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Bacteriostatic Effect of Anogeissus leicarpus Methanolic Leaves Extract

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ABSTRACT

This study was carried out to investigate the in-vitro antimicrobial activity of A. leiocarpus extract against clinical isolates performed by cup-plate agar diffusion method in five concentrations (100- 6.25 mg/ml) against 3 Gram negative bacteria: Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumonia and 1 Gram positive bacteria: Staphylococcus aureus. The methanolic extract exhibited inhibitory effects against most of the tested microorganisms with zone of inhibition ranging from (21-8 mm). The largest inhibition zone were obtained methanolic extract of A. leiocarpus (leaves) against the Gram negative Escherichia coli(21±0.5mm) in 100 mg/ml concentration, comparison with (32 mm) Gentamicin 40 mgc. and Staphylococcus aureus (11 mm) in 100 mg/ml concentration comparison with (35mm) Gentamicin 40mgc. The methanol extract of A. leiocarpus was considered for further exploration of isolation active compounds analysis.

Keywords: *Anogeissusleicarpus, antimicrobial, methanolic extract*

INTRODUCTION

Microbial infections are major public health problems in the developed and developing countries. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistances in human pathogens is increasing [1]. Today infectious diseases account for one-third of all deaths in the world; the World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases. The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind [2]. The development and spread of resistance to currently available antibiotics is a worldwide concern, the increasing phenomenon of acquisition of resistance among microorganisms to antimicrobial drugs is attributed to the indiscriminate and improper use of current antimicrobial drugs [3]. Natural resources like plants are currently used all over developed and under developed countries of the world as traditional home remedies and are

promising agents for drug discovery as they play crucial role in traditional medicine [4].

Medicinal plants have been used over years for multiple purposes, and have increasingly attract the interest of researchers in order to evaluate their contribution to health maintenance and disease's prevention [5]. Recently between 50,000 and 70,000 species of plants are known and are being used in the development of modern drugs. Plants were the main therapeutic agents used by humans from the 19th century, and their role in medicine is always topical [6]. The effects of plants extracts on Bacteria have been studied by a very large number of researchers in different parts of the world [7].

Anogeissusleiocarpus is one of the important species of the genus *Anogeissus*, (Combretaceae). It is an evergreen tree widely distributed in Africa and well known in African traditional medicine for treating of many diseases [20]. Bark powder is used on wounds, sores, boils, abscesses and diabetic ulcers and gives good results. Bark powder has also been added with "green clay" and used as a face mask to treat dangerous black heads [8]. It is used as a cough medicine, as pulped roots are placed on wounds and ulcers; bark powder is also used to relieve toothache on the periodontal, it is also used as anti-worms and boiled leaves are used for washing and fumigation [9]. *A. leiocarpus* is also known for its use to treat inflamed wounds in humans and animals [9]. There are also many traditional uses of the plant. In Sudanese traditional medicine, the decoction of the barks is used against cough. Rural populations of Nigeria use sticks for orodental hygiene, the end of the sticks are chewed into fibrous brush which is rubbed against teeth and gum [10]. This paper seeks to investigate whether the methanolic extract of *A. leiocarpus* could inhibit four clinical isolates microorganisms.

MATERIALS AND METHODS

Plant Materials

The plant used in this study was collected from AlSunut Jungle in the middle of Khartoum State central of Sudan collected between January and February 2018. The specimens were taxonomically identified by the member of Herbarium in Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Centre for Research, Khartoum, Sudan. A voucher specimen was deposited at the herbarium of the institute. The leaf was air-dried and coarsely ground to powder.

Preparation of Crude Extract

Extraction was carried out for the Leaves of *Anogeissusleiocarpus* by using overnight maceration techniques according to the method described by (Altúzar-Molina *et al.*, 2011). About 50 g were macerated in 250 ml of methanol for 3 hours at room temperature with occasional shaking for 24 h, the supernatant was decanted and clarity field by filtration through a filter paper, after filtration, the solvent was removed under reduced pressure by rotary evaporator at 55°C. Residue was weighed and the yield percentage was calculated then stored at 4°C in tightly sealed glass vial ready for use.

Test Microorganisms

The methanolic Leaves extract of *A.leiocarpus* plant were tested against four bacterial species: one Gram-positive bacteria *Staphylococcus aureus*, three Gram-negative bacterial strains

Escherichia coli, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, The bacterial strains used in the study were obtained from the Department of Microbiology, Faculty of Medical Laboratory Science, International University of Africa, Khartoum, Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37°C for 24h and then used for the antimicrobial test.

In Vitro Testing of Extracts for Antimicrobial Activity

The cup-plate agar diffusion method described in (Fagere and Al Magbou, 2016) [21] was used adopted with some minor modifications to assess the antibacterial activity of the prepared extract. One ml of the standardized bacterial stock suspension (between 10^8 and 10^9 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Moller Hinton agar which was maintained at 45°C. 20 ml aliquots of the inoculated Moller Hinton agar were distributed into sterile Petri-dish plates. The agar was left to set and in all of these plates 5 cups (6 mm in diameter) was cut using a sterile corkborer (No. 4) and agar discs were removed. Each cup was filled with 0.1 ml sample of the methanolic extract using an automatic pipette, and thereafter the extract was allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 24 hours. Two replicates were carried out for the extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured and averaged. The mean values were tabulated.

