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Original article

Pharmacognostic Characteristics and Mutagenic Studies of *Alstonia boonei* De Wild.

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Abstract

Background and objectives: The bark of *Alstonia boonei*, known as stool wood, is sold in large quantities on the Ghanaian market. It is used for the treatment of numerous ailments including snake bites, worm infestation, malaria and rheumatic pains. For safety reasons, it is necessary to control the raw materials by setting simple but relevant parameters to ensure identity, purity and quality which have been the aim of the present study to authenticate the plant materials. **Methods:** The macroscopic, microscopic, physico-chemical, phytochemical, UV-visible, fluorescence, HPLC and elemental characteristics were evaluated. Mutagenicity was also investigated with the Ames test. **Results:** The leaf characteristics can be employed to preliminary confirm the identity of the plant. The greyish-green outer bark and inner cream bark is rough, short and splintery with a bitter taste. The powdered stem bark showed microscopic prismatic calcium oxalate crystals, scalariform xylem vessels and lignified brachysclereids. Phytochemicals present were alkaloids, saponins, tannins, flavonoids, glycosides and terpenes. The bark fluoresced reddish-brown in 50 %v/v H_2SO_4 under UV light of λ 254 nm and contained traces of Cd, Fe, Zn and As, which were within recommended limits. HPLC fingerprint showed peaks at 254 nm, and UV analysis in various solvents showed spectral shifts on ionization. *A. boonei* demonstrated mutagenicity in Ames test. **Conclusion:** The characteristic macroscopic, microscopic, physico-chemical and chemical parameters evaluated for the plant sample can be used in rapid identification, authentication and establishment of the quality of raw materials. This will improve the quality and hence efficacy. The mutagenicity suggests the need for further safety evaluation.

Keywords: *Alstonia boonei*; Ames test; quality control; simple methods

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Introduction

The past three decades have seen a tremendous increase in the use of herbal medicines with many people across the world relying on them for some part of their primary healthcare [1]. Estimates reveal that 80 % of patients in Africa and 70 % in Ghana use traditional medicine for their primary healthcare [2]. *Alstonia boonei* De Wild, of the family Apocynaceae, stem bark has been sold in large quantities daily on the Ghanaian market [3], is used for treating a myriad of diseases. *A. boonei* is a large tropical deciduous medicinal plant originating from West African [4,5]. It is commonly found in the forest zones of Ghana and is popularly known as stool

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wood, God's tree or "Onyame dua", "Sinduro" in Twi, , "Sinu" in Ga-Adangbe, "Bakunin" in Nzema, and "Siaketekre" in Ewe [4,5]. It grows up to 35 m high and 1.2 m in diameter with a rough greyish-green or grey bark. Its white latex is copious. The leaves are arranged in whorls at the nodes, oblanceolate shape and the apex is rounded to acuminate. The lateral vein is prominent almost at right angle to midrib. The flowers are white coloured with lax terminal cymes. The fruits are occur in pairs with slender follicle which may grow up to 16 cm long with brown floss at each end [5]. *Alstonia boonei* is commonly prepared as decoctions and tinctures. Preparations of the stem bark is traditionally used for snakebites, venereal diseases, malaria, measles, boils, wounds, arterial hypertension, arthritis, cataracts, placenta retention, worm infestation, rheumatic pains and for muscle relaxation [6]. This plant is contraindicated in pregnant women, lactating mothers and liver dysfunction [4]. Therapeutically, the stem bark was shown to have antipyretic, antirheumatic, anti-inflammatory, analgesic/pain-killing, antimalarial/antipyretic, antidiabetic (slightly hypoglycaemic), anthelminthic, aphrodisiac, antimicrobial and antibiotic properties [4,5,7-10]. Extracts of *A. boonei* have potential anthelminthic effects by the ability to inhibit glutathione S-transferase in parasitic nematodes [11].

Adversely, high doses of this plant extract are known to cause Steven-Johnson's syndrome. Various species of *Alstonia* are rich in alkaloids, steroids, triterpenoids and phenolic compounds, and these compounds are known to contribute to their toxicity [12,13].

A major obstacle for using of these herbal medicines is the lack of available and applicable quality control measurements [14]. This is because herbal medicines have distinct characteristics. They contain more than one active principle which is frequently unknown. This calls for appropriate authentication and standardization of the source materials [15]. Quality assurance of herbal materials are necessary to ensure that they contain adequate levels of active ingredients and have no inorganic impurities such as toxic metals, sand, pathogenic microbes, mycotoxins, pesticides, adulterants, etc. Methods employed to ensure the quality of plant materials encompass simple macroscopic and microscopic identification, physico-chemical analysis such as thin layer chromatography (TLC) and many more current sophisticated methods [16]. However, the most current methods are extremely lacking especially in developing countries; hence, there is a need to use simple, available but relevant means to ensure the quality of such widely used herbs.

