Anticancer and antibacterial potentials of methanolic extracts of the leaf and stem bark of *Afzelia africana*

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Received: 30 July 2021
Revised: 13 October 2021
Accepted: 26 October 2021
Published: 7 March 2022

Egyptian Pharmaceutical Journal 2022, 21:40–45

Context
Afzelia africana is one of the most important woody fodder plants in many parts of Africa.
Aims
This study evaluated the anticancer and antibacterial potentials of *A. africana* leaf and stem bark in Nigeria.
Patients and methods
The methanolic extracts were filtered and concentrated *in vacuo* according to standard procedures. The extracts were screened for phytochemical properties in accordance with standard methods. The antibacterial potentials of the plant extracts against 10 selected clinical bacterial isolates were evaluated using the agar well diffusion method. Anticancer potential using Human Embryonic Kidney cells and therapeutic index (TI) of the plant extracts were determined by standard procedures.
Statistical analysis used
The χ^2 test was used to determine the correlation between the antibacterial activities exerted by different concentrations of the stem bark and leaf extracts.
Results
Both plant parts contained alkaloids, anthraquinones, flavonoids, glycosides, saponins, tannins, triterpenes, and xanthoproteins. The anticancer test with Human Embryonic Kidney cells showed that the leaf and stem bark extracts elicited cytotoxic activity. The leaf extract had TI ranging from 0.23 to 0.97 whereas that of the stem bark ranged from 0.24 to 0.75, against the selected test organisms. There was no significant difference (P=0.077) in the TI between the leaf and the stem bark extracts. There were no significant differences between the antibacterial activities exerted by the leaf and stem bark extracts and within the antibacterial activities exerted by the control antibiotics (P=0.073).
Conclusions
This study revealed that the stem bark and leaf extracts of *A. africana* possess anticancer and antibacterial properties.

Keywords:
Afzelia africana, antibiotics, anticancer, *Fabaceae*, phytochemicals, therapeutic index

Egypt Pharmaceut J 21:40–45

1687-4315

Introduction

Medicinal plants are extensively employed in the treatment of various diseases and disorders all over the world. Historically, the use of herbal medicine dates back to antiquity. In African traditional medicine, several plant species have been claimed to possess medicinal properties and are therefore employed in the treatment of many ailments [1]. *Afzelia africana* Sm., an African legume tree in *Fabaceae* family, is used in traditional medicine for the treatments of pain, malaria, gonorrhea, and leprosy; is known for its socio-economic importance; and is well distributed in West African savannahs and forests [2]. The leaves are used in human feeding and forage for livestock. The wood is overexploited and commercialized for various industrial purposes, whereas the barks have several medicinal properties [3,4]. The stem bark decoction of *A. africana* is used for treatments of malaria, rheumatism, paralysis, and constipation. The maceration is used for the treatment of leprosy. The bark is burnt, and its ash is mixed with shea butter for soap, which is used to treat lumbago (back pain). Its root is blended with millet-beer for the treatment of hernias, or the root decoction with pimento is taken as treatment for gonorrhea and stomachache. The mixture of *A. africana* and *Syzygium guineense* leaves plus *Xylopia* fruit is used to treat edema [4]. This study, therefore, was designed to investigate

the anticancer and antibacterial properties of the leaves and stem bark of *A. africana*.

Materials and methods

Collection and authentication of plant samples

The fresh leaf and stem bark of *A. africana* were collected at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife.

Preparation of methanolic extracts of leaf and stem bark of *Afzelia africana*

The plant parts were air-dried and separately ground into powder. Each sample material (200 g) was soaked into 80% methanol for 72 h and then filtered. The filtrates were concentrated *in vacuo* to obtain crude methanolic leaf and stem bark extracts [5].

Phytochemical screening of the plant extracts

The plant extracts were screened for phytochemical constituents according to the methods of Harbones [6], Trease and Evans [7], and Sofowora [8].

Flavonoids: a 5-ml portion of the extract was added and mixed with 0.1 g of metallic zinc and 7 ml of concentrated sulfuric acid. The appearance of red color was indicative of flavonols [6].

Tannins: this was determined using the Braymer's test. Three drops of 5% ferric chloride were added to a mixture containing 2 ml of the extract and distilled water. The appearance of brownish green or dark-blue coloration indicated the presence of tannins [7].

