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Antimicrobial Activity of Leaf Extract of *Anogeissus Leiocarpus* (African Birch) On Some Selected Clinical Isolates.

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Abstract: *The comparative study on the antimicrobial activity of ethanolic and aqueous extracts of Anogeissus leiocarpus (African birch) leaf against Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans was evaluated in this research. The phytochemical analysis of both ethanolic and aqueous leaf extracts of Anogeissus leiocarphus (African birch) revealed the presence of saponine, tannins, steroide, cardiac glycoside, flavonoide and anthraquinone. There was antimicrobial activity for ethanolic extract against the test organisms with higher zones of inhibition on Pseudomonas aeruginosa ranging from 14mm-18mm than Staphylococcus aureus which ranged from 11mm-17mm, followed by Streptococcus mutans which ranged from 10mm-15mm. However, the aqueous extract revealed higher activities (zones of inhibition ranging from 10mm-23mm) against the test bacteria .The Minimum Inhibitory Concentration (MIC) for both ethanolic and aqueous against bacteria had a range of 200g/ml -400g/ml and 100mg/ml-200mg/ml respectively. The Minimum Bactericidal Concentration (MBC) showed potency against the organisms mostly at 400mg/ml except for P.aeruginosa . The MIC and MBC values observed for both ethanolic and aqueous extracts of Anorgeissus leiocarpus (African birch) revealed effectiveness against infections caused especially by S. aureus, and S.mutans at high concentration. The plant extract and phytochemicals that have potentials should be purified and developed into agents which can be used as a preventive or treatment therapies against the test organisms in a challenging economy.*

Key words: *Anorgeissus leiocarpus extract, efficacy, test bacteria*

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I. Introduction

The use of medicinal plants as therapeutic agents in treatment and prevention of diseases has increased over the years, probably due their easy access and economic reasons (Sha et al., 2006. They also contribute predominantly to the health care system especially among the rural populace (Omoregie and Osagie, 2012).

Nigeria as one of the developing countries is a rich source of medicinal plants that are raw materials of many pharmaceuticals (UNESCO,1998). Research has revealed that the market share of the USA alone rose from 18.4 % of the world total in 1976 to over 52 % in 2000 while in low-income countries (Nigeria inclusive) the share of pharmaceuticals consumed fell from 3.9 % of the total in 1985 to 2.9 % in 1999 (WHO,2001) . This could due to the increase usage of medicinal plants by citizens of low-income countries and the need to explore these resources as a way of growing the economy while improving the pharmaceutical needs of the citizens (Bukar et al.,2016) in this depressed economy of ours.

Medicinal plants of great importance to the health of individuals and communities in general. Plants provide an alternative strategy in search for new drugs. There is a rich abundance of plants which continue to be a valuable source of new and improve drugs (Shah et al, 2006). Traditional medicinal plants are therapeutic resource used by the population of the continent specifically for health care, which may also serve as starting material for drugs (Sofowora, 1993). WHO, (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fraction, purification plant concentration or other physical or biological process which may be produced for immediate consumption or as basis for herbal products.

The medicinal value of plants has in chemical substances that produce a definite physiological action in the human and animal body. The therapeutic values of these plants have been linked to their biologically active secondary metabolites such as flavonoids, alkaloids, tannins, polyphones, saponins etc. the plants bioactive agents have been reported to possess diverse biological roles in combating disease (Okwu, 2005).

Anogerssus leiocarpus leaf is among many plant species which have been reported to have ethno medicinal uses and have been widely use in traditional system of medicine (Mann, e tal, 2003).

Anogeissus leiocarpus (African birch) commonly called Axle-wood tree is a plant that is widely used in Nigeria; it belongs to the family combretaceal (Combretordeae). It is a very grateful tropical tree which grows up to 28-30m height, typically 15-18m with light green foliage. The base of the trunk is wide and occasionally

striped. It has a dense crown and often drooping branches. The colour of the bank is grey and it's fibrous with thin scales. It has finely pubescent stems and alternate to sub-opposite, elliptical to oval leaves which is 2-8cm long growing in drier areas tending to smaller leaves and hairier flowers (Arbonnier, 2004).

Anogeissus leiocarpus is one of the major plants commonly used as chewing stick in Nigeria, which is believed to kill harmful bacteria in the oral cavity. The use of A. leiocarpus in the treatment of oral disease, such as thrush and black tongue has been reported in many studies, A. leiocarpus has many application, leaves, roots and trunk bark of the tree are used by traditional practitioners for treatment of helminthiasis (infection of the human body with a parasitic worm such as roundworms and pin worms, trypanosomiasis (sleeping sickness malaria and dysenterie syndrome. It is also used in traditional medicine as a remedy for many admen's of livestock and man which include schistosomiasis (disease caused by parasitic worms) leprosy, diarrhoea and psoriasis. Some members of the combretacea have high concentration of bioactive substance such as flaronoids, terpenoids, tannins or polyphenolic compounds (Mann 2008). Therefore, this research was aimed at determining the efficiency of A. leiocarpus as useful regimen in infection treatment.

