

Phytochemical screening and antimicrobial activity of the root of *Anogeissus leiocarpus*

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Abstract:

Traditionally, medicinal plants have been used to heal and treat a wide range of health conditions, including pain, inflammation, skin disorders, digestive troubles, and more. The primary emphasis of this investigation was the Phytochemical screening and antibacterial activity and of *Anogeissus leiocarpus* (*A. leiocarpus*) root crude extracts. Phytochemical screening was carried out using standard methods and antibacterial sensitivity test was carried out using agar diffusion method. The methanol extract contains screening Phytochemicals such as tannins, alkaloids, phenols, terpenoids, saponins, and cardiac glycosides. On the other hand, saponins, tannins, alkaloids, flavonoids, terpenoids, phenols, and cardiac glycosides are found in water crude extract. *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and yeast were the four bacterial strains used in the Agar diffusion method antimicrobial tests of both extracts. The methanol extract was shown to have a significant effect on the other strains, however it was less effective against *Salmonella typhi* (200 mg/ml, only 10 mm), *Staphylococcus aureus* (200 mg/ml, only 24 mm), *Escherichia coli* (200 mg/ml), and yeast (200 mg/ml, only 26 mm Zone of inhibition) ZOI. At the lowest concentration of 6.25 mg/ml for all microorganisms in the water extract, no ZOI was seen; nevertheless, the extract was susceptible to other strains, with the yeast strain exhibiting approximately 28 mm ZOI at 200 mg/ml. This confirms traditional healers' folkloric assertions and attests to the presence of bioactive chemicals in *A. leiocarpus* root that may have therapeutic value. As such, it may be a promising target for medication development.

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1. Introduction

Medicinal plants are extremely valuable for both human and animal healths because of the compounds that they contain that have specific physiological effects [1]. Many of the current conventional medications are derived from plants, and plants are a rich source of a wide variety of secondary [2]. It is known that plants' secondary metabolites might differ depending on where they are found. Consequently, other phyto-compounds with significant biological activity that may not be present in the same plants but gathered from different sites may be discovered through comparative phyto-chemical investigations of medicinal plants obtained from various locations. According to Sutaryo, *et al.* [3], of the 119 plant-derived medications recognized by the World Health Organization, 74% were found through chemical research that isolated the phyto-chemical components that gave rise to their traditional medicinal uses. The use of medicinal plants as a medication is merely based on a customary folk use that has been carried down through many generations, where low-income rural and urban inhabitants rely on these plants to treat various ailments without really understanding what the active ingredient in these plants [4]. Although plant-based

medications are safe, there have been numerous reported dangerous and deadly adverse effects [5]. A comprehensive scientific safety assessment of the medicinal plants is required in light of the growing interest in herbal medicines [3]. Medicinal plants are used extensively to treat illnesses all over the world. This may be because they contain a wide variety of phyto-constituents, including cardiac glycosides and tannins [6, 7].

New therapeutic agents are in high demand because they can treat infectious disorders that are resistant to drugs [8]. Although there has been progress in the development of antimicrobial medicines, drug-resistant bacteria and the concealment of unidentified microbial infections remain significant public health concerns. Global health concerns about microorganism resistance to one or more antimicrobial treatments have prompted researchers worldwide to create chemotherapeutic drugs that can be used to treat diseases caused by pathogenic microorganisms [9, 10]. Scholars that are intrigued by the therapeutic properties of medicinal plants are not only interested in establishing the plants' traditional usage, but also in identifying the active ingredients that possess antimicrobial resistance inside the plants [11, 12].

