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Phytochemical Screening, DPPH radical scavenging activity and Antibacterial Sensitivity of Stem-bark Extracts of *Anogeissus leiocarpus* Grown in Kwaya-Kusar LGA, Borno State

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Abstract

The phytochemical screening of stem bark of *Anogeissus leiocarpus* was carried out according to standard procedure. The extraction of powdered stem bark sample (200 g) was done in methanol, ethanol and distilled water using maceration. The percentage yield revealed a relatively low yield in ethanol (4.9%), methanol (4.8%) and water (3.2%). The results revealed the presence of flavonoids, glycosides and steroids in all extracts. Tannins, Phenols, Terpenoids, alkaloids were present in ethanol and aqueous extracts. Terpenoids, Alkaloids, and saponins were present methanol extracts. Saponins and Tannins were also present in aqueous extracts, respectively. The antibacterial sensitivity tests of the three extracts on *E. coli* and *S. aureus* were conducted. The results showed that *E. coli* was not sensitive to all tested concentrations of the aqueous extract but sensitive to ethanol and methanol extracts at higher concentrations. *S. aureus* was sensitive to methanol and aqueous extract at 250 to 1000 mg/ml but not sensitive at very low doses. *S. aureus* was sensitive to ethanol extract at 500 and 1000 mg/ml but not sensitive at lower concentrations. Preliminary assessment of the extracts ability to scavenge the stable DPPH free radical as basis for antioxidant activity was conducted. The results showed the percentages of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity as pilot antioxidant assay for the three (3) extracts. The methanol extract recorded 87.65% inhibition at 1000 mg/ml while ethanol extract recorded 90.82% inhibition and aqueous extract recorded 82.50% inhibition. The three crude extracts upon purification could revealed more potent activity.

Keywords: Phytochemical, DPPH, free radical, antibacterial, *Anogeissus leiocarpus,* Antioxidant.

INTRODUCTION

Plants are chief sources of bioactive compounds with varied physiological roles credited to their phytochemical constituents. They are extensively use for the management of different ailments in traditional medicine for countless years [1]. In rural areas, people more often use medicinal plants to treat various ailments due to availability and ease accessibility of medicinal plants [2]. In most instances, herbal preparations are taken orally in form of decoctions, infusion or powder or through topical applications [1]. Many phytochemicals have offered a diversified pharmacological activity which could allow for the development of novel or lead drugs for application in modern medicine [3,4]. There are numerous medicinal plants being processed into different finished plant-based products of various efficacy for use as commercial products for export [5]. More than 70,000 plant species were used worldwide for the treatment or management of various ailments [6]. The current increased researches in natural products are clear justification of their promising potentials to address health challenging needs particularly in developing countries like Nigeria. Furthermore, they will facilitate sustainability, discover diverse therapeutic uses and advance scientific understanding of herbal medicine. The rising incidence of antimicrobial resistance has called for urgent and well-thought researches in order to explore most safer and effective alternatives infection management [7]. The therapeutic potentials of *Anogeissus leiocarpus* stem bark remains largely unexplored despite its traditional use in various medicinal applications [1]. The plant as a natural source of herbal preparations in many African cultures there is a need to screen the bioactive compounds from the stem bark of *Anogeissus leiocarpus* grown in the research area and bridge the gap between traditional and scientific knowledge to validate the folkloric uses and provide scientific data for further research. *Anogeissus leiocarpus* also “African birch” in English is commonly known as "fever tree," or chewing-stick. It is a native African tree. In Northern Nigeria the plant is called “Marke” in the native Hausa language [1]. The plant is commonly found in the farms and abundant lands or by roadsides but the plant is not yet cultivated despite its medicinal values. Although the plant suffers from indiscriminate cutting and over utilization from both cattle breeders and reapers mostly local people for firewood as well as farming activities [1]. It belongs to a genus *Anogeissus* which consists of eight (8) species, five (5) species from South Asia, two (2) species from the South Arab Peninsula and one (1) species from Africa [1,8]. *Anogeissus leiocarpusis* is a typical tree of woodlands and savannas of the Sudanese regions [9]. It has large ecological distribution ranging from the boarders of Sahara up to the out layer humid tropical forests. In West Africa, it distributed from Senegal to Nigeria, Cameroon and extends to Ethiopia and East Africa. It grows in dry forests and gallery forests [10]. It is a deciduous tree species that can grow up to 15–18 m tall and 1m in diameter [9]. The Bark is usually greyish and scaly with branches often drooping and slender. The leaves are alternate, ovate–lanceolate in shape, 2-8 cm long and 1.3-5 cm across. They are acute at the apex, attenuate at the base and pubescent beneath. The flowers are yellow; bisexual and petals are absent. Fruits are globose, cone-like heads, each fruit is broadly winged, dark grey and 3cm across. It

can reproduce by seeds as well as vegetative propagation [10].

