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IN-VITRO ANTIBACTERIAL ACTIVITY *OF ANOGEISSUS LEIOCARPUS* DC (STEM BARK) EXTRACTS AGAINST *ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS*

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ABSTRACT
Antibacterial secreening of ethanolic, aqueous and chloroform extracts of Anogeissus leiocarpus was carried out against Escherichia coli and Staphylococcus aureus. The result shows that all the extract exhibited antibacterial activity against the test organisms with the exception of the lowest concentration of aqueous and chloroform extract. Ethanolic extracts at concentration of 300ug/ml showed greater activity against E. coli (16mm) and Staphylococcus aureus (15mm) than the remaining extracts. Activity was greater against E.coli in response to all fractions (16mm, 15mm, and 12mm respectively at the highest concentrations than the Staphylococcus aureus (14mm, 11mm, and 9mm). Results showed that, antibacterial activity of the extracts was dose - dependant. Augmentin and gentamicine were the antibiotics used as the positive control discs against the test organisms. These antibiotics showed greater activity than the crude extracts respectively. The bioactive compounds detected in the extracts were alkaloids, tannins, flavonoids, glycosides and saponins. As there is growing interest in obtaining sample of plant materials with a view to explore the possibilities for medicinal products and in addition the current global upsurge of bacterial resistance to antibacterial drugs, the plant may be used as promising candidate for drug development.
Keywords: Antibacterial, Extracts, Concentration, Bioactivity, Inhibition. Anogeissus leiocarpus

INTRODUCTION

plants are used medicinally in different countries and are sources of many potent and powerful drugs (Kubmarawa *et al,* 2009). Most of the developing countries have adopted traditional medical practice as integral part of their culture. The use of plants and herbal products as major component of therapeutic agents and source of chemical diversity in drugs discovery programmes is no longer in doubt. Recently there is growing interest in obtaining samples of plant materials and or/ethno botanical uses of plants with a view to explore the possibilities for commercial medicinal products. This developed interest in the healing power of plants has been attributed to the current global upsurge of bacterial resistance to antimicrobial drugs (Sale, and Doughari, 2008). This has brought considerable progress in understanding the nature of valuable component in plants for the treatment of various diseases.

Anogeissus leiocarpus (DC), belong to the family combretaceae, its common name is Axle wood tree, is a tall evergreen tree native to savannah of tropical Africa**.** It is the sole West African species of the genus *Anogeissus*, a genus otherwise distributed from tropical central and East Africa through the tropical South East Asia. Axle wood tree has many applications in Nigeria. It is used medically for the treatment of ascaricides, gonorrhoea, general body pain, asthma, coughing (Mann, *et al;* 2008). Information obtained from the Yorubas and South Eastern people of Nigeria illustrate that the plant is also used as an antimicrobial agent against bacterial infections (Dweeck, 1996). The infusion and decoction are used as cough medicine, the powdered bark is also rubbed to reduce tooth ache on gums and the leaves decoction is used for washing and fumigation (Mann *et al,* 2008). In Nigeria it is also used as a chewing stick. This paper seeks to investigate antibacterial activity of the plant extracts against *E. coli* and *S. aureus.*

MATERIALS AND METHODS

SECREENING FOR ANTIMICROBIAL ACTIVITY

Processing of plant materials

The plant material was collected from Kaburma of Dawakin Tofa local government area of Kano state Nigeria. The plant was identified and confirmed in the herbarium of Forestry Department Audu Bako College of Agriculture, Thomas, Dambatta. The plant material used was stem bark. This was dried under shade and grounded into powder using clean mortar and pestle. The powdered material was also sieved and fine powder was obtained (Bukar *et al;* 2009).

Extraction method
Plant extracts were obtained by cold percolation method (Veeramuthu, 2006). Ethanol, water and chloroform were the solvents used in the extraction method. For all the solvents 50g of the dried plant materials were percolated in 500ml of the solvents in a 750ml capacity flask. The mixtures were agitated intermittently and kept for two weeks for ethanol and chloroform, while aqueous was kept for only one week. The mixtures were filtered through Whatman No.1 filter paper. The filtrate were evaporated to dryness using a water bath at 40°C for water and ethanolic extract, while chloroform extract was allowed to dry overnight at an ambient temperature. The residues were stored at 4°C for subsequent use.

Preparation of sensitivity disc
Whatman No.1 filter paper was punched using paper puncher of which discs of 6.0mm in diameter were obtained. These were placed in sterile capped bijou bottles and sterilized in a hot air oven at 160°C for one hour.

Preparation of concentrate for sensitivity test
Two gramme (2g) of each plant extract was dissolve in 2ml of appropriate diluents to arrive at 1.0g/ml (1000000ug/ml) which serves as stock solution (Shamsuddeen *et al;* 2008).
From the stock four concentrations were prepared. 0.3ml was added to 0.7ml of diluent, 0.2ml to 0.8ml, 0.1ml to 0.9ml, 0.01ml to 0.99 making 1ml each. 100 sterilized discs were placed into each bottles which gave disc potencies of 3000ug/disc, 2000ug/discs, 1000ug/discs, and 100ug/disc respectively

Test organisms
The test organisms used in the research were bacterial isolates obtained from Aminu Kano Teaching Hospital (AKTH). The isolates were *Escherichia coli* and *Staphylococcus aureus.* The organisms were confirmed in the microbiology lab of Biological Sciences Bayero University Kano. The stock culture were maintained on nutrient agar at 40°c in a refrigerator in accordance with procedure by Scott (1989).

Standardization of inoculum
Each culture of the isolates were standardized by culturing on nutrient agar for 24hrs at 37°C. The overnight culture were diluted in normal saline (0.5 w/v) until turbidity marched with 0.5 Macfarland standard to give a mean of $3.3*10^6$ Cfu/ml (Deeni and Hussain, 1991).