Reports of the effectiveness of a natural drug often creates a high demand. To meet growing demands, the natural drug can be adulterated with inferior quality material [17] which could eventually result in treatment failure or increased toxicity. In view of the above, it is essential to lay down pharmacognostic specifications peculiar to each medicinal plant [17]. This study therefore aimed to evaluate pharmacognostic parameters of *A. boonei* leaves and stem barks which can serve as simple means to estimate the quality control of the plant material before they are used for treatment and manufacturing of herbal medicines. In the present study, the mutagenic study was also conducted because only a small percentage of traditionally used medicinal plant materials have been thoroughly investigated for such activities [18]. Plant materials are often assumed to be safe as a result of their long history of use; however, reports have shown that a number of plants used in traditional medicine may have in vitro mutagenicity and 90% of such mutagens are carcinogens capable of inducing cells to undergo abnormal growth and genetic defects which could result in cancers [19-22].

Materials and Methods

Ethical considerations

This study was approved by the Proposal and Ethical Committee of the School of Pharmacy, University of Ghana (ID 2019/7/10) dated 27th October, 2018.

Plant material

The leaves and stem barks of *A. boonei* were collected from the botanical garden of the University of Ghana. The sample was authenticated at the Ghana Herbarium, Department of Plant and Environmental Biology, University of Ghana. Herbarium voucher (number PSM67/19) has been kept at the Herbarium of the Department of Pharmacognosy and Herbal Medicine, University of Ghana, Legon. Fresh leaves to be used for microscopic evaluation were stored in containers filled with glycerin prior to analysis. The stem bark was air

dried for three weeks, milled into coarse powder and kept in well-labelled air-tight containers for further analysis.

Macroscopic evaluation

The macroscopic characteristics, such as colour, odour, texture and fracture were recorded for the stem bark. The shape, colour, arrangement, apex base, texture, margin and venation were determined for the fresh leaves which could aid in future identification of the plant.

Microscopic evaluation

Qualitative and quantitative microscopy was performed with the Leciad compound light microscope. The microscopic characteristics of the samples were studied using standard procedures according to WHO guidelines on Quality control methods for herbal materials, 2011 [23].

Physico-chemical analysis

The ash values, extractive values, moisture content, swelling index, foaming index and foreign organic matter were determined for the air dried stem bark. The total ash, acid insoluble and water soluble ash values were estimated and extractive values of petroleum ether, 50 % ethanol and water were also determined [23].

Preliminary phytochemical screening

Preliminary phytochemical screening was performed to detect the presence of alkaloids, saponins, tannins, flavonoids, glycosides and terpenes [24,25].

Fluorescence studies

Fluorescence analysis of the powdered stem bark was carried out to determine the characteristic colour in specific solvents according to methods described by Ranjith, 2018 [26]. Observations were made under visible day light and UV light of short wavelength (λ 254 nm) and UV light of long wavelength (365 nm) for their characteristic colours [26]. The solvents employed were distilled water, 1N HCl, 1N NaOH, 50 % H_2SO_4, methanol, glacial acetic acid, nitric acid and chloroform.

HPLC analysis

Fifty grams of the pulverized stem bark of *A. boonei* was extracted with 500 mL of 50% ethanol by ultra-sonication for 15 min followed by centrifugation at 6000 rpm for 10 min. The clear supernatant was collected and the residue again extracted with another 50 mL. The combined extracts were concentrated under vacuum at 40^{o}C and lyophilized. The 50 %v/v ethanol extract was dissolved in methanol to prepare a solution of 1 mg/mL and filtered. The samples were then analysed using KNAUER HPLC (KNAUER Products, Berlin, Germany) and the ClarityChromVR Software (Vertex Plus C_{18}-KNAUER, Berlin, Germany). HPLC fingerprint was developed under the chromatographic conditions: Injection volume: 10 µL, mobile phase: methanol: water (5: 95); Flow rate: 1 mL/min; Detection wavelength: λ 254 nm, Stationary phase: Luna C18 reverse-phase column (250 × 4.6 mm).