Several researches were conducted on different parts of *Anogeissus leiocarpus* from quite different geographical locations including biological activity and screening of secondary metabolites. The inspiring potentials of medicinal plants in recent phytomedicine researches has triggered searching plants from different locations in the same regions for possible improved biological activity due to varied phytochemical constituents [11]. In fact, phytochemical analyses of medicinal plants collected from different area might lead to identification of some phytochemicals with significant biological activity that might not be present in the same plant species growing in different areas. Since many variations of secondary metabolites in plants from place to place were reported [12].

Anogeissus leiocarpus is rich in flavonoids, tannins, and other phenolic compounds, the stem bark exhibits significant antioxidant properties. These antioxidants help scavenge free radicals, protecting cells from damage and oxidative stress linked to chronic diseases like cancer, heart disease, and neuro degenerative conditions. The presence of phytocompounds with anti-inflammatory and pain-relieving effects could be beneficial potential for managing conditions like arthritis, rheumatism, and headaches. Research indicates that the stem bark extract may help protect the stomach lining from ulcers and gastritis. This could be due to its anti-inflammatory properties, as well as its ability to stimulate mucus production, which acts as a protective barrier. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and thus prevent disease. Antioxidants have the ability to prevent, delay or ameliorate many of the effects of free radicals. During certain diseased state, as well as during aging, there is a need to boost the antioxidant abilities, thereby potentiating the immune mechanism. The antioxidants preserve and stimulate the function of immune cells against homeostatic disturbances [13]. Antioxidant activity refers to the ability of plant compounds to neutralize harmful free radicals in the body, which can cause oxidative damage leading to various diseases. Antioxidants can be found in many foods, including fruits, vegetables, nuts, and seeds. Evaluating the antioxidant potentials of plants helps in identifying natural antioxidants that could have health benefits [14].

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary hydroquinone (TBHQ) are commonly employed as preservatives or additives in pharmaceutical, cosmetic, and food companies. The free radicals are known to be scavenged by these synthetic antioxidants. However, reports on the involvement of synthetic antioxidants in chronic diseases and their adverse side effects leading to carcinogenicity have restricted their use in foods. Therefore, international attention has been focused on natural antioxidants mainly from plant sources [15] and the search for natural and effective natural antioxidants is utter most necessity [16]. Several plants extractives have been reported to protect free radical-induced damage in various experimental models. In recent times, focus on plant research has increased all over the world and a lot have been collected to show the immense potentials of medicinal plants in various traditional systems [17]. Majority of

these plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, anthraquinones, quinones, coumarins, alkaloids, amines, betalains and other metabolites, which are rich antioxidant agents.

The therapeutic values of *Anogeissus leiocarpus* stem bark remains largely unexplored despite its versatile use in local medicinal applications particularly those grown in the research area. Moreover, it might bridge the gap between traditional and scientific knowledge to validate the folkloric uses and also provide scientific data for further research. Therefore, this research was aimed at preliminary investigation of stem bark extracts of Anogeissus leiocarpus grown in southern Borno particularly in Kwaya-Kusar LGA, Borno State.

MATERIALS AND METHODS

Sample Collection

The stem barks of *Anogeissus leiocarpus* was collected in April, 2023 from a near-by farmland along Gombe-Biu Road in Kwaya town, Kwaya Kusar Local Government Area, Borno State, Nigeria. The stem bark sample was transported in a clean dry polythene bag to Nigerian Army University. The sample was identified and authenticated by Sanusi Kabir in the Department of biology, Faculty of Natural and Applied Sciences, Nigerian Army University Biu (NAUB). The voucher specimen of sample was deposited in the herbarium.

Methodology

Sample Preparation

The stem barks of *Anogeissus leiocarpus* were dried under shade at room temperature for a month and grinded into powder using mortar and pestle in the Chemistry Laboratory, Department of Chemistry, NAUB. 200 grams of finely powdered stem bark of *Anogeissus leiocarpus* was extracted with methanol, ethanol and distilled water each using maceration method for 7 days with gentle agitations. The extract was decanted, filtered and evaporated to dryness on rotary evaporator at 40 °C; the yield of the methanol extract was measured. Furthermore, Successive maceration was also carried out for Ethanol (200 ml) and water (200 ml). Extracts were concentrated on rotary evaporation.

