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RESEARCH ARTICLE

Efficacy of Anogeissus leiocarpus as a Therapeutic Agent for Some Pathogenic Bacteria

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Abstract

Background: This study examines the folkloric potential of the leaf extract of*Angogeissus leocarpus* and it is aimed at the effectiveness for possible treatment of infections caused by some pathogenic bacteria.

Methodology: Solvent extract of the leaf of*A. leocarpus* was processed for phytochemical and antibacterial effects. Clinical and type bacterial cultures were tested for susceptibility to the quotes.

Results: The phytochemicals screened from the ethanol leaf extract were saponin, flavonoids, steroids, alkaloids, tannins, glycosides and phlobatannins while from the aqueous extract, it was flavonoids, tannins, saponin and steroids. The ethanol leaf extract provided the higher bioactive compounds for inhibition (10-36 mm) of the tested bacteria species. The aqueous leaf extract inhibited the bacteria species with zones that ranged from 10-25 mm. The ethanol leaf extract's minimum inhibitory concentration (MIC) was effective at 12.5 mg/mL and minimum bactericidal concentration (MBC) at 50 mg/mL. The aqueous MIC was effective with the 50 mg/mL concentration and MBC with the 100 mg/mL concentration. Promising values were recorded in the proximate and mineral contents analyzed. The hydroxyl radical scavenging values of the ethanol and aqueous extracts are 0.56±0.60 and 73±0.4, FRAP (1.86±0.11 and 1.24±0.2), DPPH (1.79±1.3 and 1.61±0.1) mg of ascorbic acid/1 g dry leaves sample respectively.

Conclusion: *A. leiocarpus* extracts effective in bacteria inhibition and nutrition could be a good source for drug development to treat diseases hence it could have a space of recognition among the few medicinal plants which have been studied for their medical properties with novel therapeutic values.

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Introduction

Infectious diseases have continuously been identified as one of the most serious threats to world health. The many ways of their prevention and cure with folklore have helped immensely in recent times. In 2013, the World Health Organization (WHO) stated that infections killed 61.7 per cent of the 9.6 million people who died in Sub-Saharan Africa (Dhama *et al*., 2014). Despite the success of antibiotic discovery, infectious diseases remain the world's second biggest cause of death and antibiotic resistance is one of the century's most serious issues (Harriet et al., 2022). In 2019, the WHO stated that the top causes of death accounted for 55% of the 55.4 million deaths worldwide where lower respiratory infections and diarrheal disease were ranked in the top ten causes of death worldwide (WHO, 2022). Organisms that cause respiratory infection such as *Staphylococcus aureus*, *Streptococcus pneumoniae* as well and *Pseudomonas aeruginosa* (Aylana and Gili, 2016) and Enterobacteriaceae, which cause diarrhoea and urinary tract infections are now resistant to nearly all previous antibiotics (Breijyeh *et al*., 2020). This resistance is majorly caused by the indiscriminate use of medications to treat these infections (Spellberg and Gilbert 2014). Microorganism resistance to one or more antimicrobial medicines is a global health issue that has prompted scientists all over the world to develop chemotherapeutic medications to treat diseases caused by microbial infections (Hammuel *et al*., 2011). The use of plants and their constituents in primary health care has an ancient history as old as human beings (Rahmani and Ally, 2015) and to date are routinely used in traditional medicine to manage diseases. The majority of urban and rural duelers are extremely in the practice of using the accessible and available herbal remedies within their localities in combating diseases. This type of practice has become phenomenal, hence herbal plants have bioactive compounds that can be effective in disease management (Akharaiyi and Okafor, 2021)

The synthesized aromatic substances in medicinal plants are the values responsible as defensive weapons against microorganisms (Akharaiyi and Boboye, 2010). Some of the synthesized aromatic substances in plants such as flavonoids, alkaloids, carotenoids, phenolic compounds, and tannins are distributed widely in plants and they exert multiple biological effects which include antioxidant, anti-carcinogenic, anti-inflammatory and free radical scavenging potentials among others (Nirmaladevi *et al*., 2010).

Anogeissus leiocarpus has a common name as the Axlewood tree and it belongs to the family of Combretaceae. The plant has so much medical importance in the treatment of various diseases of non-microbial origins (Akanbi, 2012). It has also been reported to be effective in the treatment of infections caused by bacteria (Victor, 2013). The decoction of the leaves has also been recommended for the treatment of skin diseases and in the itch of psoriasis. The powdered bark is applied

to wounds, sores, boils, cysts and diabetic ulcers with good results. Decoctions and concoctions for the healing of various diseases have been confirmed without toxic effects and so can be confirmed as plant food for safe consumption (Victor, 2013).

The purpose of the work is to determine the effectiveness of the plant extracts for possible treatment of infections caused by some pathogenic bacteria.

Material and methods

Leaves sample collection and plant extract preparation

Fresh leaves of *Anogeissus leiocarpus* were harvested from the forest around Edo State University Uzairue and authenticated by Dr. Odologie Imarhiagbe of the Department of Biological Sciences, Edo State University, Nigeria. A voucher specimen number with FD 1257 was deposited in the University herbarium. The leaves were rinsed in distilled water and air-dried for three weeks at a temperature of between 25 ^{o}C to 27 ^{o}C. The dried leaves were ground to powder with the aid of an electric blender. 100 g each of the leaves powder was extracted with 500 mL each of ethanol and water for 24 hours at room temperature (27±2 ^{o}C) and filtered. The ethanol extract filtrate was concentrated in vacuo while the aqueous extract was concentrated in a water bath regulated at 50 ^{o}C. The semi-solid extracts were kept in glass vials and refrigerated before use.