Ultraviolet/visible spectrometric analysis

An aqueous extract was prepared by weighing 10 g of coarsely powdered air-dried stem bark of *A. boonei* in 100 mL of distilled water. The plant material was sonicated three times for intervals of 15 min each time. The solution was filtered into a round bottomed flask and concentrated using a rotary evaporator set at 45 ^{o}C. The residue was freeze dried to obtain the crude aqueous extract. An amount of 20 mg of this extract was accurately weighed and separately dissolved in 20 mL of distilled water, dilute hydrochloric acid (0.1M), dilute sodium hydroxide (0.1M), methanol, acidified methanol and basified methanol. Each of these preparations was sonicated for 10 min to ensure complete dissolution of the extracts. The sonicated solutions were allowed to cool to room temperature and filtered using membrane filter of 0.45µm mesh size. Dilutions of 1 in 10 of each of the solution was made in the respective solvents. They were filtered and analyzed using a 1 cm quartz cell. All samples were scanned over a wavelength range of 200-600 nm (Ultraviolet/ visible range) using a single beam Jenway 7315 UV- Vis spectrophotometer.

Heavy metal analysis

An Olympus Vanta M Portable ED-XRF (VMR) analyzer (USA) equipped with 4-Watt x-ray tube with application optimized anode material rhodium (Rh) and tungsten (W), 50kV x-ray tube and large area silicon drift detector was used to analyze the sample for heavy metals. Calibration of the XRF was done using the SARM 2711A,

certified reference material from the manufacturer. Twelve g grams (12 g) of the stem bark powder was sieved with a 180 µm mesh size sieve into a fine powder and kept in a dry well-labelled container for analysis. The loose sample was irradiated following the manufacturer's protocol. Simultaneous measurement of the levels of heavy metals present was done.

Mutagenic studies

Sample preparation

Fifty grams (50 g) of pulverized material was boiled in 500 mL of distilled water for 20 min. The decoction was centrifuged at 6000 RPM for 10 min. The supernatant was collected and concentrated under vacuum at 40 ^{o}C and lyophilized to obtain dried crude extracts. The extract was stored at -20 ^{o}C for subsequent use. The samples were prepared and diluted with sterile water on the day of the assay to a concentration of 10 µg/mL and sterile filtered using a 0.22 µM membrane filter.

Induction of mutation

The Muta-ChromoPlate™ two strain kit (manufactured by Environmental Bio-Detection Products Inc, Canada) which works on the principle of the Ames test was employed to determine the mutagenicity potential of the extracts [27]. The experiment was carried out in accordance with protocol provided by the manufacturer. *Salmonella typhimurium* TA98 was grown overnight for 14 h at 37 ^{o}C in 10 mL nutrient medium. The metabolic reaction mixture consisting 4% S9 fraction (metabolic enzyme), 1% 0.4 M $MgCl_2$, 1% 1.65 M KCl, 0.5% 1 M D-glucose-6-phosphate disodium, 4% 0.1 M NADP, 50% 0.2 M phosphate buffer and 39.5% sterile distilled water were prepared into sterile 50 mL Falcon tubes. A 2.5 mL aliquot of the reaction mixture was added to each tube. Sterile water or sample material to be tested or 100 µL of the positive control (nitrofluorene) were added to the respective tubes. Five microliters of the bacteria suspension was added to each tube, except for the blank tube. The mixture was vortexed for 15 min and contents of each tube poured into sterile reagents boats and 200 µL was dispensed into each well of a sterile 96-well plate and incubated for 5 days at 37 ^{o}C. Mutagenicity was detected as an increase in the number of histidine revertants with reference to scores provided in the test kit to determine whether the mutation was significant or not. If a reverse mutation occurs, the bacteria in the colony have the ability to synthesize histidine and will continue to grow, turning the colour in the well from purple to yellow. The number of positive wells scored in the 96 well plates determined the significance of mutataion in the fluctuation test as described by Gilbert, 1980 [27]. The statistical table provided in the kit was used to compare the natural background rate of reverse mutation to the rate of reverse mutation within a sample assay. Based on these samples, p values of 0.001 were classified as highly mutagenic, p values of 0.01 moderately mutagenic and p values of 0.05 weakly mutagenic.

Statistical analysis

The statisitcal tool used to detected te level of significance was the t-test .