Phytochemical screening

Qualitative phytochemical tests of the stem bark sample were conducted in accordance to reported standard procedures [1].

Test for flavonoids (Alkaline reagent test)

The filtered extract (2 ml) was mixed with a few drops of 20% NaOH. The formation of intense yellow colour was detected. Then, a few drops of 70% diluted hydrochloric acid was added, and the yellow colour disappeared. The formation and disappearance of yellow color indicate the presence of flavonoids [18].

Test for phenols (Ferric chloride test)

2 ml of filtered sample will be mixed with 2 ml of 5% aqueous $FeCl_3$. The formation of the blue color points out the occurrence of phenols [19].

Test for tannins (Ferric chloride test)

2 ml of filtered sample was added to 10% of alcoholic $FeCl_3$. The formation of the black/brownish blue indicates the presence of tannins [20].

Test for alkaloids (Dragendorff's Test)

2 ml of filtered crude extract was dissolved separately in dilute Hydrochloric acid and filter. The filtrate was then treated with Dragendorff's reagent (potassium bismuth iodide solution). The formation of a red precipitate indicates the presence of alkaloids [20].

Test for terpenoids (Chloroform test)

2 ml of the crude extract was added into 0.5 ml chloroform, 0.5 ml of acetic anhydride, and a few drops of concentrated sulfuric acid. The formation of reddish-brown precipitate shows the presence of terpenoids [18].

Test for steroids

2 ml of crude extract was mixed with potassium hydroxide solution. The blood-red color shows the presence of anthraquinones [22].

Test for saponins (Foam test/ Frothing test)

2 ml of the crude extract was added into 4 ml of distilled water. The solution was mixed well and shake vigorously. If the foam is persisted for ten (10) minutes then saponins are present [18].

Test for glycosides (Keller-Kilian Test)

2 ml of crude extract was mixed with 0.5 ml glacial acetic acid, 3 drops of 1% aqueous $FeCl_3$ solution, and 0.5 ml H_2SO_4 concentrated. The formation of brown ring between the layers indicates the presence of cardiac steroidal glycosides [23].

Antibacterial activity

Test Organisms

Escherichia coli and *Staphylococcus aureus* were obtained from the research laboratory of the Department of medical microbiology and Parasitology, General Hospital, Biu. The isolated colonies were obtained and maintained on Muller-Hinton agar (MHA) (Oxford, UK).

Agar Disc diffusion method

Stem bark extracts were tested for antibacterial activity by the disc diffusion method. A single colony was aseptically transferred with an inoculating loop to about 20 ml of the prepared nutrient agar. Filter papers are cut out with a diameter of 10mm. The filter paper is then transferred to the oven and sterilized for one hour. Using sterilized forceps, the filter papers were then transferred to the various extracts at a concentration of 1000 µg/ml. The filter papers were left in the extracts for about 20 minutes so as to soak the extracts very well. The filter papers were then transferred to cultured agar plates. The plates are then incubated at 37ºC for 24 hours in the incubator. Amphiclox was used as standard antibiotic drug and Acetone was used as a negative control.

Antioxidant activity of Stem-bark Extracts

Samples were prepared by dissolving 0.5 g of the stem bark extracts in 250 ml of solvent (2000 ppm stock solution). Solution of following varying concentrations. (1000, 500, 250, 100, 50 mg/ml) were obtained by serial dilution. Antioxidant activity (DPPH free radical scavenging activity) of the extracts was measured based on the scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as described by Brand-Williams [24]. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard. 2 ml of 0.002% of DPPH solution in methanol was mixed with

2 ml of plant extract solution of varying concentrations. Corresponding blank samples were prepared and L-Ascorbic was used as reference standard. Mixture of 2 ml methanol and 2 ml DPPH solution was used as control. These solutions were kept in dark for 30 min, and optical density measured at 517 nm using UV-Vis spectrometer, LT-290 Labtronics model. The reaction was carried out in duplicate and the decreased in absorbance was measured at 517 nm. The DPPH radical scavenging activity (S %) was calculated using the equation

$$S\% = \frac{(\text{A control} - \text{A sample})}{A\ control} \times 100$$

A control is the absorbance of the blank control (containing all reagent except the solution extract) and A sample is the absorbance of the sample.

