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Original Research Article

Phytochemical Evaluation and Herbal Formulation of the Stem bark of *Anogeissus leiocarpus* (DC) Guill. & Perr. (Combretaceae)

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Abstract

Purpose: *Anogeissus leiocarpus* (Combretaceae) is a medicinal plant traditionally used by the Hausa tribe of Northern Nigeria for managing respiratory tract infections. Its antibacterial and antifungal properties have gained attention, especially during the COVID-19 pandemic. This study focuses on the phytochemical screening and formulation of tea from the powdered stem bark of *A. leiocarpus*. The study aimed to evaluate the phytochemical composition of the aqueous stem bark extract, formulate loose and bagged teas, and assess their quality and safety.

Method: Phytochemical screening was conducted to identify bioactive compounds. Loose and bagged teas were formulated using standard procedures. Quality assessment included uniformity of mass and optimization of extraction. Acute toxicity was evaluated in Wistar rats using OECD guideline 425.

Results: Phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, tannins, steroids, and triterpenes. Tannins had the highest concentration (0.117 mg/ml), while saponins had the lowest (0.030 mg/ml). The formulated tea bags passed the uniformity of mass test, with optimal extraction achieved by infusing a tea bag in 200 mL of boiled water for 10 minutes. The acute toxicity study indicated a wide margin of safety, with an LD_{50} greater than 5000 mg/kg body weight.

Conclusion: The aqueous stem bark extract of *A. leiocarpus* is rich in phytochemicals and can be safely formulated into loose and bagged teas. The findings support its traditional use and potential for further development as a therapeutic agent.

Keywords: Phytochemicals, tea, Stem bark, Formulations

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INTRODUCTION

Anogeissus leiocarpus (DC) Guill. & Perr. (Combretaceae) is a deciduous tree species that can grow up to 15 -18 metres in height and measures up to 1.0 metre in diameter [1]. The plant has been reported to contain several phytochemical constituents namely flavonoids, tannins, phenolic acids and triterpenes which are believed to have important pharmacological activities [2]. It is used in several traditional medicines in Africa to treat various diseases [3]. *Anogeissus leiocarpus* possesses antibacterial, antifungal, anthelmintic, antiplasmodial, trypanocidal, Leishmanicidal, hepatoprotective and antioxidant activities [4]. It is used by the Hausa tribe of Northern Nigeria traditionally for the management of respiratory tract infections and that it was later used during the COVID-19 pandemic [5]. The plant is also used for the treatment of diabetic ulcers, general body pain, blood clots, asthma, coughing and tuberculosis [6].

Herbal preparations are one of the most important traditional medicine (TM) therapies and remain the mainstay of about 80% of the world's population, mainly in developing countries for primary health care [7]. It has been estimated that 25% of modern medicines are made from plants that were first traditionally used. The reasons for this are complex, and may have probably stemmed from the ability of plants to produce structurally diverse molecules; these molecules are made from raw, renewable resource by an eco-friendly process [7]. Among several factors contributing to the potential use of phytomedicines are safety, lack of adverse reactions and minimal side effects which have been mostly found to significantly influence the use of such medicines in developed countries [8]. In rural areas there are additional cultural factors that encourage the use of herbal preparations; people believe that where an area gives rise to a particular disease, it will also support plants that can be used to treat it. Additionally, hundreds of primary health care centres intended to serve rural areas lack staff, diagnostic facilities, and adequate supplies of medicines [9]. Traditional herbal medicines in different formulations such as teas are getting significant attention in global health. This paper seeks to investigate the phytoconstituents of *A. leiocarpus* stem bark, its safety margin, and the formulation of its powder into tea preparations.

MATERIALS AND METHODS

Plant Collection, Identification and Preparation

Anogeissus leiocarpus consisting of the leaves and flowers was collected at Samaru (11.0855° N 7.7190° E), Zaria in April, 2023. It was identified and authenticated by Mr. N Sunusi of the Herbarium unit, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria and a voucher specimen number - ABU 01738 was assigned to it for future reference.

Preparation of Herbal Material

The bulk stem bark of *A. leiocarpus* was collected and scrapped to remove sand clumps, rotten and fibrous sections. The stem bark was washed several times with potable water. It was then cut into smaller pieces with a clean, sharp cutlass and pulverized using a thoroughly washed and clean grinder. The wet mass of the powdered stem bark was dried in an oven set at 50^{o}C and screened through an appropriate sieve. The dry powder was packed in a waterproof bag and stored in a cool dry place until used [10].

Extraction of *A. leiocarpus* Stem Bark Powder

The prepared stem bark powder (0.5 Kg) was macerated for 72 hours with distilled water. The resultant aqueous extract was freeze dried and then kept and subsequently used for the phytochemical screening, quantitative analysis and toxicity testing [11].

Experimental Animal

Wistar rats of both sexes weighing (100 - 150 g) were used for the acute toxicity evaluation. They were obtained from the Animal House, Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria. The animals were maintained in a ventilated room, fed on standard animal feed and granted access to water. Ethical approval for the experimental protocols was sought from the Ahmadu Bello University Animal Use Ethics Committee; the ethical approval number was ABUCAVC/2023/069.