Results and Discussion

Pharmacognostic studies involve a number of different evaluation techniques which are widely employed to determine the quality of natural products. These evaluations focus on organoleptic, macroscopic, microscopic, physico-chemical, fluorescence and chemical characteristics of the crude drug [14] which are very important in establishing the identity, the purity and the quality of plant samples. The very first step in pharmacognostic analysis involves the organoleptic evaluation which employs the use of sensory organs. For example visual inspection provides the simplest and most rapid means by which authenticity can be established. If a plant material is found to be significantly different from the specifications in terms of its colour, texture, odour or taste, it is considered as out of specification [28]. The present evaluation showed that the outer stem bark of *A. boonei* was greyish-green, while the inner bark was light yellow or cream in colour. The bark had a characteristic odour, bitter taste and rough texture. Figure 1 is a photograph of the leaves and stem bark of *A. boonei*. A summary of the organoleptic and macroscopic characteristics of these leaves and stem barks of *A. boonei* have been provided in tables 1 and 2, respectively.

Qualitative microscopic analysis of the leaves, which could serve as a means of verifying the identity of the plant gave leaf constants of stomatal number, epidermal cell number, vein islet number, veinlet termination number and stomatal index which have been provided in table 3.

Figure 1. Leaves and stem bark of *Alstonia boonei*

Table 1. Macroscopic characteristics of *Alstonia boonei* leaves

Characteristic	*Alstonia boonei* leaves
Shape	Obovate
Colour	Dark green
Arrangement	Palmately compound
Venation	Reticulate
Apex	Mucronate
Margin	Entire
Surface	Glabrous
Texture	Papery

Table 2. Macroscopic characteristics of *Alstonia boonei* bark

Characteristic	*Alstonia boonei* bark
Condition	Dried
Outer bark colour	Greyish-green
Inner bark colour	Light yellow / cream
Odour	Characteristic
Taste	Bitter
Texture	Rough
Fracture	Short and splintery

Microscopic examination of *A. boonei* leaves showed normal features of such as vein islet, veinlet termination and paracytic stomata (figures 2). Powder microscopy of the stem bark however had prismatic calcium oxalate crystals, scalariform xylem vessels and lignified brachysclereids (figure 3). The physico-chemical analysis has been shown in table 4.

The observed total ash, water soluble and acid insoluble ash indicated the approximate measure of chemical constituents [29]. The total ash (9.42 %w/w) represented the total amount of material remaining after ignition was 9.42 %w/w, while acid insoluble ash which indicated the amount of silica present, especially in the form of sand and siliceous earth was 4.9 %w/w and water-soluble ash which is the difference in weight between the total ash and the residue after treatment of the total ash with water, was almost 9.4 %w/w and this was almost the same as the total ash.

Cold extractions revealed that the 50 % ethanol solvent yielded the highest extract (6.13 %w/w), followed by petroleum ether (2.48 %w/w), and water (2.4 %w/w) (table 5). This result indicated that *A. boonei* stem bark has a lot more middle-polar phyto-constituents than the non-polar and polar constituents

Table 3. Leaf constants of *Alstonia boonei*

Parameter	*Alstonia boonei*
Stomatal Number	15.7
Epidermal Cell Number	73.1
Vein islet Number	18.9
Veinlet termination number	17.6
The stomatal index [stomata per square mm of epidermis]	16.89

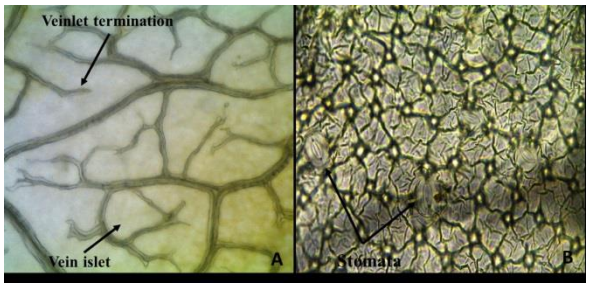

Figure 2. *Alstonia boonei* leaf showing A: vein islets and veinlet terminations; B: paracytic stomata

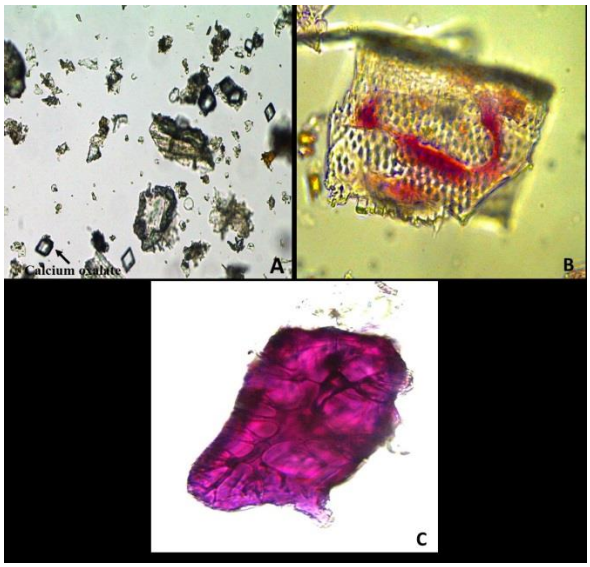

Figure 3. Powder microscopy of the stem bark of *Alstonia boonei*; A: prismatic calcium oxalate crystals; B: scalariform xylem vessels; C: lignified brachysclereid.