Alkaloids: the Mayer's test was used to determine the presence of alkaloids in the extract. A 1-ml portion of the Mayer's reagent was added to 1 ml of the extract. The production of a whitish or cream colored precipitate indicated the presence of alkaloids [6].

Saponins: 2 g of the sample was added to 20 ml of distilled water and boiled in a water bath and filtered. Five milliliters of distilled water was added to 10 ml of the filtrate and shaken vigorously. The appearance of a stable froth signified the presence of saponins [6,8].

Steroids: the extract (1 ml) was dissolved in 2.0 ml of chloroform in a test tube, and then 1 ml of conc. H_2SO_4 was added. Formation of reddish-brown color at the interphase confirms the presence of steroids [8].

Cardiac glycoside: this was determined using the Keller-Kilani test. A 5-ml portion of the sample was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This mixture was underlaid with 1 ml of concentrated H_2SO_4. The appearance of a brown-ring interface was indicative of a deoxysugar characteristic of cardenolides [7].

Triterpenes: a 20-mg portion of the extracts was suspended in 10 ml of chloroform. This was warmed and then filtered. A 4-ml portion of concentrated sulfuric acid was mixed to the chloroform filtrate and mixed properly. The formation of red coloration indicated a positive result.

Xanthoproteins: a few drops of nitric acid were mixed with 1 ml of aqueous extract. Some drops of ammonia solution were added, and the mixture was observed for the formation of reddish or light brown precipitate, indicating the presence of xanthoproteins. Three drops of 10% (v/v) of ammonia solution were introduced to the mixture and observed for the formation of pink, violet, or red coloration in the ammonia layer to signify the presence of xanthoproteins in the extract.

Anthraquinones: a 0.5 g portion of the extract was mixed in 2 ml of diluted sulfuric acid, boiled, and filtered while still hot. About 3 ml of benzene was added to the filtrate, mixed thoroughly, and the benzene layer separated.

Phlobatannins: a 0.5 g portion of the extracts was heated with 10% (v/v) HCl in a boiling water bath. The formation of red precipitate was observed signifying the presence of phlobatannins.

Anticancer test: XTT cytotoxicity assay

The anticancer activity of the plant extracts was tested on Human Embryonic Kidney (HEK) 293 cells as reported by Zanariah *et al.* [9]. Exactly 100 µl of HEK cell suspension (1×10^5 cells/ml) was added to the inner walls of a 96-well plate and 200 µl of incomplete medium (Dulbecco's modified eagle's medium) to the outer wells. The plates were incubated for 24 h to permit adherence of the cells to the base of the wells. Serial dilution of the plant extracts, which had already been dissolved in dimethyl sulfoxide, the negative control (DMSO), and positive control (Actinomycin D), and with the complete medium (10% fetal bovine serum +Dulbecco's modified eagle's medium+penicillin/streptomycin) was done in a 24-well plate. This was carried out to give eight different concentrations of each sample and to obtain a final volume of 1 ml per well. Then, 100 µl of each concentration from the 24-well plate was added to the 96-well plate in triplicate, and there was a triplicate medium control and a DMSO control for each extract. To account for the color of the plant extracts, a reference plate was considered. This

plate comprised the media and plant extracts in duplicate without cells. The plates were incubated for 72 h, after which 50 µl of XTT reagent (Roche, Charles Avenue, Burgess Hill RH15, UK) was added to all of the wells, which were incubated for about 2 h and 30 min. The plates were then read on an ELISA plate with KC junior software to read the absorbance at 450 and 690 nm as the reference wavelength.

Source of microorganisms

The microorganisms used for this study, most of which have been implicated in infectious diseases, were collected from National Collection of Industrial Bacteria (NCIB) and Locally Isolated Organisms (LIO). These include *Klebsiella pneumoniae* (NCIB 418), *Escherichia coli* (NCIB 86), *Pseudomonas aeruginosa* (NCIB 950), *Staphylococcus aureus* (NCIB 8588), *Bacillus subtilis* (NCIB 3610), *Bacillus cereus* (NCIB 6349), *Bacillus stearothermophilus* (NCIB 8222), *Enterococcus faecalis* (NCIB 775), *Proteus vulgaris* (LIO), and *Shigella* sp. (LIO).