Bacteria species

Clinical bacterial isolates, *Escherichia coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginisa*, and *Klepsiella pneumoniae*, were collected from the Microbiology Department of the Federal University of Technology Akure. The American-typed cultures include *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 49619. The bacterial isolate was purified by sub-culturing on Nutrient agar and incubated at 37 ^{o}C for 24 hours. Therefore, the pure isolates were Gram-stained and characterized with biochemical tests for verification and authenticity.

Preparation of inoculums

The purified bacteria isolates were sub-cultured in peptone water for 18 hours. The McFarland standard of 10^{7} was obtained by adding sterile saline solution to the cultures

The extracts phytochemical qualitative analysis

Chemical methods in addition to the weight of the extract and timing for instant results were used to determine qualitatively the presence of phytochemicals contained in the plant extracts (Harborne and Williams, 2000; Trease and Evans, 1989). The phytochemicals tested are saponins, glycosides, tannin, flavonoids, alkaloids, steroids, phlobatannins,

and terpenoids

The plant extracts quantitative phytochemical analysis

Total Phenolic Compounds Determination

One hundred milligrams of the extract was mixed with 100 mL of distilled water. 1 mL was pipetted into a test tube and mixed with 0.5 mL of 2 N Folin-ciocalteu and 1.5 mL of Na_2CO_3 solution. It was made up of 10 mL of distilled water. The mixture was vigorously shaken and allowed to stand for 2 hours after which the absorbance was taken at 765 nm. The obtained data were used for the estimation of the total phenol value with the standard calibration curve that was from the various garlic acid concentrations (Adusei *et al*., 2019; Siddiqui *et al*., 2017)

Determination of total flavonoids

100 µL of 20% aluminium trichloride in methanol was added to 5 mL of extract. The mixture was dispensed in a cuvette and allowed to stand for 40 min. It was placed on spectrophotometer and the absorbance was read at 415 nm. From 100 µL of the extract, blanks were prepared and 1 drop of acetic acid was added and further diluted with 5 mL of methanol. The absorbance of standard rutin was measured (Zhishen *et al*., 1999).

Determination of total alkaloids

Two hundred millilitres of 10% acetic acid in ethanol was used to mix 5 g of extract. The mixture was covered for 4 hours. It was filtered and concentrated in a water bath to obtain one-quarter of the initial volume. Thereafter, concentrated ammonium hydroxide was added to the mixture until completion of the precipitation. The mixture was allowed to settle to enable the collection of the precipitate. The collected precipitate was washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed as alkaloids (Devi *et al*., 2020).

Determination of Tannin

Each of the plant extracts was boiled with distilled water for 1 hour. Colour development was done with Folin-Denis reagent and sodium carbonate solution. Absorbance was measured at 750 nm spectrophotometrically. From the standard of tannic acid, the value of tannic acid was evaluated in the extract (Elgailani *et al*., 2014).

Saponins determination

Saponins were extracted for 2 hours in a reflux condenser containing pure acetone. Exhaustive re-extraction over heating mantle with methanol in the soxhlet apparatus was done for 2 hours. The extract was weighed after allowing methanol to evaporate. The amount of saponin was evaluated as a sample percentage (Nahapetian and Bassiri, 1974).

Steroids determination

A few drops of acetic acid were used to dissolve 1 g of each extract in a test tube. The mixture was warmed and cooled under slow-running tap water. After this, one drop of concentrated sulphuric acid was added gently to the mixture. The turning of the mixture to a green colour is an indication of steroids present in the extract (Satheesh *et al*., 2012)

Glycoside determination

To 5 mL of each extract, 2 mL of glacial acetic acid that contained a drop of ferric chloride was added. The mixture was underlaid with 1 mL of concentrated hydrochloric acid. A deoxy-sugar characteristic of cardenolide will be indicated with a brown ring at the interface. However, a velvet ring could be formed below the developed brown ring. A gradual greenish ring could be formed all through the layer.

Antibacterial test of plant extract

The inhibitory effect of the extract was determined by the agar well diffusion method described by CLSI, (2018). Mueller Hinton agar culture plates were prepared and allowed to gel. The test bacteria species with a concentration of 10^7 colony-forming units were streaked on the surface of the agar and left for one and a half hours for the seeded bacteria species to be well established in the culture medium. The plates were punched with sterile cork borer (diameter of 4 mm) to create open wells which were filled with 0.004 ml of extract. The plates were kept at room temperature for 2 hours to permit extract diffusion and bacteria establishment in the culture medium before incubating, at 37 ^{o}C for 24 hours. Zones of inhibition were measured and recorded as degrees of sensitivity.

Minimum inhibitory concentration (MIC) determination

The MIC of the extract was determined by broth dilution method using Mueller Hinton broth. The tubes were incubated at 37 ^{o}C for 24 hours and observed for turbidity by checking the first tube in the serials that showed no visible trace of growth. The first tube in the serials with no visible growth after the incubation period was taken as the MIC.