Table 4. Phisico-chemical parameters of *Alstonia boonei*

Parameters	*Alstonia boonei* (% w/w)
Total Ash	9.42
Acid insoluble ash	4.98
Water soluble ash	9.40
Moisture content	4.51
Foreign organic matter	Nil
Foaming index	Less than 100
Swelling index	Nil

The moisture content of the air dried sample was determined to be 4.51 % w/w. It is important to estimate the moisture content in order to control the presence of water in dried plant samples. This is necessary because excessive moisture could result in degradation of the plant material [29].
Phytochemical screening of the 50 % ethanol extract of *A. boonei* showed presence of alkaloids, tannins, glycosides, saponins, flavonoids and terpenes.

Table 5. Extractive values of *Alstonia boonei*

Parameters	*Alstonia boonei* (%w/w)
Petroleum ether	2.48
50 % ethanol	6.13
Water	2.4

The foaming index was less than 100, indicating that there could be low or no saponins present in the sample. Swelling factor was also zero, indicating that the plant may probably not contain gums and mucilage nor appreciable amounts of pectins or hemicelluloses which could be of importance to the pharmaceutical industry [23]. The phytochemical investigation revealed the presence of alkaloids, tannins, saponins, glycosides, flavonoids and terpenoids which have been verified in other studies [4,30]
Analysis for characteristic fluoresces of powdered *A. boonei* bark at short and long wavelengths of λ 254 nm and λ 365 nm respectively, showed varying colours in different solvents (table 6). This could provide qualitative studies for both identity and quality of the stem bark of *A. boonei*. For example, there was a red fluorescence in nitric acid. Fluorescence analysis is a very useful for preliminary identification of different types of constituents present in natural products. These constituents fluoresce under UV light but may not show such a characteristic when observed in day light. This phenomenon may be due to the materials themselves or fluorescent derivatives formed after treatment with specific reagents [14]. Fluorescence behavior could be used to further authenticate the plant sample and detect some adulteration that may affect the fluorescent behavior of pure plant drugs. Therefore, the observed red fluorescence in nitric acid and blue fluorescence in several solvents of *A. boonei* under UV light of both long and short wavelength could be used as a parameter for quality assessment. Plant materials that contain phenolic groups, e.g. simple phenolic compounds, flavonols, flavonoids etc., show spectral shifts on ionization. Spectral shifts were observed when UV analysis of the stem bark extract was done in distilled water and also under acid and basic conditions. UV spectra of the aqueous extract showed a hypsochromic shift and a hyperchromic effect of absorbance at λ 220 nm in both acidic and basic conditions. The reduction in absorbance is due to the decrease in concentration of the UV active principle in the extract.
The HPLC fingerprint chromatogram showed seven peaks (figure 4), with the largest peaks having retention times of 1.6 and 1.9 min. This fingerprint chromatogram is representative of the phyto - constituents present in the stem bark which could be essential for future quality control of the plant material.
The lambda max (λmax) of the extract was 220 nm in distilled water. There is a slight shift to a longer wavelength for components in the basified media (figure 5). This characteristic effect on ionization can be used as a simple mean to further determine the purity and identity of this plant sample.
Elemental analysis of the stem bark of *A. boonei* detected cadmium, iron, zinc and arsenic (table 7). Lead was not detected. Contamination of medicinal plant materials with heavy metals such as Pb, Cu, Ni , Cd, Fe, Zn and As [31] is rampant and can be attributed to environmental pollutants and traces of pesticides [29].
There is a need to control the levels of these heavy metals in highly consumed plant materials, because they could result in serious adverse effects including cancers, organ damage etc. in humans [32].