Antimicrobial susceptibility
Disc diffusion method was employed to screen for antimicrobial activity of the plant extracts. Muella Hilton agar plates were used for the inoculation of organism. The test organisms were streaked evenly on the surface of the agar plates with the use of sterilized wire loop. With the aid of sterile pair of forceps, impregnated paper discs containing the extracts of materials at different concentrations 3000µg/discs, 2000 µg /discs, 1000 µg /disc and 100 µg /disc were arranged radially and pressed slightly and firmly to the inoculated agar surface to ensure even contact. The plates were incubated at 35°C for 24hrs. The degree of sensitivity was determined by measuring the diameter in millimetre of the visible zone of inhibition of the microbial growth produced by the diffusion of the extracts (Stockes and Ridgeway 1980).

Phytochemical screening
Phytochemical screening of the extracts was carried out according to the methods of Abalaka *et al;* (2010) and Sofowora (1993). Active compounds tested were alkaloids, tannins saponins, flavonoids and glycosides.
Alkaloids : One ml of hydrochloric acid was added to 3ml extracts in a test tube, about two drops of Meyers reagent to one ml of the extracts was added, creamy and turbid precipitate was an indication of the presence of alkaloids.
Tannin : Ferric chloride was added to 1ml of the extract, presence of blue black and blue green precipitate indicated the presence of tannin
Saponins : this was carried out by frothing test, 2ml of the extracts was vigorously shaken in the test tube for two minutes, presence of frothing in the test tube indicated positive test.
Flavonoids : a piece of magnesium ribbon was added to 1ml of the extract followed by the addition of hydrochloric acid drop wise, a magenta colouration indicated the presence of flavonoids.
Glycosides : Ten ml of H_2SO_4 was added to 1ml of the extracts and the mixture was heated in boiling water for 15 minutes. 10ml Fehlings solution was added and the mixture boiled. A brick red precipitate was confirmatory for the presence of glycosides.

RESULTS
Table 1 shows the diameter of the zone of inhibition of the extract of the plant material. The result shows that all the extract exhibited antibacterial activity with the exception of the lowest concentration of aqueous and chloroform extract. Ethanolic extracts showed greater activity against *E. coli* (16mm) at 3000ug/ml and *Staphylococcus aureus* (15mm) at 3000ug/ml than the remaining fractions. Activity was greater against *E.coli* in all the fractions (16mm, 15mm, and 12mm) at the highest concentration than *Staphylococcus aureus* (14mm, 11mm, and 9mm).

Results shows that, antibacterial activity of the extracts was enhanced by an increase in the concentration of the extracts i.e the higher the concentration of the plant extracts the greater the zone of inhibition. Augmentin and gentamicine were the antibiotics used as the control discs against the test organisms. These antibiotics showed greater activity than the crude extracts 30mm and 27mm respectively. The bioactive compounds detected in all the extracts were alkaloid, tannin, flavonoids, glycosides and saponins.

Table 1: Zone of inhibition (mm) of ethanolic, aqueous and chloroform extracts of *Anogeissus leiocarpus* at various concentrations

		Concentrations (µg/disc)				
Extract	**organisms**	**3000**	**2000**	**1000**	**100**	***control**
Ethanolic	*Escherichia coli*	16	15	13	9	30
	Staphylococcus aureus	14	12	10	7	27
Aqueous	*Escherichia coli*	15	13	10	NI	30
	Staphylococcus aureus	11	9	8	NI	27
Chloroform	*Escherichia coli*	12	9	8	NI	30
	Staphylococcus aureus	9	8	NI	NI	27

Key: NI - No inhibition, ** Control Discs = Gentamycine (*E. coli*), Augmentin (*Staphylococcus aureus*)

Table 2: **Phytochemical constituents of ethanolic, aqueous and chloroforms extracts of the plant material**

	Extracts of the plant material		
Active compound	**ethanol**	**aqueous**	**chloroform**
Alkaloid	+	+	+
Tannin	+	+	-
Saponins	+	+	+
Flavonoids	+	+	-
Glycosides	+	+	+

Keys = + = Present, - = Absent

DISCUSSION

Results of the antimicrobial screening showed that all the extracts had exhibited antibacterial activity against the test organisms.. Ethanolic extracts and aqueous fractions showed greater activity than the chloroform extract, this may be due to better solubility of the active component in water and ethanol. Activity of the extracts of stem bark of *Anogeissus leiocarpus* against the test organisms justifies their traditional usage in ethno medicine. The activity showed by the plant extracts may be due to the presence of active compounds.

The zone of inhibition produced by the test organisms indicated the susceptibility to the plant extracts. It was observed that the zone of inhibition observed by the bacterial isolate varied. According to Prescott (2002) the effect of bioactive agent varies with target specie. Mann *et al.* (2008) also reported that the position of the zone edge (diameter of the zone of inhibition) is determined by the initial population density of the organisms, their growth rate and diffusion of the antimicrobial agents, which clearly explains the difference in the zone of inhibition observed. Augmentin and gentamicine were the antibiotics used as the control discs against the test organisms. These antibiotics showed greater activity than the crude extracts. This is not surprising because standard antibiotics are well refined industrial products so there is no doubt their activity will be more compared to crude extracts.

CONCLUSION

Stem bark extracts of *Anogeissus leiocarpus* have shown antibacterial activity against all the test organisms, this reveals that, the plant contains some bioactive compound. As there is growing interest in obtaining sample of plant materials with a view to explore the possibilities for medicinal products and in addition, the current global upsurge of bacterial resistance to antimicrobial drugs, the plant may be used as promising candidate for drug development.

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