Minimum bactericidal concentration (MBC) determination

The MBC was determined by sampling all the macroscopically cleared tubes. The tubes were gently shaken/mixed and 100 macro litre of the sample was obtained and poured plated with plate count agar (PCA). The seeded bacteria plates were left to gel before being placed in an incubator for 24 hours at a temperature of 37 ^{o}C. After incubation, the concentration of the extract that did not produce any bacterial growth on the culture medium was reported as the MBC value for the extract.

Mineral and proximate contents determination

The criteria of the Association of Official Analytical Chemists (AOAC, 1980) were adopted to attain the nutritional values of

the leaf extract/

In-vitro antioxidant assay

Hydroxyl radical scavenging assay

The method of Halliwell *et al.* (1987) was adopted. The extract was mixed with 4 nM deoxyribose, 0.3 mM ferric chloride, 0.2 nM EDTA, 0.2 mM ascorbic acid, and 2 mM H_2O in a tube. The tubes were closed tightly and incubated at 37^{o}C for 30 minutes. After which, 0.4 mL of 5% TBA and 0.4 mL of 1% TBA were added and placed in a water bath at 37 ^{o}C for 20 minutes. The appearance of pink colour was measured against the blank in a spectrophotometer at a wavelength of 532 µm. Ascorbic acid served as the positive control. The leaves extract hydroxyl radical scavenging potential was reported as % inhibition of 2-deoxy-d-ribose oxidation on hydroxyl radicals and calculation was by the below equation.

$$\% \text{ inhibition} = \frac{A_o - A_1 \times 100}{A_o}$$

Where Ao was the absorbance of the control and A_1 was the absorbance in the presence of the extract or positive control. The inhibition %.was plotted against concentration and IC_{50} was calculated from the graph. The experiment was in triplicate at each concentration

Ferric Reducing Antioxidant Assay

The method of Buriciva and Reblova. (2008) was adopted. 0.1 g of the extract was dissolved in 20 mL of H_2O and filtered to obtain a debris-free filtrate. 2.5 mL of the filtrate was added to a solution mixture with 2.5 mL each of phosphate buffer and potassium ferrocyanide. It was then incubated at 50 ^{o}C. To this mixture, 1 mL of 0.1% ferric chloride, 10% Trichloroacetic acid and 5 mL of distilled water were further added. Both the standard and sample absorbance were read at 700 µm in a spectrophotometer against a blank

Free radical scavenging activity (DPPH)

The method of Dorman *et al.* (2004) was adopted. An extract concentration of 20 mg/mL was obtained from a cuvette and reacted with 0.05 mg/mL concentration of aliquot of 0.5 mL of 0.1 mm 1, 1-diphenyl 1-2 picrylhidrazyl (DPPH) radical. The mixture was left to stand for 20 minutes. The absorbance of the mixture was measured in a spectrophotometer at 520 µm. The absorbance measure was expressed as mg/1 g of L-ascorbic of dry plant material. However, calibration was used, in which the extracts were replaced with a fresh solution of ascorbic acid concentration that ranged between 0 to 1.6 mg/mL – 100 mg/mL. The DPPH free radical percentage was calculated with the below equation.

$$\text{DPPH scavenging effect (\%)} = \frac{A_o - A1 \times 100}{A_o}$$

Where Ao was the absorbance of the control and A_1 was the absorbance in the presence of the extract.

Statistical analysis

The obtained results were expressed as mean ± standard deviation (SD) and were subjected to a one-way analysis of variance (ANOVA). The least significant difference (LSD) was performed for the pairwise mean comparisons, to determine the significant treatment dose at a 95% level of confidence. Values were then considered statistically significant at ($P<0.05$).

Results

The qualitative phytochemical analysis of the extracts showed that both the water and ethanol extracts contained several constituents of secondary metabolites that are active against bacteria inhibition. The chemicals screened from the leaf extracts qualitatively and quantitatively are flavonoids, steroids, alkaloids, terpenoids, tannins, phenol and glycoside which were not present in the aqueous extract qualitatively. However, the quantity and quality of phytochemicals were more in the ethanol extract than in the aqueous extract (Table 1).

Table 1. The qualitative and quantitative phytochemical screening of the leaf of *A. leiocarpus*

PHYTOCHEMICALS	ETHANOL EXTRACT Quality Quantity	AQUEOUS EXTRACT Quality Quantity
Saponins	+ 76.6±0.42 mg/g	+ 36.3±0.26 mg/g
Flavonoids	+ 21.0±0.07 mg/g	+ 10.8±0.32 mg/g
Steroids	+ 12.8±0.54 mg/g	+ 7.4±0.52 mg/g
Alkaloids	+ 22.5±0.41 mg/g	+ 14.3±2.4 mg/g
Terpenoid	+ 2.37±0.57 mg/g	+ 1.24±0.18 mg/g
Tannins	+ 420±0.31 mg/100g	+ 212±0.20 mg/100g
Glycosides	+ 125±0.03 mg/100g	- 15±0.16 mg/100g
Phenol	+ 4.4±0.23 mg/g	+ 3.2±0.66 mg/g