RESULTS AND DISCUSSION

Maceration was reported among the most commonly applied extraction methodologies [25] because of its effectiveness and simplicity and safety to handle. The extraction yield of stem bark of Anogeissus leiocarpus with methanol, ethanol and distilled water was presented in table 1 which showed that distilled water has the least yield. Methanol and Ethanol yields showed better yield but with insignificance difference in the percentage yields between the two solvents of course due to their functionality. The results of qualitative phytochemical tests of the crude extracts showed that stem barks of the plant contained saponins, flavonoids, Tannins, steroids, glycosides, Terpenoids, Phenol, and alkaloids. The aqueous extract contains flavonoids, steroids, saponins, glycosides and tannins but terpenoids, alkaloids, phenols were not detected. The methanol extract contains flavonoids, alkaloids, saponins, steroids, terpenoids, phenols and glycosides while tannins are not detected. The ethanol extract contains all the phytochemicals tested. The present of these phytochemicals point out the significance of *Anogeissus leiocarpus* in herbal remedies. This could also be the possible reason for its medicinal values in African traditions. The results agreed with the work of Usman and Danjani (2020), who reported the presence of alkaloids, tannins, saponins, flavonoids, steroids and Glycosides [26]. Similarly, Muhammad et al. and Hafsat et al., have reported the presence of alkaloids, glycosides, phenols, steroids, tannins, saponins, flavonoids and anthraquinones in the plant. Muhammad et al., carried out research on stem-bark powder of *Anogeissus leiocarpus* which was extracted with butanol, hexane and water using percolation and Soxhlet extraction methods [27]. The extract fractions were screened for the presence of secondary metabolites using standard procedures which revealed the presence of secondary metabolites including alkaloids, cardiac glycosides, reducing sugars, tannins, saponins, flavonoids, amino acids, anthraquinones, carbohydrates, steroids, triterpenes, monosaccharides and glycosides. Usman et al., also reported the presence of, alkaloids, steroids, saponins, tannins, flavonoids and reducing sugars [28].

Phytochemicals are reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants [29]. The antibacterial sensitivity tests of the three extracts on *E. coli* and *S. aureus* were carried out as a preliminary step for antibacterial screening. The results presented in table 3 showed that *E. coli* was not sensitive to all tested concentrations of the aqueous extract. The aqueous extract did not inhibit the growth of *E. coli* at the tested

concentrations. *E. coli* was only sensitive to ethanol extract at 500 and 1000 mg/mg but not sensitive at lower concentrations. The ethanol extract could inhibit growth of the bacterium only at higher doses. In methanol extract, *E. coli* was sensitive only at the concentration of 1000mg/ml but at the lower concentrations it was not sensitive. It means the extract inhibits the growth of *E. coli* only at the highest concentration of the extract. The aqueous extract however demonstrated a significant growth inhibition at 250 to 1000 mg/ml against *S. aureus* from the results of the sensitivity tests (table 3). *S. aureus* was more sensitive to aqueous extract than the methanol and the ethanol extracts, even though it was sensitive at 250, 500 and 1000 mg/ml of the methanol extract but it was not sensitive at lower doses. *S. aureus* was sensitive to ethanol extract at the two higher concentrations but not sensitive at lower concentrations. The results revealed that growth inhibition of the tested bacteria varied with concentrations of the extracts. The three extracts used in the study have shown effectiveness against two bacterial species with sensitivity at different doses. This is in agreed with the results reported by Dambatta [30] on *in-vitro* antibacterial activity of *Anogeissus leiocarpus* extracts against *Escherichia coli* and *Staphylococcus aureus*. Muhammad et al., [27] carried out research on stem-bark powder of *Anogeissus leiocarpus* which was extracted with butanol, hexane and water and further tested for antibacterial activity against clinical bacterial isolates of respiratory tract infections including *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella ozaenae*, *Escherichia coli* and *Pantoea agglomerans* [27]. The antibacterial effects produced by the extracts were dose dependent against *Staphylococcus aureus* and *Salmonella typhi* while *Klebsiella* Species were found to be resistant at lower tested doses. The *in vitro* anti-spasmodic effect revealed that the ethanol leaf extract has a dose dependent depolarizing effect on the rabbit ileum [31]. Usman et al., [29] reported that stem barks of *Anogeissus leiocarpus* extracts were not active against Methicillin Resistant *Staphylococcus aureus*, *Klebsiella pneumonia* and *Candida krusei*.