Preparation of bacterial cultures

The different bacterial cultures were prepared according to the method of Jorgensen *et al.* [10]. The fresh cultures of the bacterial were prepared from the original stock by subculturing each bacterial culture into freshly prepared nutrient agar and incubated at 37°C for 24 h. The fresh cultures were transferred into freshly prepared nutrient broth and standardized to 0.5 McFarland turbidity standards using a spectrophotometer to obtain the desired cell density of 1.5×10^8 (cells/ml).

Antibacterial activity of leaf and stem bark extracts using the agar well diffusion method

The sensitivity of the bacterial species to the plant extracts was tested *in vitro* following the method of Jorgensen *et al.* [10]. The 24-h-old broth was streaked homogenously on the nutrient agar medium by using a sterile glass spreader, and the plates were incubated at 37°C for 24 h. Each of the crude extracts was prepared in DMSO to obtain a final concentration of 25 mg/ml and screened for antibacterial activity. The sterile Petri dish containing the nutrient agar was allowed to solidify. Then, 0.1 ml of the test organism was smeared on the surface of nutrient agar and allowed to stand for 5 min in aseptic condition. Five wells of 6 mm in diameter each were made on the agar with a cork borer. Six drops of the working solution were transferred into each well and labeled. A prediffusion time of 30 min was allowed before incubation at 37°C for 24 h. After incubation, the plates were examined and the diameters of the zone of complete inhibition were observed and measured using a meter rule. Streptomycin and ampicillin were used as positive controls at 1 mg/ml. All the tests were aseptically performed in triplicates.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the plant extracts that exhibited antibacterial activities against the test organisms was determined using the microplate dilution method [11]. The plant extracts were separately diluted in DMSO, whereas the inocula of organisms were prepared in Mueller-Hinton broth with the turbidity adjusted to 0.5 McFarland standards in preparation of 1.0×10^8 bacterial cells/ml. Then, 150 µl of plant extract was added to each well of the 96-well microplate. The bacterial suspension (50 µl) was added to each well except the negative controls. Streptomycin and ampicillin were used as the positive controls. DMSO (10%) and plant extracts without bacterial suspension were used as the negative controls. The microtiter plates were incubated at 37°C for 24 h. The highest dilution which had the lowest concentration of the plant extract that inhibited the growth of the test microorganism was taken as the MIC.

Determination of the minimum bactericidal concentration

The wells showing no growth from the lowest concentrations in the MIC assay were selected for minimum bactericidal concentration (MBC) determination. Bacterial cells (100 µl) from the MIC test plate were subcultured on freshly prepared solid nutrient agar by making streaks on the surface of the agar. The plates were incubated at 37°C for 24 h overnight. The concentration of the plant extract in the plate that showed no microbial growth was considered to be the MBC of the extract [12]. All the tests were performed in triplicate.

Data analyses

Data were collated and statistically analyzed using IBM SPSS software, version 25. A χ^2 test was used to test the correlation of the antibacterial activities exerted by different concentrations of the stem bark and leaf extracts. The value of α was set at less than or equal to 0.05. The P value was considered statistically significant if less than the value of α.

Results

Phytochemical constituents of the stem bark and leaf extracts of *Afzelia africana*

Both stem bark and leaf extracts contained alkaloids, anthraquinones, flavonoids, glycosides, saponins, tannins, triterpenes, and xanthoproteins (Table 1).

Anticancer potentials, therapeutic index, and effective concentration (EC_{50}) of the stem bark and leaf extracts of *Afzelia africana*

The anticancer test with HEK cells showed that the leaf and stem bark extracts elicited cytotoxic activity at 0.65 and 0.54 µg/ml. The leaf extract had TIs of 0.23, 0.97, 0.25, 0.27, and 0.41 against *S. aureus*, *E. coli*, *Klebsiella oxytoca*, *Salmonella enterica*, and *Shigella sonnei*, respectively. Similarly, the stem bark had TIs of 0.35, 0.75, 0.24, 0.32, and 0.57, respectively, for the same organisms. There was no significant difference (P=0.077) in the therapeutic index (TI) between the leaves and the stem bark extracts (Table 2).