Keys: + = Present, -- =Absent

The bacteria inhibition of the ethanol and aqueous leaf extracts of*A. leiocarpus* was determined on some clinical bacteria isolates and type bacteria cultures. The extracts were potent to all the bacteria tested except *S. aureus* which was resistant to the aqueous extract. Antimicrobial activity was estimated by measuring the diameters of the formed inhibition zones around the created wells. The ethanol leaf extract exhibited the highest inhibition on *E. coli* ATCC 35218 by a zone of 36 mm, followed by *K. pneumoniae* ATCC 49619 been inhibited by a zone of 32 mm. while the least inhibition of 10 mm was on *Escherichia coli*. The highest exhibition with aqueous extract was on*K. pneumoniae* ATCC 49619 by a zone of 25

mm, followed by *E. coli* ATCC 35218 being inhibited by a zone of 22 mm and least inhibited *Pseudomonas aeruginosa* by a zone of 10 mm. Observation from this study proved ethanol extract of higher potency than aqueous extract except on *P. aeruginosa* where a higher inhibition zone was observed with aqueous extract (Table 2). The minimum inhibitory concentration (MIC) of ethanol extract was effective at 12.5 mg/mL on all tested bacteria isolates, while the minimum bactericidal concentration (MBC) was effective at the concentration of 50 mg/ml except for *K. Pneumoniae* ATCC 49619, *K. pneumoniae* and *E. coli* ATCC 35218 which were initially most inhibited with the crude extract of ethanol, had MBC at 12.5 mg/mL The MIC on the tested isolates was at 50 mg/mL except on *E. coli* ATCC 35218 where it was 12.5 mm. The MBC was effective on the tested bacteria species at concentration of 100 mg/mL, exceat for *E. coli* ATCC 35218 and *Staphylococcus aureus* ATCC 25923 on which it was at 50 mg/mL. (Table 2).

Table 2. Antibacterial activity of water and ethanol extract of *A. leiocarpus*

Bacteria species INHIBITIONS DETERMINED Water extract (mg/ml) ethanol extract (mg/ml)						
WEI (mm)	MIC	MBC	EEI (mm)	MIC	MBC	
E. coli ATCC 35218	22	12.5	50	6	12.5	12.5
E. coli	15	50	100	10	12.5	50
S. pneumoniae	20	50	100	26	12.5	50
S. aureus ATCC 25923	17	50	50	28	12.5	50
S. aureus	-	-	-16	12.5	50	
P. aeruginosa ATCC 27853	13	50	100	25	12.5	50
P. aeruginosa	10	50	100	20	12.5	50
Kpneumoniae ATCC 49619	25	50	100	32	12.5	12.5
K>pneumoniae	16	50	100	23	12.5	12.5

***Legend**: WEI = Crude water extract inhibition, EEI = ethanol extract inhibition, MIC= Minimum inhibitory concentration, MBC = Minimum bactericidal concentration.*

A. leiocarpus ethanol leaf extract was bactericidal on *S. pneumoniae, K. pneumoniae, P. aeruginosa, and E. coli,* but that effect was not extended to *S. aureus* with the aqueous extract concentrations.

The lipid contents of the ethanol leaf extract were 4.18±0.4 and 3.11±0.2 for the aqueous extract. Crude protein content was 15.36±0.5 and 15.36±0.5 for ethanol and aqueous extracts respectively. The ash contents were 6.24±1.5 and 5.28±0.4 for ethanol and aqueous extracts respectively. The carbohydrate content for ethanol was 68.16±1.2 and 56.27±0.6 for the aqueous extract. The crude fibre content was higher in the aqueous extracts (7.34±1.4) than in the ethanol extract (6.56±1.6) (Table 3).

Table 3. Proximate composition (%) of the leaves of *A. leiocarpus*.

Parameters	Contents ALEE ALAE
Lipid	4.18±0.4 3.11±0.2
Crude Protein	15.36±0.5 15.36±0.5
Ash	6.24±1.5 5.28±0.4
Carbohydrate	68.16±1.2 56.27±0.6
Crude fibre	6.56±1.6 7.34±1.4

Legend: ALEE = *Angogeissus leocarpus Ethanol Extract, ALAE = Angogeissus leocarpus Aqueous Extract*

The amount of nitrogen content in the ethanol extract was 4.16 while it was 4.12 in the aqueous extract. Calcium content was 1.25 and 1.20% for ethanol and aqueous extracts respectively. Sodium was 0.62% in the ethanol extract and 0.58% in the aqueous extract. The magnesium content in the extracts was 0.64% and 0.62% for the ethanol and aqueous respectively. Phosphorus measured 1.54% and 1.53% for the ethanol and aqueous extracts respectively. The potassium content of the extracts was 2.03% in the ethanol and 2.01% in the aqueous (Table 4).

Table 4. Mineral contents (%) of the leaves of *A. leiocarpus*

N ALEE ALAE	Ca ALEE ALAE	Na ALEE ALAE	Mg ALEE ALAE	P ALEE ALAE	K ALEE ALAE
4.16 4.12	1.25 1.20	0.62 0.58	0.64 0.62	1.54 1.53	2.03 2.01

Legend: *N = Nitrogen, Ca = Calcium, Na = Sodium, Mg = Magnesium, P = Phosphorus, K = Potassium,* ***ALEE =*** *Angogeissus leocarpus Ethanol Extract, ALAE = Angogeissus leocarpus Aqueous Extract*

Values of 0.56±0.60, 1.18±0.11, and 1.76±1.3 were recorded for HRS, FRAP and FRAS respectively in the ethanol extract, while it was 7.3±0.4, 1.24±0.2, and 1.61±0.1 for HRS, FRAP and FRAS respectively in the aqueous extract. The ethanol extract of the plant suggests more antioxidant quality than the aqueous extract (Table 5).