A. leiocarpus, a huge deciduous tree of the combretaceae family, is also known as African birch, *Marke* in Hausa, and *Tava* in Marghi (TəwnaTəm). It originated in sub-Saharan Africa and is now extensively found in nations including Ghana, Nigeria, Cameroon, Sudan, and the Ivory Coast. Traditionally, it has been known that using the root of *A. leiocarpa* as a chewing stick has antibacterial effects on *Lactobacillus* species [13]. About 163 different plant species are utilized as chewing sticks in the Benin Republic, according to Yedomonhan, et al. [14]. While the stem and roots are both employed as chewing sticks, the therapeutic properties of the root make them more popular. The plant's extract and that of *Xanthoxylumgilletti* combined with citrus juice showed promise against HIV-related opportunistic illnesses and their sequelae [15]. The plant is used by traditional healers in the Ivory Coast to treat dysenteric syndrome, trypanosomiasis, malaria, and helminthiasis [16]. According to recent research, the plant demonstrates a range of pharmacological properties, such as leishmanicidal [17], antioxidant and hepatoprotective [18, 19], and antiplasmodial [20, 21]. Little or no work in recent pass has been done on this medicinal plant for it Phytochemical and antimicrobial activity. Thus, the purpose of this work is to examine the Phytochemical and antibacterial properties of *A. leiocarpus* root extracts prepared using methanol and water.

2. Material and methods

2.1 Sampling and sample preparation

A. leiocarpus plant roots were gathered from an uncultivated location in Hong, Adamawa state, Nigeria. Mr. Sunday Zira, a taxonomist from the Department of Forestry at Modibbo Adama University, Yola, recognized the roots. For two weeks, the root was air dried in the Modibbo Adama University, Chemistry Department in Yola. The dry portion was broken into bits and manually pounded into a fine powder using a sterilized, clean pestle and mortar. The material that had been powdered was gathered and labeled in sterile cellophane packets. The sample was stored until it was needed in a cold, dry location. For the extraction, the sample that had been pulverized was utilized.

2.2 Extraction Process

2.2.1 Soxhlet Extraction

Using this procedure, the Soxhlet apparatus's thimble chamber held the crushed sample. After being heated in the bottom flask, the extraction solvent, methanol, vaporized into the sample thimble, condensed in the condenser, and then began to drip back. The procedure was repeated after the liquid content reached the siphon arm and poured into the bottom flask [22].

After weighing about 30 g of the dried sample powder, 250 ml of methanol is used in the Soxhlet apparatus for 48 hours to complete the extraction procedure. After eight hours of evaporation at 70 °C, the extract was dried and concentrated. Prior to Phytochemical screening, the concentrated extract was prepared as a gel and kept at room temperature [23].

2.2.2 Maceration Extraction

A beaker containing 30 g of the powdered plant sample was filled with 150 ml of water to allow it to percolate. After properly sealing the beaker with aluminum foil, it was left for a full day. After that, the extract was extracted by filtering the mixture via a funnel that was placed within filter paper. A rotary evaporator operating at 40°C was used to concentrate the extracted material. Before being used, the concentrated extract was kept in a universal container and chilled at 4 °C [24].

2.3 Phytochemical Screening

Qualitative analyses of methanol and water of *A. leiocarpus* root. The extracts underwent a preliminary phytochemical examination to check for the presence of alkaloids, flavonoids, saponins, cardiac glycosides, and terpenoid, as reported by [25, 26, 27]

2.3.1 Test for Saponins

Foam Test: To create stable and long-lasting foam, 10 milliliters of the filtrate were combined with 5 milliliters of distilled water and aggressively agitated. Saponins were present because froth formed [26].

2.3.2 Tesfor Alkaloids

Mayer's Test: Two milliliters of 10% HCl and two milliliters of Meyer's reagent were applied to two milliliters of the extract, respectively. The presence of alkaloid is shown by the production of an orange precipitate [27].

2.3.3 Test for Tannins

Iron (III) chloride Test: Three drops of 0.1% ferric chloride was added to 5 cm³ of the filtrate. Tannins are indicated by a blue-black or brownish-green hue [26].

2.3.4 Test for Phenol

Iron (III) chloride Test: A small amount of 10% FeCl₃ was added after 2 milliliters of distilled water were added to 1 milliliter of plant extract. The development of a blue-green tint indicates the presence of phenol [26].