II. Materials And Method

The leaves of the plant were collected from apparently healthy plant from Qua'an-Pan LGA Southern Zone of Plateau State, Central Nigeria. As soon as it was collected it was rinsed with clean water to reduce dust load. It was identified and authenticated in the Drug Development Unit of NVRI, Vom by the botanist in charge of the unit. The leaves were air dried for at least 4 weeks and pounded to fine powder with pestle and mortar and extracted using:

2.1Aqueous extraction

The extract of the plant was prepared by suspending 50g of the leaf powder separately in 500cm^3 of distilled water, which was shaken intermittently for 6 hours and it was allowed to stand for 48 hours in a refrigerator to avoid fermentation and was filtered through a What'sman No.1 filter paper.

The filtrate was concentrated to dryness at 50^0c under reduce pressure in a hot air oven and stored in refrigerator at -4^0c until required.

2.1.1 Soxhlet extraction

The soxhlet ethanolic extraction was carried out using Redfern *et al*.(2016) method. The concentrate was dried at 50^0c under reduce pressure in a hot air oven and stored in a refrigerator at -4^0c until required.

2.2 Phytochemical screening

The phytochemical screenings were carried out in the Biochemistry Division of NVRI, Vom, for both aqueous and ethanolic plant extracts using Qualitative Standard Procedure (Soforowa, 1993;Trease and Evans, 1983).

2.3 Clinical isolates

Staphylococcus aureus, Pseudomonas aeruginosa were obtained from the stock cultures in NVRI, Vom while *Streptococcus mutans* was isolated from a decayed tooth collected from the Dental Centre of Plateau State Hospital Management Board Jos by the following procedure:10 samples were collected, 5 in nutrient broth and the other 5 in Brain Hearth Infusion Broth (BHIB). As soon as a decayed tooth was removed it was collected into the media and incubated for 24hours at 45^0c, and sub-cultured into Blood agar plate and incubated anaerobically for 24 hours.

The different colonies gotten from the samples were Gram stained, tested for sugar fermentation and bio-chemicals for the isolation and identification of *Streptococcus mutans*.

2.4 Antimicrobial sensitivity bioassay

The antimicrobial assay was performed by using the well diffusion method (Habamu *et al.*, 2010). Wells of 4mm in diameter were made into previously seeded nutrient agar. Each well was filled with 0.1ml of the extract at 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml concentration. The same quantity of sterile distilled water without plant extract served as negative control while ciprofloxacin was used as positive control .The plates were pre-incubated for 2 hours to allow diffusion of the extract before the plates were incubated overnight at 37^0c and blood agar plates containing *Streptococcus mutans* was incubated anaerobically.

The diameter of clear zone of inhibition was measured in mm using protractor and well calibrated meter rule.Triplicate plates were prepared for each extract and control.

2.5 Determination of Minimum Inhibitory Concentration (MIC).

The MIC of the extracts were determine using the method described by Vinothkumar *et al.*, (2010), by diluting the extracts in double fold starting from 400mg/ml to 1.56mg/ml, with nutrient broth at 1% concentration in a series of bottles and to each of the bottles 10µl volume of the test organism was added and incubated at 37^0c for 24 hours. Control was prepared by inoculating tubes with test organisms without extract as control .The tubes were then examined for the presence of turbidity after the incubation period of 24hours at 37^0c. The least concentration with no observable bacterial growth when compared with the control was considered as the Minimum Inhibitory Concentration (MIC).

2.6 Determination of Minimum Bactericidal Concentration(MBC)

The suspension from the minimum inhibitory concentration tubes that showed no growth were inoculated on nutrient agar and incubated over night at 37^0c for bacteria .The least concentration from the MIC tubes that showed no bacterial growth was considered as the minimum bactericidal concentration (MBC).

III. Results

The phytochemical screening revealed that *Anogeissus leiocorpus* (African birch) leaf contained; saponins, tannins, steroids/terpenes, gurdiac glycosides, alkaloids, flavonoids and anthraquinones (Table I). The ethanolic extract against the test organisms showed higher zones of inhibition against *Pseudomonas aeruginosa* ranging from 14mm-18mm than *Staphylococcus aureus* which ranged from 11mm-17mm, followed by *Streptococcus mutans* which ranged from 10mm-15mm. However, the aqueous extract revealed zones of inhibition ranging from 10mm-23mm against the test bacteria (Table2).The Minimum Inhibitory Concentration (MIC) for both ethanolic and aqueous against bacteria had a range of 200g/ml -400g/ml and 100mg/ml-200mg/ml respectively (Table3 and 4). The Minimum Bactericidal Concentration (MBC) showed potency against the organisms mostly at 400mg/ml except for *P.aeruginosa* (Table5).