Table 5. *In-vitro* antioxidant profileof *A. leocarpus*

HRS	FRAP (mg of ascorbic acid/1 g dry plant material	DPPH (mg of ascorbic acid/1 g dry plant material
ALEE ALAE	ALEE ALAE	ALEE ALAE
0.56±0.60 7.3±0.4	1.86±0.11 1.24±0.2	1.76±1.3 1.61±0.1

Legend: *Angogeissus leocarpus Ethanol Extract, ALAE = Angogeissus leocarpus Aqueous Extract*

4. Discussion and Conclusion

Phytochemicals such as saponin, terpene, flavonoids, tannin, steroids and flavonoids have been reported present in*A. leiocarpus* (Ogunjobi *et al*., 2020). The presence of these phytochemicals in reasonable amounts in plants has pharmacological effects on the health care system. Several plants known to possess such phytoconstituents have been reported in several kinds of literature and are employed in folklore medicine. Dhama *et al*. (2014), reported that many plants develop these chemicals that are inhibitory to the infections caused by microorganisms and prevention of predators which include man and animals. The phytochemical screened from the ethanol and aqueous extracts represent the bioactive compounds known for bacterial inhibitions that can be relied upon. The quantity of chemicals screened from the ethanol extract could result from the antibacterial potency it exhibited over the aqueous extract in this study. This denotes that ethanol may extract more of the bioactive compounds in plants than water. The screening of plant chemicals from the leaves of *A. leiocarpus* becomes a vital step required for its investigations in order to ascertain the constituents of bioactive compounds for effectiveness on microbial susceptibility and the *in vitro* antioxidant for the physiological changes it might manifest in the human system. These are of importance hence the level of bioactive constituents in medicinal plants can be used to pivot or extrapolate its potency and possibly drug formulation. Apart from the medical importance of chemicals derived from plants, they are also valued in other human life, such in their domestic use and in the industries for various products (Malinowska *et al*., 2020). Though the chemicals present in plants can be extracted with different solvents, the solvents with high polarity often dictate the pace of the quality and quantity of phytochemicals to be extracted. This was pertinent in this study where ethanol was found to be a transcendent extracting solvent than water. The most common organic solvents usually employed for extracting bioactive compounds from plants include acetone, ethanol and methanol (Tinky *et al*., 2020). Based on the plant's chemical constituents screened, this study was extended to investigate the antibacterial tendency of *A. leiocarpus* leaf extract as a potent plant which could be used to prevent bacteria invasion in the body. Some plants have been proven to possess the chemicals required for effective therapy and physiological attributes for the treatment of several illnesses. Consequent to this, the presence of phytochemicals in plants denotes a key source for the remedy of effective medication (Radha and Vijayakumari, 2013).

Many of the species of the tested bacteria have developed resistance to the antibiotics for their treatment, while the surveys for medicinal plants like *A. leiocarpus* with various and strong chemical compounds could be important to combat the infections caused by these pathogenic bacteria.

Mann *et al*. (2015); Alhassan *et al*. (2016), have reported the inhibitory potential of*A. leiocarpus* against some pathogenic bacteria species such as *Klebsiella species, E. coli,* and *P. aeruginosa*. This signifies that the plant's leaves contain active phytochemicals which clearly demonstrate the antibacterial activity of the extracts. This observation is in line with the findings of Usman *et al*. (2020). Ntiejumokwu and Alemika, (1991) have reported various sections of the plant parts have been able to inhibit the growth of some hospital isolates. Mann, (2012), found that *A. leiocarpus* has a significant effect against some of the bacteria implicated in the pathogenesis of human infections, such as *Pseudomonas* species that causes inflammation in the respiratory tract, *Salmonella* species that causes typhoid fever and *E. coli* which is the causative agent of lung and intestinal infections. The inhibition of these bacteria species with ethanol and aqueous leaf

extracts of *A. leiocarpus* is, therefore, also established in this study.

For meaningful findings in any research that involves the analysis of proximate compositions, the required parameters in such substances will include crude protein, crude fat, ash, crude fibre and carbohydrate (Ekwumengbo *et al.*, 2014). The appreciable value of the crude fat in this research has proved the plant a good source of fat that may not be harmful to the body system. Lipids being fatty compounds play several roles that are beneficial to the human body like regulating the substances that enter and go out of the body, absorption of vitamins, and movement and storing of energy. The plant is also valued for its protein contents hence, protein is good for human health maintenance. The crude protein of any substance is a determinant of the quantity of protein it possesses. In this study, we have found the leaves of *A. leiocarpus* to possess the amount of protein that will be valued for its use in herbal remedies. The determined amount of crude fibre in the leaves projected it for use as a traditional medicine because it will assist so well in the digestion and production of intestinal bacteria. Also, the crude ash result obtained becomes of interest hence the substances to be absorbed as minerals are part of the good functions it will add to the human body. The high carbohydrate content of the leaves make it a plant for utilization hence carbohydrate is essential for providing the body with the required glucose for energy production. Whether the carbohydrate contains more than one sugar, hence it is in drug to be drunk, it will be broken down into monosaccharides by digestive enzymes during food digestion in the stomach. The carbohydrate contained in the leaves, if used as a therapeutic agent, will aid in the structure and formation of cells to replace the dead ones, and the strengthening of weak organs and tissues of patients (Cummings and Stephens, 2007).