Antibacterial sensitivity of test organisms to plant extracts

The antibacterial activity of stem and leaf methanol extracts of *A. africana* was dictated by the sizes of the zones of inhibition (mm) shown by the test pathogens. The zones of inhibition exerted by the stem bark extract against the test pathogens ranged from 15 to 20 mm, whereas that of leaf extract ranged from 15 to 29 mm. Streptomycin and ampicillin exerted inhibiton zones ranging from 15 to 29 mm and 15 to 21 mm, respectively, against the test pathogens. There were no significant differences (P=0.073) between the antibacterial activities exerted by the leaf and stem bark extracts and within the antibacterial activities exerted by the antibiotics (Table 3).

Table 1 Phytochemical constituents of the stem bark and leaf extracts of *Afzelia africana*

Phytochemical	Stem bark	Leaf extract
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	+
Saponins	+	+
Steroids	–	–
Glycosides	+	+
Triterpenes	+	+
Phlobatannins	–	–
Xanthoproteins	+	+
Anthraquinones	+	+

+, present; –, absent.

Minimum inhibitory concentration and minimum bactericidal concentration of extracts against test organisms

The MIC of stem bark and leaf methanol extracts of *A. africana* against the test pathogens ranged from 0.391 to 1.562, whereas that of leaf extract ranged from 0.271 to 1.987. The MBC of the stem bark and leaf extracts of the plant ranged from 0.391 to 6.25 and 0.232 to 6.36, respectively, against the test pathogens, and there were no significant differences (P=0.065) between MICs of the plant parts (Table 4).

Discussion

For a drug substance or herbal preparation to be adopted in clinical applications, it must be selectively toxic to the target microorganisms or must, at least, interfere directly with a reaction pathway with minimal or no toxic effects to the host cells [13]. The antibacterial and anticancer potentials of *A. africana* detected in this study give a fresh insight into the application of these plant parts with the intent of discovering new leads for fighting infectious diseases including cancer.

Table 2 Cytotoxicity and therapeutic index of the leaf and stem bark extracts of *Afzelia africana*

Extracts	Cytotoxicity (µg/ml)	Therapeutic index				
		Staphylococcus aureus	*Escherichia coli*	*Klebsiella oxytoca*	*Salmonella enterica*	*Shigella sonnei*
Leaf	0.65	0.23	0.97	0.25	0.27	0.41
Stem bark	0.54	0.35	0.75	0.24	0.32	0.57

P=0.077.

Table 3 Antibacterial activity of stem and leaf methanol extracts of *Afzelia africana* against selected pathogens

Bacterial strains	Diameter of zone of inhibition (mm)			
	Stem	Leaf	Streptomycin	Ampicillin
Bacillus cereus (NCIB 6349)	20	15	15	17
Proteus vulgaris (NCIB 67)	15	19	19	19
Escherichia coli (NCIB 86)	19	20	21	20
Serratia marcescens (NCIB 1377)	20	21	15	16
Bacillus subtilis (NCIB 3610)	21	19	29	20
Pseudomonas aeruginosa (NCIB 950)	20	15	20	15
Staphylococcus aureus (NCIB 8588)	17	19	25	19
Micrococcus luteus (NCIB 167)	19	21	22	20
Vibrio fluvialis (LIO)	20	15	20	21
Klebsiella pneumoniae (NCIB 418)	17	29	25	19

LIO, Locally Isolated Organisms; NCIB, National Collection of Industrial Bacteria. P=0.073.

Table 4 Minimum inhibitory concentration and minimum bactericidal concentration of stem bark and leaf methanol extracts of *Afzelia africana*

Bacterial strains	MIC (mg/ml)		MBC (mg/ml)	
	Stem bark	Leaf	Stem bark	Leaf
Bacillus cereus (NCIB 6349)	1.562	1.438	0.391	0.287
Proteus vulgaris (NCIB 67)	1.562	1.673	0.391	0.375
Escherichia coli (NCIB 86)	1.562	1.987	0.781	0.539
Serratia marcescens (NCIB 1377)	1.562	1.432	1.562	1.239
Bacillus subtilis (NCIB 3610)	1.562	1.894	6.25	6.35
Pseudomonas aeruginosa (NCIB 950)	0.391	0.271	1.562	1.568
Staphylococcus aureus (NCIB 8588)	0.391	0.465	1.562	1.673
Micrococcus luteus (NCIB 167)	0.781	0.892	0.391	0.783
Vibrio fluvialis (LIO)	1.562	1.432	0.391	0.232
Klebsiella pneumoniae (NCIB 418)	1.562	1.643	6.25	6.36

LIO, Locally Isolated Organisms; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; NCIB, National Collection of Industrial Bacteria. P=0.065.