2.3.5 Test for Flavonoids

Ammonia solution Test: Five centiliters of a 1.0M diluted ammonia solution and five centiliters of concentrated tetraoxosulphate (VI) acid were added after precisely ten centiliters of the extract's aqueous filtrate were added. The presence of flavonoids is shown by the yellow hue that initially emerged and then vanished upon standing [25].

2.3.6 Test for Cardiac Glycosides

Keller-Killani test: In a test tube, one milliliter (1 ml) of concentrated H₂SO₄ was made. A mixture of 5ml of the plant material samples' aqueous extract and 2ml of glacial CH₃CO₂H with one drop of FeCl₂ is combined. Carefully adding the mixture above to the 1 ml of concentrated H₂SO₄ resulted in the concentrated H₂SO₄ being positioned underneath the mixture. The presence of the cardiac glycoside component is indicated by the formation of a brown ring [25].

2.3.7 Test for Terpenoids

Salkowski test: To create a layer, 5cm³ of the extract was mixed with 2cm³ of chloroform and 3cm³ of concentrated tetraoxosulphate (VI) acid. Positive evidence for the presence of terpenoids is the reddish-brown coloration near the contact [26].

2.4 Determination of Antimicrobial Activity

At Modibbo Adama University's microbiology laboratory in Yola, clinical isolates of *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, and yeast were obtained. On nutritional agar medium and sabour and dextrose agar media, respectively, the bacterial and fungal cultures were kept alive.

Using agar diffusion techniques, the antibacterial properties of the *A. leiocarpus* crude root extracts in methanol and water were ascertained. Nutrient agar that has been sterilized was added to a Petri plate and let to set. The test-inoculated nutritional broth was added to the pre-set Petri plate and evenly dispersed. The control was added in the middle, given two hours to diffuse at room temperature, and then incubated for twenty-four hours at 37°C. Using a clear ruler, the zone of inhibition (ZOI) caused by the different reactions of the test organisms at different extract concentrations was calculated. This determined the sensitivity level [29].

3. Result and Discussion

Table 1 show the percentage yield of *A. leiocarpus* root using methanol and Water as solvent and soxhlet and maceration method of extraction respectively.

Table 1: Percentage (%) yield

Solvent Used	Method of Extraction	Duration	% Yield
Methanol	Soxhlet	48 Hours	21.11
Water	Maceration	3 days	4.00

The *A. leiocarpus* root was extracted using two different methods: maceration (which used water as a solvent) and soxhlet extraction (which used methanol as a solvent). The yield percentage achieved with soxhlet extraction is 21.11%, whereas the yield percentage through maceration of water is 4.0 %. These arise from the solvent's polarity as well as the extraction method's effectiveness. Water and methanol are both polar compounds, but they have different capacities for dissolving and extracting Phytochemicals. Given that water is more polar than methanol, it is evident that soxhlet extraction is more effective than the maceration approach. Table 2 displays the results of the phytochemical examination of the *A. leiocarpus* root's methanol and water extracts. The antibacterial effectiveness of the water and methanol extracts against the investigated microorganisms is displayed in Table 3.

Table 2: Phytochemical screening of methanol and water extract from roots of *A. leiocarpus*

Phytochemicals	Test Type	Methanolic Extract	Water Extract
Saponins	Foam	+	+
Tanins	Iron (iii) Chloride	+	+
Flavonoids	Ammonia Solution	-	+
Alkaloids	Meyer's	+	+
Terpenoids	Salkowski	+	+
Phenols	Iron(iii) Chloride	+	+
Cardiac glycosides	Keller-kiliani	+	+

Key: + Present
- Absent

Table 3: Antimicrobial efficacy of methanol extract from roots of *A. leiocarpus* (Zone of inhibition in mm)

Microorganisms	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
<i>Salmonella typhi</i>	10	6	4	0	0	0
<i>Staphylococcus aureus</i>	24	22	18	16	12	10
<i>Escherichia coli</i>	22	20	18	16	14	8
Yeast	26	20	18	10	8	4