The analyzed minerals become important in human health though in small amounts but necessary in controlling body fluids inside and outside the cells, converting food to energy, and building strong bones. The consumption of *A. leiocarpus* leaf as food may help some people get the required amount of minerals needed for health maintenance. For example, adequate calcium is needed in cells to be able to perform its functions in blood clotting (Hall, 1991), and muscle contraction (Naveh-Many *et al.*, 1995). Sodium is part of what controls blood in the human body (Cogswell*et al.*, 2016). However, a moderate amount is needed to avoid its increase which may lead to irritability, oedema, weakness and lethargy (Kalogeropoulos *et al.*, 2015; Xi *et al.*, 2015). Magnesium is involved in membrane function, enzyme activation, and intracellular signaling (Castiglioni, 2021; Volpe, 2013). Potassium is necessary in the body for the normal function of the heart, plays a role in the proper function of skeletal muscle, and is required for the reaction of many enzymes (Weaver, 2013). Phosphorus plays a role in bone and teeth formations, UTP, ATP, and GTP, and is also a component of DNA and RNA (Arai and Sakuma, 2015). In this study, these minerals were determined with an emphasis on judging the leaves of *A. leiocarpus* as a medicinal plant of importance in the traditional medicine or possibly for drug formulation.

There was a significant correlation between the antioxidants measured in the ethanol and aqueous extracts. The results from the three methods to evaluate the antioxidant value in the leaves of *A. leiocarpus* were based on the several studies which have ascertained that some plants of medical importance possess phenol compounds and polysaccharides that are effective in eradicating free radicals and for such, are been classified as natural antioxidants (Konate *et al.*, 2014). The appreciable *in vitro* antioxidant potential of *A. leiocarpus* in this study may be the reflection of the quantity of its phenolic contents.

The obtained results revealed that it has quality antioxidants that could be helpful in defending the body system against any effect that might be caused by reactive oxygen species (ROS). ROS are produced in nature by cells after undergoing stress, however, they could also be caused by some other conditions such as psychological stress, smoking, and microbial toxins as reported by Onoja *et al.* (2014). Antioxidants have been proven to eradicate oxidative damage from reactive oxygen species by their nature of scavenging oxygen. In certain conditions, high an accumulation of ROS or low antioxidants that leads to chronic diseases could result in humans but the quality of antioxidants measured in *A. leiocarpus* in this study could serve a purpose to avert chronic diseases and also to be employed in the traditional health system. There exists a correlation between the reducing power ability and radical scavenging potentials of the extracts. The combination of these antioxidant, could act as a free electron for a conversion that will be more of therapeutic value in the prevention of chronic diseases. In a study on *Hyptis suaveolens* leaf extracts, compared results found *A. leiocarpus* to be significantly ($P<0.05$) higher in antioxidant values (Akharaiyi *et al.*, 2023).

Conclusion

The present investigation shows the vital roles the plant extracts could be involved in the treatment of some bacterial diseases and its traditional use for herbal formulations in curing various illnesses. As a result, the plant could be a good source for drug development to treat diseases caused by human pathogens. The plant could therefore have a space of recognition among the few medicinal plants which have been studied for their medical properties with novel therapeutic value and of little or no side effects.

Statements and Declarations

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Conflict of interests

We declare there is no conflict of interest among us the authors of the research.

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Authors' contribution

The research idea, design and the first draft was by AFC, Literature review was managed by ATA and OM, and all the data obtained were analyzed by ATA. The final draft approval of the research was by all authors before submission for

consideration.

References

- Adusei, S., Otchere, J. K., Oteng, P., Mensah, R. Q., & Tei-Mensah E. (2019). Phytochemical analysis, antioxidant and metal chelating capacity of *Tetrapleura tetraptera*. *Heliyon*, *5(11)*, e02762. https://doi.org/10. 1016/j. heliyon. 2019. e02762
- Akanbi, O.M. (2012). The antiplasmodal activity of *Anogeissus leiocarpus* and its effect on oxidative stress and lipid profile in mice infected with *Plasmodium bergheii*. *Parasitology Research*, *110(1)*, 219-226
- Akharaiyi F.C, & Boboye, B. (2010). Antibacterial and phytochemical evaluation of three medicinal plants.*Journal of Natural Products*, *3*, 27-34
- Akharaiyi, F.C., Ehis-Eriakha, C.B., Olagbemide, P.T., & Igbudu, F.H. (2023). *Hyptis suaveolens* L. leaf extracts in traditional health care system. *Foods and Raw Materials 11(2)*, 293-299. https://doi.org/10.21603/2308-4057- 2023-2-577
- Akharaiyi, F.C., & Okafor, A.C. (2021). Effect of *Spathodea campanulata* Ethanol Leaf Extract on Hematology and Liver Function of Salmonella infected and Paracetamol-induced Swiss Albino Mice. *FABAD Journal of Pharmaceutical Science, 6(3)*, 251-260
- Alhassan, D.A., Uba, A.I., Muhammad, A.U., & Muhammad, Y.Y. (2016). Phytochemical Screening and Antimicrobial Activity of Crude Stem Bark Extracts of *Anogeissus leiocarpus*. *European Journal of Medicinal Plants, 11(2)*, 1-7.
- Arai, H., & Sakuma, M. (2015). Bone and nutrition. Bone and phosphorus intake.*Clinical Calcium. 25(7)*, 967–972
- Aylana, Reiss-Mandel. & Gili, Regev-Yochay. (2016). *Staphylococcus aureus* and *Streptococcus pneumoniae* interaction and response to pneumococcal vaccination: Myth or reality?. *Human Vaccines & Immunotherapeutics*, *12(2)*, 351-357
- Breijyeh, Z., Jubeh, B., Karaman, R. (2020). Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. *Molecules 16 25(6)*, 1340. https://doi.org/10.3390/molecules25061340
- Buricova L, Reblova Z. (2008). Czech medicinal plants as possible sources of antioxidants.*Czech Journal of Food Sciences, 26(2)*, 132-138
- Castiglioni< S. (2021). Editorial of Special Issue “Magnesium in Human Health and Disease”*Nutrients, 13*, 2490. http://doi.org/10.3390/nu13082490
- CLSI. (2018). Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute.
- Cogswell, M.E., Mugavero, K., Bowman, B.A., & Frieden, T.R. (2016). Dietary sodium and cardiovascular disease risk—measurement matters. *Northern England Journal Medicine,. 375(6)*, 580
- Cummings, J.H., & Stephens, A.M. (2007). Carbohydrate terminology and classification. *European Journal of Clinical Nutrition, 61*, S5-S18.
- Devi, Sisca., Ropiqa, Meri., Murti, Yosi Bayu., Nugroho, & Akhmad, Kharis.. (2020). Screening of Extraction Process and The Estimation of Total Alkaloids in Carica papaya Linn. Leaf. *Traditional. Medical. Journal, 25(2)*, 90-93.