Phytochemical Screening

Phytochemical screening of the aqueous extract was carried out using the methods described by Evans [12] and Sofowora [13].

Quantitative Evaluation

The quantitative phytochemical evaluation was carried out using UV Spectrophotometer (1001 Plus Milton Roy USA) using the following methods.

Determination of total phenolic content (TPC)

Estimation of total phenol content was measured spectrophotometrically by using Folin - Ciocalteu colorimetric method, using Gallic acid as the standard and expressed as Gallic acid equivalent

(GAE) per gram of sample in accordance with Ainsworth and Gillespie [14].

Determination of total flavonoid content (TFC)
The TFC was determined by using aluminium chloride colorimetric assay. It was determined as mg quercetin equivalent per gram of sample [15].

Determination of total alkaloid content (TAC)
The TAC was quantified by spectrophotometric method. This method is based on the reaction between alkaloid and bromocresol green (BCG). It was determined as mg Atropine equivalent per gram of sample [16].

Determination of tannin Content
The tannin content was determined by Folin - Ciocalteu method. The tannin content was expressed in terms of mg of Gallic acid equivalent (GAE) per gram of sample [17].

Determination of Saponins Content
Total saponins content was determined by using spectrophotometric method. The total saponins concentration was expressed as mg diosgenin equivalents (DE) per gram dry weight (DW) of the sample in accordance with Makkar et al. [18].

Acute Toxicity Evaluation
An acute toxicity study was conducted using the Organization for Economic Co-operation and Development (OECD) Test Guideline [19] in Wistar rats. The limit dose test procedure was adopted to evaluate the acute toxicity of the aqueous extract of *A. leiocarpus* via oral administration in Wistar rats. A total of 3 rats were used. Prior to treatment rats were fasted overnight but allowed free accesses to water. One rat was dosed with 5000 mg/kg of the aqueous extract of the plant. The rat was observed during the first 30 minutes and one-hour post dosing. After 24 hours, two additional rats were dosed and observed for a period of 14 days for signs of toxicity namely convulsion, hyperactivity, dullness, diarrhoea, tiredness, weakness and excessive urination and consequent mortality.

Formulation of Herbal Teas
Appropriate quantity of previously processed dry powder of *A. leiocarpus* stem bark (100 g) was then put into clean small plastic container, tightly closed and labelled as the loose herbal tea. Another batch of the previously processed stem bark powder (100 g) required to prepare 20 tea bags was weighed, such that each tea bag contains 5 g of the processed powder was then dispensed into the tea bags, appropriately sealed and labelled as the bagged herbal tea. [20,21].

Uniformity of Mass of Formulated Tea Bags
Ten (10) individual tea bags were selected at random and weighed. Each tea bag was weighed in its entirety, then emptied completely, ensuring no tea residue remained. The weight of the empty tea bag was subtracted from the initial weight to determine the net weight of the tea content. This process was repeated for all twenty tea bags. The average weight of the twenty tea bags was calculated. By analyzing the variation in these weights, an assessment of the tea bag's mass uniformity was made. [22]

Optimization of Extraction Method of Aqueous Extract of the Tea Bag
A tea bag, weighing 5 g net, was steeped in 150 mL of freshly boiled water for 10 minutes. The infusion was then filtered, and both the solid residue (tea leaves) and the liquid extract were measured. This extraction process was repeated using 200 mL, 250 mL, and 300 mL of freshly boiled water. Each extraction volume was tested in triplicate to ensure accuracy and reliability [23].

RESULT AND DISCUSSION

The plant was found to be rich in important phytochemical constituents namely alkaloids, flavonoids and tannins (Table 1). When these phytochemicals were quantified, tannin content was the highest indicating that this plant was rich in tannins (Table 2). This was supported by previous findings by Elegami [24], where a preliminary phytochemical screening of the *Anogeissus leiocarpus* stem bark for the major secondary constituents showed that, the plant was rich in tannins and having appreciable quantities of flavonoids, terpenes and saponins, however it was devoid of alkaloids and anthraquinones. Some of the identified phytoconstituents of this plant were reported to have antimicrobial activities and cytotoxic effects [24,25].

Oral administration of the aqueous stem bark extract of *A. leiocarpus* at a limit dose of 5,000 mg/kg body weight in Wistar rats did not produce any visible sign of toxicity over a period of 14 days. Therefore, the LD_{50} in the Wistar rats was estimated to be above 5,000 mg/kg. The aqueous stem bark extract of *A. leiocarpus* was found to have a wide margin of safety when administered orally in rats making it a potentially good herbal preparation for human consumption. This was supported by previous research that was conducted by Agaie [26], where investigation of oral acute

toxicity of the aqueous leaf extract of the plant in rats revealed no deaths with oral doses of up to 3,200 mg/kg body weight; however, the rats showed signs of depression and in appetence. Histopathological changes were not observed in any organs except the lungs, which suggested that the aqueous leaf extract of the plant should be used with some degree of caution, especially when administered.