The reports of Sutaryo, et al.[3], Namadina, et al. [28], and Barku, et al. [30], indicated the presence of saponins, tannins, alkaloids, phenols, flavonoids, and cardiac glycosides are consistent with table 2 above. It contradicts the study by Olajide [18], which indicated that terpenoids, glycosides, and alkaloids were absent. According to Kubmarawa, et al. [31], the presence of saponins, alkaloids, and tannins is significant as an antibiotic for treating pathogenic strains. The most significant applications of cardiac glycosides are in the treatment of congestive heart failure, as well as their anticancer and rhinovirus-inhibitory properties [3]. The plant may have biological activity, including antifungal, antiviral, and antioxidant qualities, based on the presence of saponins. Immunomodulatory effects

are another possible outcome [3]. According to Saxena, et al. [32], terpenoids possess medical advantages such as anti-ulcer, antibacterial, and sesquiterpenoid antimalarial properties, in addition to the diterpenoid anticancer medication taxol. Two benzene rings and flavones are the precursors of flavonoids. They demonstrate anticancer pharmacological actions and biological features include antibacterial, cytotoxic, and anti-inflammatory effects [32]. Many plant extracts contain tannins, which have the ability to scavenge free radicals [29]. They are thus employed as antioxidants.

The isolates of *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*, and yeast were subjected to antimicrobial activity. The zone of inhibition of Methanol

extract of *A. leiocarpus* on the isolated organisms at varying concentrations is displayed in Table 3. Table 4 indicates that *A. leiocarpus* Methanol extract has a greater antibacterial action on yeast, *E. coli*, and *Staphylococcus aureus* than on *Salmonella typhi*. At 200 mg/ml, MLE displayed a high ZOI of 26 mm on yeast. There was only a 4mm zone at 6.25

mg/ml. *Staphylococcus aureus* was found to have a 10 mm ZOI at a low concentration of 6.25 mg/ml, which is equivalent to the 200 mg/ml ZOI for *Salmonella typhi*. According to Usman *et al.* [32], the antibacterial effect of *A. leiocarpus* MLE on *E. coli* is demonstrated, which contradicts the findings of Mann [8].

Table 4: Antimicrobial efficacy of water extract from roots of *A. leiocarpus* (Zone of inhibition in mm)

Microorganisms	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
<i>Salmonella typhi</i>	8	5	2	-	-	-
<i>Staphylococcus aureus</i>	20	16	14	10	4	-
<i>Escherichia coli</i>	24	18	12	8	6	-
Yeast	28	20	16	12	6	-

The Water extract is susceptible to *Salmonella typhi* but more sensitive to *Staphylococcus aureus*, *Escherichia coli*, and yeast, according to Table 4's notable ZOI against *Salmonella typhi*. Given that MLE does not exhibit any discernible susceptibility to *Salmonella typhi* in Table 3, there is a strong link between the sensitivity of methanol extract and water extract to *Salmonella typhi*. For all test pathogen, no ZOI was seen at a concentration of 6.25 mg/ml. The extracts' susceptibility to organisms varies based on concentration.

With regard to antifungal activities, the plant outperforms the other test species. Regarding isolated yeast, the zone of inhibition is greater at high doses than it is for other microorganisms in Tables 3 and 4. This suggests that the plant can treat illnesses caused by fungi. This is consistent with the [31] report. Tables 3 and 4 suggest that *A. leiocarpus* may be susceptible to *Salmonella typhi* in its synergistic action.

4. Conclusion

The investigation verified that *A. leiocarpus*'s Methanol extract and Water extract included terpenoids, flavonoids, alkaloids, saponins, and cardiac glycosides. These Phytochemicals exhibited significant antibacterial action against yeast, *E. coli*, and *Staphylococcus aureus*. This suggests that *A. leiocarpus* may be beneficial in treating some strains. For this plant to effectively combat *Salmonella typhi*, it must work in concert. In contrast to prior research on *A. leiocarpus*, this one demonstrates how phytochemicals vary with geographic location and how pathogen sensitivity.

Conflict of Interest

The authors declare no conflict of interest exist

Reference

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