Statistical Analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program 2010.

RESULTS AND DISCUSSION

The methanolic extract against test organisms showed higher zones of inhibition against *E. coli* ranging from 21mm-11mm than *K. pneumonia* which ranged from 17mm-10mm, followed by *Streptococcus aureus* which ranged from 11mm-8mm. However, The Minimum Inhibitory Concentration (MIC) for methanolic against bacteria had 12.5mg/ml for *E. coli* and 25mg/ml for *K. pneumonia.*

Comparison of the observations given in Tables 1 showed that the methanolic extract of *A. leiocarpu* sinhibited *Klebsiella pneumoniae* less than 5µg/ml exhibited of Gentamicin. It also inhibited *E.coli* higher than that of 5µg/ml Gentamicin. It inhibited *E. coli* less than that of 10µg/ml Gentamicin. Moreover, the methanolic extract showed ineffective results against *Staphylococcus aureus* in 100mg/ml and 50 mg/ml comparing with Gentamicin results that showed higher activity.

Table 1: *Antimicrobial activity of A. leiocarpus extract against tested microorganisms.*

Sr. No.	Tested Microorganisms	Concentrations (mg/ml)				
		100	50	25	12.5	6.25
		Zone of Inhibition in mm ± (SD)				
1	*Escherichia coli*	21±0.5	16±0.5	14±1	11.33±0.5	00
2	*Staphylococcus aureus*	11±0.7	8±0.9	00	00	00
3	*Klebsiella pneumonia*	17.6±0.5	15.6±0.5	10±0.5	00	00
4	*Pseudomonas aeruginos*	10±0.2	00	00	00	00

Table 2: *Antibacterial activities of reference drug against the tested microorganisms.*

Drugs	Concentrations (mg/ml)	Tested bacteria used (M. D. I. Z mm)			
		S. a	***E. c***	***K. p***	***Pa. a***
Gentamicin	40	35	32	26	23
	20	33	30	24	22
	10	30	17	21	21
	5	28	-	20	19

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 [11]. 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 [12].

Furthermore, the finding showed that potency of the plant extracts increased as concentration increased. The above results indicate that the leaves extract of the plant is more bacteriostatic than bactericidal. Mann *et al*. (2015), Elsiding*et al*. (2015), Timothy *et al*. (2015) and Alhassan*et al*. (2016) [13] [14] [15] and [16] have also reported the potential effect of *Anogeissus leiocarpus* against pathogenic microorganisms such as *S. aureus*, *Klebseilla*species, *C. albicans*, *E. coli*, *S. dysenteriae*, *Aspergillusniger* and *P. aeruginosa*. Mann (2012) reported wide range of activity of this plant sample against *S. pyogenes* and *B. subtilis.* The zones of inhibition range from 18-30 mm which indicates strong zone of clearance by the extracts against the microbes. This indicates that this plant can be used in the management of infections caused by the tested pathogens in this research as reported by Alhassan*et al*. (2016) [16]. This result has confirmed the claims of other researchers that the plant possesses antimicrobial activity against many pathogens. 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. According to Prescott (2002) [17], the effect of an agent varies with target species. Hugo and Russell (1998) [19] also reported that the position of the zone edge (diameter of zone of inhibition) is determined by the initial population density of the organism, their growth rate and the rate of diffusion of the anti-microbial agent. This explains the differences in the zones of inhibition observed. The combination of the various parts of *A. leiocarpusa* and extracts from the root and stem bark were found to exhibit antibacterial activity against the test organisms. This justifies their traditional usage as medicinal plant this may be due to the presence of the active principles observed. Plant secondary metabolic compounds are an important source of microbicides, pesticides and many medicines [9] [18]. The MIC range from 5-20 mg/mL and the MIC in this research is higher than the MIC reported by Mann (2012) and Timothy *et al*. (2015) [22] [23]. Umar and Mohammad (2015) [24] reported higher MIC of methanol extract of 90 mg/mL against *S. aureus* and *P. aeruginosa*. Ethyl acetate extract had the least MIC of 5mg/mL against *S. aureus*, *C. ulcerans*, *K. pneumonia* and *C. stellatoidea* and higher concentration of n-hexane extract was needed to inhibit the pathogens at 10-20 mg/mL. The methanol extract at 10mg/ml inhibited more of the pathogens and only *B. subtilis* was

inhibited at 5 mg/mL by the methanol extract. This could be as a result of adequate and inadequate presence of active compounds extracted by these solvents. The inhibition of the pathogens by these extracts shows the plant has the potential in the management of diseases caused by these pathogens.

CONCLUSION

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 extract. The present results indicate significant antimicrobial potentials and this suggests that traditional medicine could be used as guide in the continuous search for new antimicrobial agents.

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