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Determination of antibacterial activity, total phenolic, flavonoid and saponin contents in leaves of *Anogeissus leiocarpus* (DC.) Guill and Perr

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ABSTRACT

Objective: To ascertain the antibacterial property and quantify the contents of the total phenolics, flavonoids and saponins in leaves of *Anogeissus leiocarpus* (DC.) Guill and Perr.

Methods: Preliminary phytochemical screening was performed by the method described by Harborne in 1999. Kirby-Bauer disc diffusion method was used for assaying the antibacterial activity. Folin-Ciocalteau reagent and aluminium chloride colorimetric methods were employed for the estimation of the total phenolic and flavonoid contents, respectively. Vanillin-acetic acid and perchloric acid mixture was employed in the determination of the total saponin content.

Results: The preliminary phytochemical screening revealed the presence of glycosides, tannins, flavonoids, saponins, terpenoids and steroids in the methanolic extract while alkaloids were absent. Only steroids were identified in the *n*-hexane extract. The methanolic leaf extract was active against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris* while the *n*-hexane was inactive. The total phenolic content obtained was (47.75 ± 0.12) mg gallic acid equivalent/mg extract, the total flavonoid content was (121.50 ± 0.11) mg quercetin equivalent/mg extract, while the total saponin content was (63.42 ± 0.15) mg ginsenoside Rb 1 equivalent/mg extract.

Conclusions: The leaves of *Anogeissus leiocarpus* have high contents of saponins and flavonoids.

1. Introduction

Bacterial infections have become a common phenomenon and the resistance of bacteria to available antibiotics is a major health issue worldwide. There is the need to develop drugs that are capable of combating infectious bacteria. Apart from microbial infections, oxidative stress is another health challenge that is gaining ground and needs to be tackled with all seriousness. It is the result of the imbalance between antioxidants and reactive oxygen species leading to cellular damage[1]. Oxidative stress is very significant in the initiation and development of many disease conditions such as inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, arteriosclerosis and aging. Synthetic drugs are losing their values because microbial resistance and side effects are associated with them. An alternative route is natural products which can be obtained from plants.

Nearly 80% of the world's population relies on traditional medicines for primary healthcare, most of which involve the use of water or ethanolic plant extracts[2]. Since ancient times, people have been exploring plants to search for new drug candidates. Researchers have therefore resorted to study medicinal plants which are referred to as nature's drug store so as to identify, evaluate, isolate and quantify some of the bioactive phytochemicals found in medicinal plants[3]. Such phytochemicals have been categorized into classes such as alkaloids, flavonoids, tannins, saponins, *etc*. Many of the identified secondary metabolites have found in the pharmaceutical industries because they possess both pharmaceutical and biological properties[4-6]. They have been shown to be non-

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phytotoxic, more systemic and easily bio-degradable[7].

Anogeissus leiocarpus (*A. leiocarpus*) is a plant found in most tropical African countries around the Savannah region. It is a member of Combretaceae family of plants. In Nigeria, the plant is referred to as a multi-purpose plant. The decoction and maceration of the stem bark are used for the treatment of anorexia, constipation, malaria, jaundice, fatigue, itching, eczema, psoriasis, carbuncles, wounds, sores, boils, cysts, hepatitis, ulcers, schistosomiasis, leprosy, diarrhoea, amoebic dysentery, trypanosomiasis, cough and tuberculosis. It also possesses antiproliferative and anthelmintic activities[8]. It is a popular chewing stick used in ridding the mouth of microbial flora such as *Staphylococcus aureus* (*S. aureus*)[9]. The aim of the present study was to determine the antibacterial activity of the leaf extracts, phytochemicals that are present and absent in the plant leaves and also evaluate the total phenolic, flavonoid and saponin content of the leaves of *A. leiocarpus*.

2. Materials and methods

2.1. Sample collection and preparation

Gallic acid and quercetin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol, methanol, hexane, sodium hydroxide, sodium carbonate, aluminum hydroxide, Folin-Ciocalteau reagent and sodium nitrite were obtained from E. Merck (Darmstadt, Germany).

Genesys 10S vl. 200 2l7H311008 spectrophotometer was used for absorbance measurements.