Table 1: Phytochemical Constituents from the Aqueous Stem Bark Extract of *A. leiocarpus*

Constituent	Test/Reagent	Observation	Inference
Carbohydrates	Molisch	Red ring at interface	Present
	Fehling	Brick red precipitates	Present
Anthraquinones	Borntrager	No observable colour change	Absent
	Modified Borntrager	No observable colour change	Absent
Cardiac glycosides	Keller-Kiliani	No observable colour change	Absent
	Kedde	No observable colour change	Abesnt
Saponins (Steroids/triterpenes)	Frothing	Honey comb froth	Present
	Haemolysis	Haemolysis of Red blood cells	Presnt
	Lieberman-Burchard	Blue-green supernatant & reddish ring interface	Present
	Salkowski	Cherry red colour	Present
Flavonoids	Ferric chloride	Greenish black colour	Present
	Sodium hydroxide	Yellow solution turns colourless	Present
	Shinoda	Reddish colour	Present
Tannins	Ferric chloride	Blue-green precipitates	Present
	Lead acetate	Whitish yellow precipitate	Present
Alkaloids	Mayer	Whitish precipitates	Present
	Dragendorf	Orange red precipitates	Present
	Wagner	Brown precipitates	Present

Loose and bagged teas were successfully formulated from the processed stem bark powder of *A. leiocarpus* as shown in Figure 1, suggesting that the plant can be suitably used in traditional herbal preparations. This is because herbal preparations in the form of teas have been reported to be one of the most suitable forms for the intake of herbal medicines in traditional practice. Herbal teas are indeed a presentation of herbal medicines. They consist of mono or poly-herbal materials that are used as decoction or infusion that are consumed for their therapeutic benefits [27].

The formulated tea bags underwent uniformity of mass and optimization of extraction assessment as part of quality evaluation of the plant's tea bags. The formulated *A. leiocarpus* tea bags recorded an average net weight of 5 ± 0.05 g. It also passed the uniformity of mass test since no tea bag deviated by 10 % or more and none deviated by more than 20 % from the mean weight. The successful passing of the uniformity of mass test by the formulated tea bags could be attributed to even filling of the tea bags. Uniformity of mass ensures consistent dosing of the medicament. This is because the correct weight of a tea bag increases the likelihood of achieving the right therapeutic dose, ensuring the desired therapeutic outcome upon administration. The extent of water extraction of tea depends on factors such as constituents of the tea, infusion temperature, water-to-tea ratio and particle size of the tea[23]. To achieve maximum extraction from the tea bag, an

optimization of extraction test was conducted. Optimum extraction was achieved by infusing a tea bag in 200 mL of freshly boiled water for 10 minutes (Table 3). This information will help patients and prescribers make informed decisions on how to prepare this tea to maximize phytochemical extraction and achieve an optimal therapeutic outcome.

a) Formulated Loose Herbal Tea *b) Formulated Bagged Herbal Tea*

c) Formulated Bagged Tea in a Container

Figure 1: Samples of the Formulated Loose and Bagged Herbal Teas from the *A. leiocarpus* Stem Bark Powder.

Table 2: Concentration of the Different Phytochemical Constituents in Aqueous Stem Bark Extract of *A. leiocarpus*

Constituent	**Wavelength (nm)**	**Absorbance**	**Concentration (mg/ml)**
Alkaloids	470	0.164	0.033
Flavonoids	510	0.213	0.062
Saponins	544	0.081	0.030
Phenols	765	0.411	0.103
Tannins	725	0.032	0.117

Table 3: Results of Optimization of Extraction from *A. leiocarpus* Tea Bags

Weight of tea bags used for extraction (g)	Volume of water used in extraction (mL)	Volume of filtrate obtained (mL)	Total extract in filtrate (g)	Total solid residue (% w/v)
5	300	295 ± 3	1.12 ± 0.05	0.38 ± 0.001
5	250	240 ± 4	1.06 ± 0.02	0.44 ± 0.001
5	200	185 ± 2	1.20 ± 0.01	0.65 ± 0.000
5	150	125 ± 3	1.10 ± 0.02	0.88 ± 0.001

n = 3

In recent times, there has been an apparent increase in the popularity and use of herbal teas, [28 29] which may be related to their acclaimed benefits in managing many chronic diseases. [30]. Thus, herbal teas belong to a rapidly expanding market of wellness beverage [31]. It has also been reported that the herbal materials used in these recipes are often characterized by the presence of various phytoconstituents responsible for their pharmacological activities and health benefits. Nowadays, many folk recipes, especially those used for managing chronic diseases, are presented as herbal teas[32,33].

CONCLUSION

The aqueous stem bark extract of *A. leiocarpus* was found to be rich in phytochemicals; the stem bark powder was formulated into loose and bagged teas and the extract was found to have a wide margin of safety. Future research on *A. leiocarpus* should explore its bioactive compounds, mechanisms of action, and long-term safety through pharmacological and toxicological studies. Clinical trials are needed to confirm its efficacy and optimal dosage, while standardization will ensure consistent quality. Alternative formulations such as capsules or tinctures can enhance bioavailability, and synergistic studies with other herbs may improve therapeutic effects. Finally, assessing its commercialization potential will support large-scale production and market distribution.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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