Table 1:The Phytochemical Analysis

Extracts/ Phytochemical Constituents	Ethanolic	Aqueuos
saponin	+	+
tannins	+	+
steroide/terpenes	+	+
gardiac/glycoside	+	+
alkaloids	+	+
flavonoid	+	+
anthraquionon	+	+

Key: - = Absence; + = Presence

Table2:Antimicrobial Effect of *Anogeissus leiocorpus*(African birch)

Organisms	Extracts	Conc(mg/ml) /zone of inhibition (mm)					Cipro.
		400	200	100	50	25	
Staphylococcus aureus	E.E	17		13	13	11	28
Staphylococcus aureus	A.E	23		19	15	13	29
Pseudomonas aeruginosa	E.E	18		17	14	14	25
Pseudomonas aeruginosa	A.E	19		17	16	15	26
Streptococcus mutans	E.E	15		14	13	10	30
Streptococcus mutans	A.E	17		14	13	11	25

Key: E.E = Ethanolic extract,Conc(concentration) A.E = Aqueous extract, Cipro. = ciprofloxacin(control)

Table 3: Minimum Inhibitory Concentration (MIC) of Ethanolic Leaf Extract of African Birch.

Organism	Extract concentration (mg/ml)									
	400	200	100	50	25	12.5	6.25	3.12	1.25	MIC
SA	-	-	+	+	+	+	+	+	+	200
PA	-	+	+	+	+	+	+	+	+	400
SM	-	-	+	+	+	+	+	+	+	200

Key: SA = *Staphylococcus aureus,* PA = *Pseudomonas aeruginosa,* SM = *Streptococcus mutans,* - = No turbidity, + = presence of turbidity

Table 4:Minimum Inhibitory Concentration (MIC) of Aqueous Leaf Extract of African Birch.

Organism	Extract concentration (mg/ml)									
	400	200	100	50	25	12.5	6.25	3.12	1.25	MIC
SA	-	-	-	+	+	+	+	+	+	100
PA	-	-	+	+	+	+	+	+	+	200
SM	-	-	-	+	+	+	+	+	+	100

KEY: SA = *Staphylococcus aureus,* PA = *Pseudomonas aeruginosa,* SM = *Streptococcus mutans,* - = No turbidity, + = presence of turbidity

Table 5: Minimum Bactericidal Concentration of Ethanolic Leaf Extract of African Birch.

Organism	Extract concentration (mg/ml)									
	400	200	100	50	25	12.5	6.25	3.12	1.25	MBC
SA	-	+	+	+	+	+	+	+	+	400
PA	+	+	+	+	+	+	+	+	+	00
SM	-	+	+	+	+	+	+	+	+	400

Key: SA = *Staphylococcus aureus,* PA = *Pseudomonas aeruginosa,*
SM=*Streptococcus mutans,* - = No growth, + = presence of growth,

IV. Discussion

The presence of the bioactive principles may support the use of plants traditionally for array of diseases including malaria, stomach disorder, skin infections, anaemia and cancer (Omoregie and Osagie, 2012). Many bioactive principles from plants have been shown to have pharmaceutical effect in treatment of some diseases, for instance component of flavonoids have been widely reported to possess antioxidant activity in antagonizing increased capillary fragility associated with diseases, reducing pains (tooth ache on gums), antibacterial, inflammatory and anti-carcinogens activities (Okwu, 2003).

Alkanoids are derivatives of amino acids and have been used to treat diseases like malaria, skin diseases cancer (Musyimi *et al.,* 2008).Tannins are polyphenolic compounds which have been known to be hepatoprotective. The zones of inhibition produced by the test organisms indicated their susceptibility to the plant extracts. It was observed that the zones of inhibition varied from one organism to another and from one solvent used for extraction to another. This could be as result of one solvent extracting more bioactive molecules than the other and also due to initial population density of the organism, their growth rate and the rate of diffusion of the antimicrobial agent(Li *et at*.,2017).

Furthermore, the finding showed that potency of the plant extracts increased as concentration increased. The above result of MBC indicates that the leaf extract of the plant is more bacteriostatic than bactericidal.

The current findings lend credence to the traditional use of this plant as medicines for infectious diseases particularly those caused by the test organisms susceptible to the extracts. The present results for both extracts indicate significant antimicrobial potentials and this suggest that traditional medicine could be used as guide in the continuous search for new antimicrobial agents.

The susceptibility of these microbes to the extracts of this plant may be a pointer to their potentials as drugs that can be used against the test organisms. These observations also suggest that constituents of the plant part could be useful in chemotherapy.

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