The plant material was collected from Omu Aran, Kwara State, Nigeria. The leaves were taken to Landmark University and were identified by Prof. S. O. Owa of the Biological Sciences Department. The leaves were then dried under laboratory condition, pulverized and stored in a dry air-tight container.

2.2. Extraction

A total of 250 g sample was soaked with methanol for three days and the solvent was changed every 24 h until no extraction was observed. The extract was concentrated by simple distillation method. Then the weight of the extract was obtained and recorded. The same procedure was carried out with *n*-hexane.

2.3. Phytochemical screening

The phytochemical screening was carried out on both extracts and fractions of the methanol extract to determine the presence of glycosides, tannins, phenols, flavonoids, alkaloids, saponins, steroids and terpenoids in accordance with the method described by Harborne in 1999[10].

2.4. Determination of the total saponin content

Twenty grams of the methanol extract was placed in a conical flask and 100 cm^3 of 20% aqueous ethanol was added into it and heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The mixture was filtered and extracted three times with 200 mL of 20% aqueous ethanol. The extracts were combined and the volume reduced to 40 mL using a water bath at about 90 °C. The concentrated extract was transferred into a 250 mL separating funnel, then 20 mL diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the process was repeated. Sixty milliliters of *n*-butanol was added to the aqueous layer. The butanol fraction was recovered and washed twice with 10 mL of 5% NaCl. The solution was heated in a water bath to evaporate off the butanol. After evaporation, the sample was dried in an oven to constant weight to recover saponin.

To prepare the recovered saponin for UV measurement, a fresh solution of vanillin-acetic acid (5% w/v, 0.2 mL) solution was prepared and perchloric acid (0.8 mL) was added and kept at 70 °C for 15 min. The solution was cooled on ice for 20 s before adding glacial acetic acid (5 mL). The solution was scanned at 550 nm using a UV spectrophotometer. The standard used was ginsenoside Rb1[11].

2.5. Determination of the total flavonoid content

Aluminium colorimetric method was used to determine the total flavonoid content of *A. leiocarpus*. Five milligrams of the extract was weighed and dissolved with 5 mL of 50% methanol and to 1 mL of this solution was added 0.7 mL of 5% (w/w) $NaNO_2$ and 10 mL of 30% (v/v) ethanol. This mixture was stirred for 5 min and 0.7 mL of 10% $AlCl_3$ (w/w) was added into it and stirred for 6 min. Five milliliters of 1 mol/L NaOH was added to the mixture and diluted to 25 mL with 30% (v/v) ethanol. The mixture was allowed to stand for 10 min and the absorbance measured at 500 nm using a UV spectrophotometer. Quercetin was used as the standard and the total flavonoid content was expressed as quercetin equivalent in mg/g extract[12].

2.6. Determination of the total phenolic content

The total phenolic content of the methanolic extract was determined by the Folin-Ciocalteau method. Five milligrams of the extract was weighed and dissolved with 5 mL of 50% methanol by using a vortex mixer. Then, 0.5 mL of this solution was pipetted into a test tube, and 3.5 mL of distilled water and 0.25 mL Folin-Ciocalteau reagent were added to it. It was left to incubate for 8 min at room temperature. Then 1 mL of 20% Na_2CO_3 was added and left to incubate for 2 h. The absorbance was measured at a wavelength of 765 nm against a reagent blank by a UV spectrophotometer. Gallic

acid was used as the standard and the total phenolic content of the extract was expressed in mg gallic acid equivalents/mg extract[13].

2.7. Determination of the antibacterial activity

2.7.1. Organisms used

The following microorganisms [*Pseudomonas aeruginosa* (*P. aeruginosa*), *S. aureus*, *Klebsiella pneumoniae* (*K. pneu*moniae), *Proteus vulgaris* (*P. vulgaris*) and *Escherichia coli* (*E. coli*)] were used for the analysis. These microorganisms were clinical isolates collected from Baptist Medical Center, Ogbomoso, Nigeria.

2.7.2. Preparation of the antibacterial medium

A total of 2.8 g of nutrient agar was dissolved in 100 mL of distilled water. It was sterilized in an autoclave at 121 ˚C for 15 min, cooled to 45 ˚C and poured into sterile Petri dishes to solidify.