Table 6. Fluorescent studies of powdered stembark of *Alstonia boonei* in various solvents

	Day light	λ 254 nm	λ 365 nm
Powdered Sample only	Brown	Dark brown	Dark brown
Distilled water	Light brown	Blue	Light Blue
1N HCl	Light brown	Blue	Light Blue
1N NaOH	Deep brown	Light Blue	Light Blue
50 % H_2SO_4	Black	Reddish Blue	Light Blue
Methanol	Light brown	Cream	Colourless
Glacial acetic acid	Light brown	Blueish green	Light Blue
Nitric acid	Red	Blue	Light Blue
Chloroform	Deep brown	Blue	Light Blue
50 % $FeCl_3$	Deep brown	Deep blue	Light Blue
95 % Ethanol	Light brown	Blue	Light Blue

Table 7. Heavy metal content of *Alstonia boonei*

Heavy metal	Quantity (µg/kg)
Cadmium (Cd)	$(2.6\pm0.2) \times 10^{-3}$
Lead (Pb)	Not detected
Iron (Fe)	$(208.9\pm0.0) \times 10^{-3}$
Zinc (Zn)	$(3.7\pm0.2) \times 10^{-3}$
Arsenic (As)	$(0.9\pm0.1) \times 10^{-3}$

A major metal pollutant, such as Pb [31] was not detectable. Cadmium (Cd), Fe, Zn and As were detected in the samples but were found to be within the WHO acceptable limits [28].

Mutagenic studies conducted on the 50 % ethanol extract of the stem bark, showed highly mutagenic (p values of 0.001) in the Ames test which was detected after three days of incubation. Hence *A. boonei* exhibits the potential of causing mutagenicity by a frame shift mutation. This could therefore suggest that this plant material could contain molecules that can intercalate between the normal bases to create mistakes during DNA synthesis. *Alstonia boonei* bark has also been reported to demonstrate a concentration dependent mutagenic effect in other studies [33].

The pharmacognostic parameters that have been determined can be collectively employed to rapidly evaluate the identity, purity and quality of *A. boonei* samples on the Ghanaian market. The mutagenic activity of the 50 % ethanol extract suggests that this plant though widely used, may need to be used with caution and further screened for toxicity.