https://doi.org/10.22146/mot.52184

- Dhama, K., Tiwari, R., & Chakrabort, S, (2014). Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: an integrated update. *International Journal of Pharmacology, 10 (1)*, 1–43.
- Dorman, H.J, Bachmayer, O., Kosar M., & HilYumen. R. (2004). Antioxidant properties of aqueous extracts from selected Laminiaceae species grown in Turkey. *Journal of Agriculture and Food Chemistry, 52*, 762-770.
- Elgailani, I. E. H., & Ishak, C. Y (2014). Determination of Tannins of Three Common Acacia Species of Sudan. *Advances in Chemistry*, *2014*, Article ID 192708, 5 pages http://dx.doi.org/10.1155/2014/192708
- Ekwumengbo, P.A., Sailua, M.S., Omoniyi, K.I., & Zubairu. S.Y. (2014). Proximate and antinutritional constituents of Abelmoschus esculentus grown in Fadama Kabanni, Zaria, Kaduna State. *Journal of Scientific Research and Reports, 3(15)*, 2015-2027. https://doi.org/10.9734/JSRR/2014/9031
- Hal, l. D., Cromwell, G., & Stahly, T. (1991). Effects of dietary calcium, phosphorus, calcium: phosphorus ratio and vitamin K on performance, bone strength and blood clotting status of pigs. *Journal of Animal Science, 69(2)*, 646–655
- Halliwell, B., Gutteridge, J.M.C., & Aruoma, O.I. (1987). The deoxyribose method: a simple test – tube assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry, 65(1)*, 215-219. https://doi.org/10.1016/0003-2697(87)90222-3
- Hammuel, C., Abdullahi, M.S. Mankilik, M., Anyim, P.B., Adesina, O.B., Inekwe, U.V., Udiba, & Batari. M.L. (2011). The phytochemical and antimicrobial activities of oil from the seed of the vetia peruviana plant. *Journal of Applied Environmental and Biological Science, 1(12)*, 597-601
- Harborne, J. B., & Williams, C.A. (2000). Advances in flavonoid research since 1992.*Photochemistry, 55,* 481-504. https://doi.org/10.1016/S0031-9422(00)00235-1
- Harriet, U. U., Obinna, C.N., Solomon, U.O., & Toluwase, H.F., Conrad, A.O. (2020.) Antimicrobial Importance of Medicinal Plants in Nigeria. *The Scientific World Journal,* Volume Article ID 7059323, 10 pages https://doi.org/10.1155/2020/7059323
- Kalogeropoulos, A. P., Georgiopoulou, V.V., Murphy, R.A., Newman, A.B., Bauer, D.C., Harris, T.B., et. al. (2015). Dietary sodium content, mortality, and risk for cardiovascular events in older adults: the health, aging, and body composition (Health ABC) study. *JAMA International Medicine, 75(3)*, 410–419
- Konte, K., Yomalan, K., Sytar, O., Zerbo, P., Brestic, M., Patrick, V.D., Gagniuc, P., & Barro, N. (2014). Free radicals scavenging capacity, antidiabetic and antihypertensive activities of flavonoid-rich fractions from leaves of *Trichilia emetica* and *Opilia amentacea* in an animal model of Type 2 diabetes mellitus.*Journal of Evidence Based Complement. Alternative Medicine*, 1–13.
- Malinowska, P. 2013. Effect of flavonoids content on antioxidant activity of commercial cosmetic plant extracts.*Herba Polonica, 59*, 63-75
- Mann, A. (2012). Evaluation of Antimicrobial Activity of*Anogeissus leiocarpus* and *Terminalia avicennioides* against Infectious Diseases Prevalent in Hospital Environments in Nigeria. *Journal of Microbiology Research, 2(1)*, 6-1.
- Mann, A., Yusuf, A., & Daniyan, S. (2015). TLC analysis and bioactivity screening of the stem bark extract of *Anogeissus Leiocarpus* against multi-resistant *Staphylococcus aureus* and Quantification of Its Phytoconstituents. *A*

Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(2), 187-203.

