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ANTIFUNGAL EFFICACY OF *ANOGEISSUS LEIOCARPUS* DC AGAINST *ASPERGILLUS NIGER* AND *RHIZOCTONIA spp.*

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ABSTRACT
The present study underscores the efficacy of aqueous and methanol stem bark extracts of Anogeissus leiocarpus against Aspergillus niger, and Rhizoctonia spp. which causes many plant diseases. The plant is reputed for its traditional use in the treatment of diseases related to microbial infection including mycosis, dermatitis, coughing and tuberculosis. Phytochemical analysis of the stem bark extracts revealed the presence of some secondary metabolites (Alkaloids, Tannins, Saponins, and Cardiac glycosides) which have been reported to have antimicrobial effects. Assays were performed using extracts concentrations of 1,000,000, 500,000, 250,000, and 125, 000µg/ml by agar well diffusion technique. Methanol extract was active against all the pathogens especially at concentration of 1,000,000µg/ml which showed greater activity against R. spp. (21mm), but A. niger developed resistance against the aqueous extract at all concentrations. Activity was greater against R. spp. in response to all extracts i.e. methanol and water (21mm, and 20mm) at the highest concentration than A. niger (18mm, and 0). The antifungal screening revealed that the extract exhibited inhibitory activities at varying concentrations against the test pathogens. These activities observed could be attributed to the presence of active metabolites contained in the extract. Further studies to isolate the lead compounds responsible for this activity are therefore advocated.

Keywords: *Anogeissus leiocarpus*, phytochemicals, herbal therapy, microorganisms, antifungal activity.

INTRODUCTION

Anogeissus leiocarpus (synonym: *Anogeissus chimperi*), locally known as ***'Marke'*** in Hausa language and commonly called African birch or axle-wood (Victor, 2013) is a typical tree of woodland and savannah of the Sudan region and center of endemism which is widely found in northern Nigeria (Mukhtar *et al.* 2017). It belongs to the family *Combretaceae* in the order of *Myrtales*. It is a deciduous tree species that grows up to 15-18m height and measure up to 1mm diameter (Andry *et al.*, 2005). *A. leiocarpus* has a large ecological distribution ranging from the boarders of Sahara up to the out layer humid tropical forest (Hennenberg, 2013; Ouederago, 2013). The plant has a wide range of values ranging from the pharmacological properties which are largely dependent upon the phytochemicals constituents of the plant, economic importance and traditional uses as well (Mukhtar *et al.* 2017; Hennenberg, 2005). In Ivory Coast, the fleshy roots are used to lessen labour pains and in Burkina Faso to accelerate wound healing (Victor, 2013). The leaves also have a wide bacterial and fungicidal activity in humans and animal, it is used medicinally for the treatment of ascaricides, gonorrhea, general body pain, asthma, coughing (Mann *et al.*, 2008).

The infusion and decoction of this plant 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). Information obtained from the Yoruba and south eastern people of Nigeria illustrate that the plant is also used as an antimicrobial agent against bacterial infections (Dweeck, 1996). Many traditional uses have been reported for the plant. In Sudanese traditional medicine the decoction of the barks is used against cough (El Ghazali *et al*., 2003). 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 (Rotimi, 1988). In Togolese traditional medicine, it is used against fungal infections such as dermatitis and mycosis, also the decoction of leaves is used against stomach infections (Batawila, 2005). The plant is also used for the treatment of diabetic ulcers general body pain, blood clots, asthma, coughing and tuberculosis (Victor, 2013).

In recent years, there has been a gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries in utilization to develop antimicrobial drugs, because plant- drive drugs have been reported to be safe and without side effects (Rai *et al* ., 2003). More so, the global emergence of antimicrobial resistant bacterial and fungal strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). In many poor countries, the available drugs are costly and beyond the reach of the common man (WHO, 2002). Strategies to improve the current situation include

research in finding new and innovative antimicrobials (Freeman, 1997) from indigenous plant materials. The aim of the present study was to investigate the antifungal activity of aqueous and methanol stem bark extracts of *Anogeissus leiocarpus* against *Aspergillus niger,* and *Rhizoctonia spp.*

MATERIALS AND METHODS

Collection and Processing of Plant Material

Fresh stem barks of *Anogeissus leiocarpus* were collected from local farm at Madobi Local Government area, Kano state, Nigeria. The plant materials were taken to the Herbarium section of Plant Biology Department, Bayero University, Kano where it was properly authenticated by a taxonomist, Buhaudeen Sa'id Adam. The voucher specimen was deposited in the herbarium with Herbarium Accession Number BUKHAN 0029. The stem barks was thoroughly washed, rinsed with distilled water and shade-dried under a room temperature of about 33^0 for two weeks. The dried stem barks was pulverized and fine powder stored in a tight sterile bottle container until needed (Bukar *et al.*, 2009).