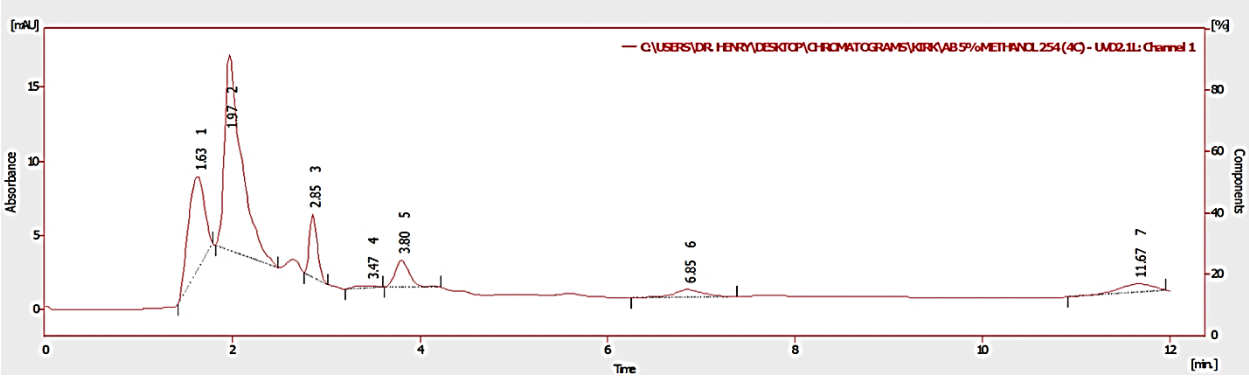

Figure 4. HPLC fingerprint of *Alstonia boonei* 50 % ethanol extract at λ 254 nm

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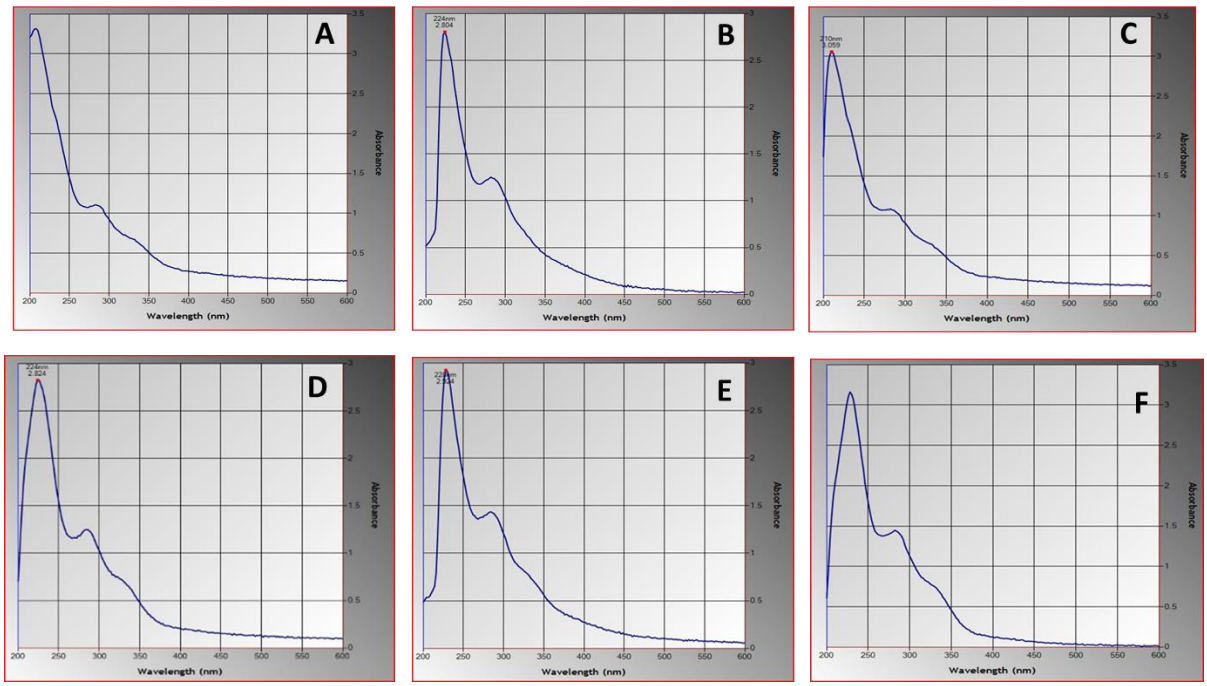

Figure 5. UV spectra of aqueous extract of *Alstonia boonei* in various solvents; **A**: distilled water; **B**: dil. NaOH; **C**: dil HCl; **D:** methanol; **E:** methanolic NaOH; **F:** methanolic HCl

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Author contributions
Emelia Oppong Bekoe conceptualized the study, coordinated the project, and conducted the physico-chemical and mutagenic analysis; Kirk B. Dodoo was responsible for the UV and HPLC analysis; Cindy Kitcher was responsible for the macroscopic and microscopic analysis; Andrew Gordon was involved in both the phytochemical and chemical analysis; Samuel Frimpong-Manso performed the elemental analysis; Gladys Schwinger was responsible for botanical identification. All authors read and approved the manuscript.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References
[1] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014; Article ID: 24454289.

[2] Boateng M, Danso-Appiah A, Turkson B, Tersbol B. Integrating biomedical and herbal medicine in Ghana - experiences from the Kumasi South Hospital: a qualitative study. *BMC Complement Altern Med*. 2016; Article ID 27388903.

[3] Van Andel T, Myren B, van Onselen S. Ghana's herbal market. *J Ethnopharmacol*. 2012; 140(2): 368-378.

[4] Science Technology and Policy Research Institute (STEPRI). Centre for Scientific and Industrial Research (CSIR). Ghana herbal pharmacopoeia. 2nd ed. Accra: CSIR-INSTI, 2015.

[5] Adotey J, Adukpo G, Opoku Boahen Y, Armah F. A review of the ethnobotany and pharmacological importance of *Alstonia boone*i De Wild (Apocynaceae). *ISRN Pharmacol*. 2012; Article ID: 22900200.

[6] Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestree Database: a tree reference and selection guide version 402009. [Accessed 2019]. Available from: http://www.worldagroforestry.org/publication/agroforestree-database-tree-reference-and-selection-guide-version-40.

[7] Olajide O, Awe S, Awe SO, Makinde JM, Ekhelar AI, Olusola A, Morebise O, Okpako DT. Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stem bark. *J Ethnopharmacol*. 2000; 71(1-2): 179-186.

[8] Osadebe P. Anti-inflammatory properties of the root bark of *Alstonia boonei*. *Niger J Nat Prod Med*. 2002; 6(6): 39-41.

[9] Taiwo O, Van Den Berg AJ, Kores B. Activity of the stem bark extract of *Alstonia boonei* De wild (Apocynaceae) on human complement and polymorphonuclear leukocytes. *Indian J Pharmacol*. 1998; 30(3): 169-174.

[10] Obiagwu M, Ihekwereme C, Ajaghaku D, Okoye F. The useful medicinal properties of the root-bark extract of *Alstonia boonei* (Apocynaceae) may be connected to antioxidant activity. *ISRN Pharmacol*. 2014; Article ID: 24592332.