2.7.3. Preparation of test samples

One milligram of each extract and the standard antibiotic, streptomycin were dissolved separately in 1.0 mL of distilled dimethylsulphoxide. The streptomycin served as the positive control.

2.7.4. Disc diffusion test

Disc diffusion method by Bauer *et al.*[14] was employed. Each tested organism was introduced on different nutrient agar plates by inoculation and sterilized Whatman No. 1 filter paper which was cut into disc form and impregnated with the extract was placed on the surface of the plates. Upon contact with the agar, the extract diffused in all directions. The zones of inhibition of the tested organisms were determined after 24 h. The incubation of the plates was carried out at 37 ˚C. The zones of inhibition obtained were expressed as mean of quadruplicate results of inhibition diameters (mm) of the plant extracts. The test was repeated using the standard antibiotic, streptomycin as the antibacterial agent.

3. Results

3.1. Phytochemical screening

In this study, the phytochemical screening of the methanolic extract revealed the presence of glycosides, tannins, flavonoids, saponins, terpenoids and steroids while alkaloids were absent. Only steroids were identified in the *n*-hexane extract as shown in Table 1.

Table 1
Phytochemical screening of extracts of *A. leiocarpus.*

Extracts	Gly	Tan	Flav	Sap	Ste	Alk	Terp
Methanol	+	+	++	+++	+	-	+
n-Hexane	-	-	-	-	+	-	-

Gly: Glycosides; Tan: Tannins; Flav: Flavonoids; Ste: Steroids; Alk: Alkaloids; Terp: Terpenoids.

3.2. Total phenolic, flavonoid and saponin contents

The results of the spectrophotometric determination of the total phenolic, flavonoids and saponins contents of the plant leaves extract are presented as follows. The total phenolic content obtained was (47.75 ± 0.12) mg gallic acid equivalent/mg extract, the total flavonoid content was (121.50 ± 0.11) mg quercetin equivalent/mg extract, while the total saponin content was (63.42 ± 0.15) mg ginsenoside Rb 1 equivalent/mg extract.

3.3. Antibacterial activity

The data for the antibacterial activity of the leaf extract are given in Table 2. The methanolic extract was active against all the screened bacteria while the *n*-hexane was inactive.

Table 2
Antibacterial activity of the crude methanolic extract [zones of inhibition (mm)].

Microorganisms	Methanol extract	*n*-Hexane extract	Streptomycin
E. coli	24.58 ± 0.13	-	00.00
K. pneumoniae	20.67 ± 0.10	-	00.00
S. aureus	23.45 ± 0.10	-	21.00
P. vulgaris	18.27 ± 0.12	-	20.50
P. aeruginosa	20.19 ± 0.12	-	30.00

4. Discussion

Medicinal plants have been shown to possess great potential uses, especially as traditional medicine and pharmacopoeial drugs. Knowledge of the chemical constituents of medicinal plants is helpful in the discovery of therapeutic agents as well as new sources of new drug candidates. Medicinal plants are also of interest in biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds. In trado-medical health system, the efficacy of the plant extracts is dependent on the proper use of the plant(s) or plant part(s). The biological efficacy of these plants in turn depends on the presence of the required quantity and nature of the secondary metabolite in the crude extract[15]. The phytoconstituents identified in the methanolic extract of *A. leiocarpus* were glycosides, tannins, flavonoids, saponins, terpenoids and steroids while alkaloids were absent. Only steroids were identified in the *n*-hexane extract. The presence of these classes of compounds could be responsible for the biological and pharmacological properties of the plant leaves. The results obtained by Mann *et al.* in 2008[16] and Sore *et al.* in 2012[17] showed that alkaloids are present in the methanolic leaf extracts of *A. leiocarpus* while the result gained by Vitor and Grace in 2013[18] is in agreement with our results. This disparity could be as a result of environmental, climatic and soil factors. All these contribute to the composition of the plant. In the evaluation of the total phenolic, flavonoid and saponin contents, the total phenolic content was determined using Folin-Ciocalteau reagent and gallic acid as the