Extraction of Plant Material

Fifty grams of the powdered stem bark of *Anogeissus leiocarpus* was soaked separately in methanol and distilled water (50g/0.5L) and the extracts decanted at an interval of 5 days. The filtrate was then evaporated to dryness in a water bath at 39^OC and stored in the refrigerator at 4^0C until required (Aliyu and Sani, 2011).

Phytochemical Screening

Phytochemical screening for the secondary metabolites was conducted using standard qualitative methods as described by Sofowara (1993) and Abalaka *et al.* (2010). Saponins, alkaloids, tannins, glycosides, and flavonoids were the major bioactive components tested in all the extracts (water and methanol)

Preparation of Extract Concentrations for Sensitivity Test

Extract concentration of the plant material was prepared using serial dilution method as described by Aliyu and Sani (2011). Two gram (2g) of the plant extract (*Anogeissus leiocarpus*) was dissolved in 2ml of appropriate diluent; Dimethyl Sulphur Oxide (DMSO) to arrive at 1.0g/ml, which serves as stock solution from which appropriate quantity of quantity of *Anogeissus leiocarpus* extracts were dissolved in 1ml of DMSO to arrive at four different concentrations: 1,000,000, 500,000, 250,000, and 125, 000µg/ml respectively.

Fungal Isolates

The fungal isolates, *Aspergilllus niger*, and *Rhizoctonia spp*. were obtained from the Plant Pathology Laboratory, Bayero University, Kano.

Preparation and Standardization of Inoculum

The isolates were sub-cultured on Potato Dextrose Agar plates and incubated at $27^OC \pm 2^OC$. Inoculum suspension were prepared from fresh, mature (3-5 days old) cultures, in some cases an extended incubation was required for proper sporulation of the isolates, the fresh colonies were covered with 10ml of distilled sterile water. The suspensions were checked to provide approximately 10^5 CFU/ml. The conidia were rubbed carefully with a sterile cotton swab and transferred to a sterile tube (Petrikkou *et al*., 2001)

Antifungal Assay/Sensitivity Test for *Anogeissus leiocarpus* Stem bark Extract

The extracts were screened for antifungal property using the agar well diffusion technique. Potato Dextrose Agar (PDA) was used for fungal cultures. Inoculum of test fungus was spread on to sterile Potato dextrose agar plate so as to achieve a confluent growth. The plates were allowed to dry and 6mm diameter wells were punched into the agar using a sterile cork borer and filled with plant extracts of different concentrations. A standard antibiotic (Mancozeb, concentration 1g/ml) and Dimethyl Sulphoxide Oxide (DMSO) were used as standard and negative controls respectively. The fungal plates were incubated for 72hours at 37^0C (Abhishek Mathur *et al*., 2011)

The degree of sensitivity was determined by observing and measuring the diameter in millimeter (mm) of the visible zone of inhibition of the microbial growth produced by the diffusion of the extracts (Stockes and Ridgeway, 1980).

RESULTS

The preliminary phytochemical screening of the stem bark extracts of *Anogeissus leiocarpus* indicates the presence of Alkaloids, Tannins, Saponin, and Cardiac glycoside (Table 1)

Table 1: Phytochemical Screening of Aqueous and Methanol Stem bark Extract of *Anogeissus leiocarpus*

Phytochemicals	Inference	
	Methanol Extract	**Aqueous Extract**
Alkaloids	+	+
Tannins	+	+
Flavonoids	-	-
Saponins	+	+
Glycosides	+	-

Key: Present = +, Absent = -

Table 2 shows the diameter of the zone of inhibition of aqueous and methanol extract of *Anogeissus leiocarpus*. Aqueous extract exhibited promising antifungal activity against *R. spp.* only (20±1.16mm, 16±0.58mm, 13±0.58mm) with the exception of the lowest concentration. Meanwhile, *A. niger* developed resistance and showed no sign of susceptibility against the extract at all concentrations

From table 2 also, methanol extract demonstrates good antifungal activity against all the test organisms, *R. spp.* (21±0.58mm, 17±0.62mm, 12±1.73mm, 9±0.58mm), and *A. niger* (18±0.68mm, 15±0.57mm, 11±1.02mm) respectively with the exception of lowest concentration. Methanol extract showed greater activity against *R. spp.* (21mm) at 1,000,000µg/ml than *A. niger* (18mm). Activity was greater against *R. spp*. in all fractions (21±0.58mm and 20±1.16mm). Mancozeb i.e. positive control, exhibited better activity (i.e. 32±2.31mm and 30±1.15) than all the extracts as shown in table 2.

