentrations are quite significant and the plant extract could be an alternative source of antibiotics. Due to reported consequence of widespread and indiscriminate use of antibiotics during the past decade, resistant enterobacteria have evolved and weakened the anti microbial potential of many antibiotics as chemotherapeutic agents. A source like this could offer an alternative.

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