Phytochemical constituents such as flavonoids, tannins, alkaloids, saponins, glycosides, triterpenes, xanthoproteins, and anthraquinones were detected in both stem bark and leaf extracts of the plant. Tannins and alkaloids have been earlier reported to possess strong antibacterial activity [14,15]. Tannins provoke antimicrobial activity by binding and precipitating extracellular and intracellular proteins. Similarly, alkaloids interrupt several molecular targets including cell membranes and nucleic acids, thus inhibiting microbial growth [16]. Selective inhibition of more than one molecular target is essential for combating multidrug and pandrug resistance among bacterial species and cancer cells. The antibiotics and anticancer activities shown by the leaf and stem bark of *A. africana* are consistent with some other plant materials [15,17].

Akinpelu *et al.* [18] also reported the presence of a number of phytochemical compounds in the crude extract of *A. africana*, and these were tannins, flavonoids, steroids, alkaloids, and saponins. These phytochemical compounds are biologically active and therefore aid the antimicrobial activities of *A. africana.* Phytochemicals utilize different modes of action to exert antimicrobial activities. For instance, tannins act not only by iron deprivation but by hydrogen bonding or interactions with specific proteins like enzymes in microbial cells. Alkaloid has also been reported in the stem bark extract of *A. africana* and has been well studied to exhibit anti-asthmatic, anti-inflammatory, and anti-anaphylactic properties [19]. Tannins and alkaloids have remarkable activity in the prevention and treatment of cancer [20]. Flavonoids, in *A. africana*, elicit analgesic, anti-inflammatory, antimicrobial, anti-allergic, cytostatic, anti-angiogenic, and antioxidant properties [21,22]. Flavonoids in human diets could prevent cancers and menopausal symptoms [23]. Saponins possess numerous pharmacological properties and had been reported in *A. africana* stem bark extract [24]. Saponins are major ingredients in traditional Chinese medicine and are associated with biological effects in medicinal plants [25]. Saponins elicit anti-inflammatory activities. The literature cited on phytochemical compounds buttressed our findings on the anticancer and antibacterial potentials of the methanolic extracts of stem bark and leaf of *A. africana.*The antibacterial activity exerted by leaf and stem bark extracts against *K. pneumoniae* and *B. subtilis* was higher than that exerted by streptomycin and ampicillin. As no significant difference occurred between antibacterial activities exerted by the stem bark and leaf extracts, it could be deduced that either of the plant parts could be used for the treatment of bacterial infections.

The anticancer test with HEK cells also showed that both the leaf and stem bark extracts elicited cytotoxic activity. Although the stem bark extract showed stronger cytotoxic activity against HEK cell lines, there was no significant difference in the TI between the two plant parts. TI represents the blood concentration at which the plant extract is relatively toxic, and the larger the TI, the safer the drug. However, in this study, the TI of the plant parts was less than 1.0, suggesting that precautions are required when using the plant remedy in disease treatment. This is consistent with the study of Igwenyi and Akubugwo [26], who also reported that *A. africana* possesses some toxicity.

Agents with cytotoxic properties were initially introduced in medicine for their ability to interrupt nucleic acid and protein synthesis in malignant cells. Subsequently, they were found to possess immune-suppressing capacities. For this reason, cytotoxic agents are now used in medicine for preventing the rejection of transplanted organs. The further realization that these

agents exhibit immunomodulatory capacities led to their use in autoimmune diseases such as systemic lupus erythematosus. Ever since their introduction, the management and prognosis of these diseases have greatly improved. As a result of their adverse effects, cytotoxic therapy is reserved only for patients with moderate to severe disease cases. Therefore, *A. africana* is found to not only elicit antibiotic activity but also cytotoxic activity, which has implicated its potential use for the treatment of autoimmune diseases and organ rejection.

Conclusion

This study revealed that the stem bark and leaf extracts of *A. africana* possess antibacterial and anticancer properties. The plant material could be exploited for alternative anticancer and antibiotic drugs discovery toward combating the global disease burden.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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