standard phenolic compound. The molybdotungstate in the Folin-Ciocalteau reagent oxidizes the phenolic compounds in the extract and yields a coloured compound which has a maximum absorption wavelength around 745–750 nm. The gallic acid calibration curve ($y = 0.021x + 0.295$) is in the range of 0.5–3.5 mg/mL with a coefficient of determination (r^2) value of 0.996. The total flavonoid content was determined using the aluminium colorimetric assay and quercetin as the standard flavonoid compound. The quercetin calibration curve ($y = 0.012x - 0.011$) is in the range of 5–25 mg/mL. It has a coefficient of determination (r^2) of 0.992. The total saponin content was determined using vanillin-acetic acid and perchloric acid colorimetric assay. Ginsenoside Rb1 was used as the standard and it gave a calibration curve of $y = 0.020x - 0.085$ with a coefficient of determination (r^2) of 0.990. The leaves of *A. leiocarpus* contain moderate amount of polyphenolics, very high concentration of flavonoids and high level of saponins. A number of pharmacological properties have been attributed to saponins. Some of the important properties include permeabilizing of cell membrane, lowering of serum cholesterol levels, stimulation of luteinizing hormone release leading to abortifacient properties and immunomodulatory potential via cytokine interplay[19,20], cytostatic and cytotoxic effects in malignant tumor cells[21], adjuvant properties for vaccines as immunostimulatory complexes[22], and synergistic enhancement of the toxicity of immunotoxins[21]. Tannins are astringents. They can hasten the healing of wounds and inflamed mucus membrane. They possess properties such as antioxidant, antimicrobial and anti-inflamatory[5]. Researches on polyphenols have shown that these compounds can protect pancreatic B-cells against glucose toxicity, and possess anti-inflamatory and antioxidant properties. They can also inhibit α-amylases or α-glucosidases thereby cause a decrease in starch digestion[23]. Polyphenolics have been shown to exhibit both biological and pharmacological activities[6]. Glycosides are important phytochemicals which have found use as antibacterial agents[24]. The cardiac glycosides can be used in the treatment of congestive heart failure and cardiac arrhythmia[25-29]. Flavonoids have been shown to possess several health benefits such as antioxidant, anti-inflammatory, antiallergic, antimicrobial and anticancer properties[30]. Another disparity was noted between our research results and that of Setzer and Setzer[19] in the area of total phenolic and flavonoid contents. While their total phenolic content was higher than ours, and their total flavonoid content was very low as compared to our results. The antibacterial analysis of the leaf extracts showed that the *n*-hexane extract is inactive against all the screened bacteria while the methanolic leaf extract inhibited *E. coli*, *K. pneumoniae*, *S. aureus*, *P. vulgaris* and *P. aeruginosa*. The plant leaves have shown inhibitory activity against both Gram-positive and Gram-negative bacteria. This shows that it has a broad spectrum of antibacterial activity. This activity was more pronounced against Gram-negative than Gram-positive bacteria. This could be as a result of the morphological differences between these micro-organisms. The Gram-positive bacteria have a thick outer peptidoglycan layer in their cell wall which is not an effective permeability barrier that makes it to be more susceptible to the extract while Gram-negative bacteria possess a thin peptidoglycan layer plus an outer phospholipidic membrane that carries the lipopolysaccharide components which make the cell wall impermeable to lipophilic solute. The inhibitory activity of the plant leaves could be as a result of the presence of the identified phytochemicals. Comparing the antibacterial activity of the methanolic leaf extract to that of the standard antibiotic, it was observed that the methanolic leaf extract was more potent against *E. coli*, *K. pneumonia* and *S. aureus* while its inhibitory activity against *P. vulgaris* and *P. aeruginosa* was close to that of the standard antibiotic. The results of Sore *et al.* in 2012[17], Kabore *et al.* in 2010[31] and Mann *et al.* in 2008[16] showed that the plant leaves are inactive against the screened bacteria which was opposed to our results while that of Sambas *et al.* in 2015[32] was in agreement with our result. This disparity could be due to the differences in the type, nature and quantity of phytochemicals present in the leaves obtained from different locations.

The leaves of *A. leiocarpus* are rich in saponins and flavonoids. These classes of compounds have been shown to possess important biological and pharmacological properties and therefore can serve as a source for new drug candidates that possess antioxidant potential. Furthermore, work is on-going to isolate and characterize the identified phytochemicals.

Conflict of interest statement

We declare that we have no conflict of interest.

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