The results of an antioxidant assay of the methanol, ethanol, and aqueous extracts was presented in figure 3. The results showed the percentage inhibition of an antioxidant assay for the three (3) different crude extracts at various concentrations. The percentage inhibition values indicate the effectiveness of each extract. Higher percentage inhibition values particularly of ethanol extracts revealed that the extracts could be more effective. The absorbance values were measured at five different concentrations of each extract and the results was presented in figure 3.

Table 1. Extraction Yield of the stem bark of *Anogeissus leiocarpus*

Mass of sample used (200 g)	**Methanol extract (g)**	**Ethanol extract (g)**	**Aqueous extract (g)**
Extraction yield	9.6	9.8	6.4
% yield	4.8	4.9	3.2

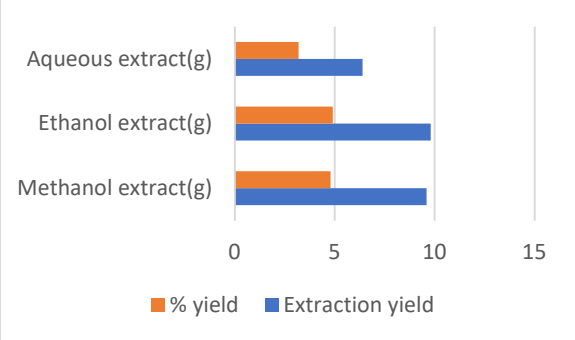

Figure 1. Solvent impact on Extraction yield of stem bark sample of *A. leiocarpus*.

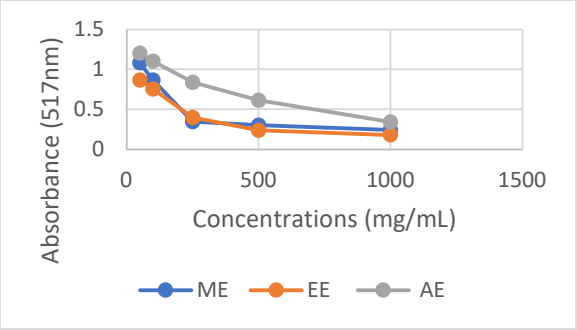

Figure 2. Absorbance of test concentrations of stem bark extracts of *A. leiocarpus*

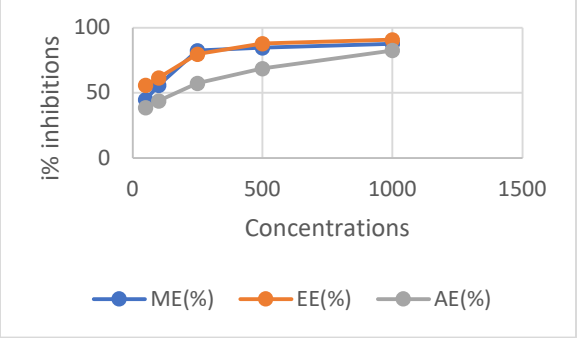

Figure 3: Percentage DPPH free Radical scavenging Activity of stem bark extracts of *A. leiocarpus*

Table 2: Antibacterial sensitivity test of stem bark extracts of *A. leiocarpus*

Extracts	Concentration (mg/mL)	Bacterial spp.	
		Escherichia coli	*Staphylococcus aureus*
ME	1000	+	+
	500	-	+
	250	-	+
	100	-	-
	50	-	-
EE	1000	+	+
	500	+	+
	250	-	-
	100	-	-
	50	-	-
AE	1000	-	++
	500	-	++
	250	-	++
	100	-	-
	50	-	-

CONCLUSION

In vitro evaluation of plants for antimicrobial property is the first step towards achieving the goal for developing eco-friendly management of infectious diseases of humans by search for new bio-molecules of plant origin. This paper reports preliminary investigation and the results so far revealed vital information on the phytochemicals constituents and bioactivity of the stem barks. The results of the investigation of stem barks of *Anogeissus leiocarpus* supported the claims of local uses of the plants in herbal remedies for management of numerous human ailments as it contained bioactive metabolites and exhibited antibacterial as well as antioxidant properties. Further research on anti-microbial and cytotoxicity studies of the plant with in-depth screening of plant is underway from our current research works on the plant.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

FUNDING

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ETHICAL STATEMENT

This work required no ethical statement.

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