- Nahapetian, A., & Bassiri, A. (1974). Changes in concentration and interrelationship of phytate, P, Mg, Cu, Zn in wheat during maturation. *Journal of Agricultural and Food Chemistry. 32*, 1179-1182.
- Naveh-Many, T., Rahamimov, R., Livni, N., & Silver, J. (1995). Parathyroid cell proliferation in normal and chronic renal failure rats. The effects of calcium, phosphate, and vitamin D. *Journal of Clinical Investigation, 96(4)*, 1786–1793
- Nirmaladevi, R., Padma, P.R., & Kavitha, D. (2010). Analyses of the methanolic extract of the leaves of *Rhinacanthus nasutus*. *Journal of Medicinal Plants Research. 4(15),* 1554-1560
- Ntiejumokwu, S., Alemika, T.O.E. (1991). Antimicrobial and photochemical investigation of the stem bark of *Boswellia dedziella*. *West Africa Journal of Pharmacology and Drug Research 10*, 00-104.
- Official Methods of Analysis (AOAC). (1980). 13th Edn, Washington DC. Association of Official Analytical Chemists
- Ogunjobi, K.M., Bdulwahab, A.S.O., Gakenou, O.F. Thompson, O.E., & Olorunfemi, O. (2020). Qualitative and quantitative evaluation of the phytochemical constituents of three wood species in Ogun State, Nigeria. *Tropical Plant Research, 7(3),* 627–633
- Onoja, S. O., Omeh, Y.N., Ezeja, M.I., & Chukwu. N.M. (2014). Evaluation of the In vitro antioxidant potentials of *Aframomum melegueta* methanolic seed extract. *Journal of Tropical Medicine*, Article ID 159343, 6 pages http://doi.org/10.1155/2014/15934
- Radha, S. R., Vijayakumari, B. (2013). Pharmocognostic studies of different plants of *Cratera magna* (Lour) *De world Journal of Pharmacy and Pharmaceutical Sciences, 2(5)*, 3462-3466
- Rahmani, A. H., & Ally S.M. (2015). *Nigella sativa* and its active constituent's thymoquinone shows pivotal role in the diseases prevention and treatment. *Asian Journal of Pharmaceutical and Clinical Research, 8*, 48-53.
- Siddiqui, N., Rauf, A., Latif, A.,. & Mahmood, Z. (2017). Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). J*ournal of Taibah University of Medical Science*, *2(4)*,360–3. https://doi.org/10.1016/j. jtumed. 2016. 11. 006
- Safiri, S., Mahmoodpoor, A., Kolahi, A- A., Nejadghaderi, S. A., Sullman, M. J. M., Mansournia, M. A., Ansarin, K., Collins, G. S., Kaufman, J. S. & Abdollahi, M. (2023) Global burden of lower respiratory infections during the last three decades. *Front. Public Health*, 10:1028525. https://doi.org/10.3389/fpubh.2022.1028525
- Satheesh, K.B., Suchetha, S.B., Vadisha, K.P., &Sharmila, P.B. (2012). Mahesh. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. *Nitte University Journal of Health Science,*2(4),34-38
- Spellberg, B., & Gilbert. D.N. (2014). The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. *Clinical Infectious Disease, 59 (Suppl 2),* S71-S75.
- Tinky, Sharma., Binjita Pandey., Bishnu, Kumar Shrestha., Gayaatri, Maiya Koju., Rojeena, Thusa., & Nabin Karki. (2020). Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. *Tribhuvan University Journal, 35(2)*, 1-11 https://doi.org/10.3126/tuj.v35i2.36183
- Trease, G.E., & Evans, W.C. (1989). Pharmacognosy 13th (ed). ELBS/Bailliere Tindall, London. 345-6,535-6,772-3.
- Usman, A. H., Ali, T., & Danjani, A. G. (2020). Phytochemical and antimicrobial studies of stem-bark extracts of *Anogeissus leiocarpus* found in Dutsin-ma, Katsina - Nigeria. *Fudma Journal of Sciences, 4(2)*, 156-167.

https://doi.org/10.33003/fjs-2020-0402-209

- Victor, Y.A. (2013). *In-vitro* assessment of antioxidant and antimicrobial activities of methanol extracts of six wound healing medicinal plants. *Journal of Natural Science Research, 3(1)*, 74-82
- Volpe, S L. (2013). Magnesium in disease prevention and overall health.*Advanced. Nutrition 4*, 378S–383S. http://doi.org/10.3945/an.112.003483
- Weaver, C. M. (2013). Potassium and health. *Advanced Nutrition 4(3)*, 368S–377S
- World Health Organization (2022). World health statistics 2022: monitoring health for the sustainable development goals (SDGs).
- Xi, L., Hao, Y-C., Liu, J., Wang, W., Wang, M., Li, G-Q., et. al. (2015). Associations between serum potassium and sodium levels and risk of hypertension: a community-based cohort study. *Journal of Geriatric and Cardiology: JGC, 12(2)*, 119
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry 64*, 555-9.