Table 2: Antifungal Activity of Aqueous and Methanol Extract of *Anogeissus leiocarpus*

Isolates	Concentration of extracts (µg/ml)					
	1,000,000	500,000	250,000	125,000	Positive control	Negative control
	Aqueous Extract Zone of inhibition (mm)					
R. spp.	20±1.16	16±0.58	13±0.58	0	32±2.31	0
A. niger	0	0	0	0	30±1.15	0
	Methanol Extract Zone of inhibition (mm)					
R. spp.	21±0.58	17±0.62	12±1.73	9±0.58	32±2.31	0
A. niger	18±0.68	15±0.57	11±1.02	0	30±0.88	0

Results are expressed as mean ± standard error of mean

Key: Positive control = Mancozeb (1g/ml), Negative control = DMSO

DISCUSSION

Phytochemicals are naturally occurring biologically active, non-nutritive chemical compounds found in plants and act as a natural defense system against various pests. Plants extracts contain different phytochemicals with various biological activities that can be of value in both medical and veterinary practice. Different plant extracts may therefore contain different phytochemicals each of which has its biological activity. Various phytochemicals have been known to processes medicinal properties and hence widely used in Nigerian system of traditional medicine (Namadina and Sani, 2018). Recently, there has been a gradual revival of interest concerning the use of medicinal and aromatic plants in developed as well as in developing countries in utilization to develop antimicrobial drugs, because plant- drive drugs have been reported to be safe and without side effects (Rai *et al* ., 2003).

Qualitative phytochemical analysis of *A. leiocarpus* stem bark extract revealed the presence of secondary metabolites of immense therapeutic properties. This finding agrees with the work of Dewanjee *et al*., (2008), Mann *et al*., (2008) and Victor (2013) who discovered that these secondary metabolites have been established to be frequently responsible for the antimicrobial properties of most medicinal plants. These phytochemicals may have caused the observed inhibitory effect either singly or in synergy with each other, and this requires further investigation to validate this claim. Earlier, Baba-Moussa *et al.* (1999) reported that Tannins present in some plant species possess antifungal property. It is hoped that the elucidation of the structure of the active principle(s) and its/their subsequent use in antifungal investigations would give better results.

Tannins also interfere with cell division, hence the presence of tannins in *A. leiocarpus* could account for the antimicrobial activity in the study, and this is in agreement with the findings of Chewonarin *et al*., (1999) and Sayyida *et al*., (2018). Tannins have been traditionally used for protection of inflamed surface of the mouth and treatment of catarrh, wound and diarrhea. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Stary, 1998; Okwu and Okwu, 2004). Also, the presence of cardiac glycosides in *A. leiocarpus*, gave clear indications that this plant can be used in the treatment of hypertension (Olaleye and Mary 2007).

It was observed that the fungal isolates used in this research exhibited varying degrees of susceptibility to the plant extracts. Thus, the values obtained for the zones of inhibition differed, for test organism. Similar result was reported by Pankaj *et al*., (2011) with methanol and butanol extracts of *Cordia dichotoma* stem bark extracts. From their findings, the growth inhibition zone ranges from 12-21mm for fungal strains (*A. niger, A. clavatus* and *Candida alloicans*). The results also corroborate the findings of Anani *et al*. (2000) and Rajakaruna *et al*. (2002). Additionally, results of this study also agree with the report of Kumar *et al.* (2009b). The difference in susceptibility observed in this study could be attributed to the inherent resistance factor of the test organisms among other factors (Ekpo and Etim, 2009). Mancozeb was the antibiotic used as the control plate against the test organisms and the plant extract showed good inhibitory activity as compared with this synthetic drug. This antibiotic showed greater activity than the crude extracts, this is not surprising because standard antibiotics are well refined industrial products, so there is no doubt its activity can be higher compared to the crude extracts It was revealed in this study, that increase in the antifungal activity was enhanced by the corresponding increase in the concentration of the extracts (i.e. the higher the concentration of the extracts, the more the activity). This finding agrees with the report of Banso *et al.* (1999), that higher concentration of antimicrobial substance showed appreciation in growth inhibition of the fungal isolates. Methanol fraction showed greater activity than the water extract, and this may be as a result of better solubility of the active components in methanol than in the water (Aliyu and Sani, 2011). Methanol is known to be highly polar solvent, and thus would extract only highly polar

compounds in a given preparation. Similar observation was reported by Khaing (2011), according to the report, the higher antifungal activity exhibited by the methanol portion may be due to the presence of substantial amounts of polar constituents in the plant material. Generally, the antifungal activities observed could be due to the presence of secondary metabolites which have been reported as active constituents responsible for antifungal activities (Salihu and Garba, 2008).

CONCLUSION

The present study showed that stem bark of *A. leiocarpus* contains some biologically active components of immense therapeutic activities. This has clearly justified its good inhibitory activity against the test organisms (*Aspergillus niger, and Rhizoctonia spp.)* used in the study. The plant may be used as promising candidate for drug development in treatment of fungal infections.

RECOMMENDATIONS

Subject to the findings of this study, further studies are required to isolate and characterized the active constituents of this plant with their clinical relevance. Although African birch has been widely reputed for its pharmacological activities, it is imperative that more work has to be done on its toxicity studies in order to determine the level of its safety.

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Conflict of Interest

The entire authors declared no any conflict of interest regarding this article.

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