[11] Fakae BB, Campbell AM, Barrett J, Scott IM, Teesdale-Spittle PH, Liebau E, Brophy PM. Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. *Phytother Res*. 2000; 14(8): 630-634.

[12] Baliga MS1, Jagetia GC, Ulloor JN, Baliga MP, Venkatesh P, Reddy R, Rao KV, Baliga BS, Devi S, Raju SK, Veeresh V, Reddy TK, Bairy KL. The evaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna (*Alstonia scholaris*) in mice and rats. *Toxicol Lett*. 2004; 151(2): 317-326.

[13] Jagetia G, Baliga M, Venkatesh P. Effect of Sapthaparna (*Alstonia scholaris* Linn) in

modulating the benzo(a)pyrene-induced forestomach carcinogenesis in mice. *Toxicol Lett*. 2003; 144(2): 183-193.

[14] Akbar S, Hanif U, Ali J, Ishtiaq S. Pharmacognostic studies of stem, roots and leaves of *Malva parviflora* L. *Asian Pac J Trop Biomed*. 2014; 4(5): 410-415.

[15] Garg V, Dhar V, Sharma A, Dutt R. Facts about standardization of herbal medicne: a review. *Chin J Integr Med*. 2012; 10(10): 1077-1088.

[16] Sahoo N, Manchikanti P, Dey S. Herbal drugs: standards and regulation. *Fitoterapia*. 2010; 81(6): 462-471.

[17] Chanda S. Importance of pharmacognostic study of medicinal plants: an overview. *J Pharmacogn Phytochem*. 2014; 2(5): 69-73.

[18] Dar RA, Shahnawaz M, Qazi PH. General overview of medicinal plants: a review. *J Phytopharmacol*. 2017; 6(6): 349-351.

[19] McCann J, Ames B. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion. *Pro Natl Acad Sci.* 1976; 73(3): 950-954.

[20] Ames B, McCann J, Yamasaki E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat Res Environ Mutagen Rel Sub.* 1975; 31(6): 347-364.

[21] Cardoso C, de Syllos Cólus I, Bernardi C, Sannomiya M, Vilegas W, Varanda E. Mutagenic activity promoted by amentoflavone and methanolic extract of *Byrsonima crassa* Niedenzu. *Toxicology*. 2006; 225(1): 55-63.

[22] Ghazali A, Abdullah R, Ramli N, Rajab N, Ahmad-Kamal M, Yahya N. Mutagenic and antimutagenic activities of *Mitragyna speciosa* Korth extract using Ames test. *J Med Plants Res*. 2011; 5(5): 1345-1348.

[23] World Health Organization. Quality control methods for medicinal plant materials. Geneva: WHO press, 2011.

[24] Harborne J. Phytochemical methods. London: Chapman and Hall Publications, 1992.

[25] Khandelwal K. Practical pharmacognosy techniques and experiments. New Delhi: Nirali Prakashan, 2002.

[26] Ranjith D. Fluorescence analysis and extractive values of herbal formulations used for wound healing activity in animals. *J Med Plants Stud*. 2018; 6(2): 189-192.

[27] Gilbert R. The analysis of fluctuation tests. *Mutat Res/Environ Mutagenesis Rel Subj*. 1980; 74(4): 283-289.

[28] World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva: WHO Press, 2007.

[29] Mehta J, Shah D, Mehta T, Patel P, Patel N. Compendial testing on herbal crude drug - a review. *Asian J Pharm Res.* 2011; 1(2): 49-52.

[30] Chime S, Ugwuoke E, Onyishi I, Brown S, Onunkwo G. Formulation and evaluation of *Alstonia boonei* stem bark powder tablets. *Indian J Pharm Sci*. 2013; 75(2): 226-230.

[31] Ogundele D, Adio A, Oludele O. Heavy metal concentrations in plants and soil along heavy traffic roads in north central Nigeria. *J Environ Anal Toxicol*. 2015; 5(6): 1-5.

[32] Jarup L. Hazards of heavy metal contamination. *Br Med Bull*. 2003; 68: 167-182.

[33] Akintonwa A, Awodele O, Afolayan G, Coker H. Mutagenic screening of some commonly used medicinal plants in Nigeria. *J Ethnopharmacol*. 2009; 125(3): 461-470.

Abbreviations

Cd: Cadmium; Pb: lead; Fe: iron; Zn: zinc; As: arsenic; UV: ultra-violet