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Phytochemical screening and antimicrobial activities of the stem bark of *Allanblackia parviflora* Chev. (Clusiaceae)

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ABSTRACT

This study has successfully explored the photochemistry and antimicrobial properties of the methanolic, aqueous and pet ether stem bark extracts of *Allanblackia parviflora* (Chevalier) (Clusiaceae). The qualitative phytochemical screening revealed the presence of alkaloids, tanins, flavonoids, cardiac glycosides, reducing sugar, triterpenoids, anthraquinones, saponins and phytosterols in the various plant samples and the absence of cyanogenic glycosides in all the samples screened. The antimicrobial assay employed Agar-well diffusion for the preliminary screening and Micro broth dilution method for the MIC determination. Ten (10) microbial strains including one fungus (*Candida albicans*), five gram-negative (*Salmonella typhi*, *Neisseria gonorrhoeae*, Escherichia coli ATCC 2592, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* ATCC 4853) and four gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus paratypi* A and *Bacillus subtilis* NCTC 10073) were employed in the assay using Ciprofloxacin as the reference drug. The methanolic extract exhibited the highest activity against *E. faecalis* with zone of inhibition 20 mm and MIC of 2.5 mg/ml. Pet ether extract on the other hand was inactive against test microbes. The results from the study therefore suggest that the stem bark of *Allanblackia parviflora* possess some phytochemicals that acts synergistically to provide the observed antimicrobial properties as claimed by traditional medicine.

Keywords: *Allanblackia parviflora*, Phytochemicals, Minimum inhibitory concentration (MIC).

INTRODUCTION

Throughout human history, there has been a constant struggle between pathogens that cause various ailments and humans. This constant struggle seems not to end, but rather, escalating at tremendous rates with the emergence of new diseases resulting from the persistent modifications and resistance of these microorganisms. For example, hepatitis, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/ AIDS) as well as Ebola, have impacted quite a substantial proportions of human lives especially African, causing a considerable rise in morbidity and mortality rates in the past years [1,2]. Although modern synthetic drugs including antibacterial, antiviral and antifungal agents have mitigated this problem in favour of the human race, the persistent use of these drugs as chemicals predispose users to the associated side effects including hypertensive allergic reaction, severe skin rash, anorexia, dizziness, diarrhea, anaphylaxis, renal toxicity, liver toxicity, eosinophilia (elevated white blood cells), peripheral neuropathy hemolytic anemia and lethargy depending on the type and class of the antibiotic. As a result of this and other reasons such as financial constraints and availability, there have been intense scientific investigations for novel antimicrobial agents or compounds devoid of or of minimal side effects but with the same or even better therapeutic activities. In this regard however, plant based natural products are highly favored due to their availability and continuing improvement in their safety and efficacy. Among the plants that have seen efficacy and safety successes in their use is the Allanblackia species.

Allanblackia parviflora (A. Chevalier 1909) remains the only allanblackia specie in Ghana out of the nine known species in Africa precisely the east, west and central regions [3, 4]. It belongs to the family Clusiaceae and nearly a threatened plant distributed in the rainforest zones in the country specifically Western, Eastern, Ashanti and Central regions [5]. Owing to the cosmopolitan nature of Ghana, the plant has different indigenous names including *Sonchi*, *Osonodokono*, *Kusieadwe*, *Kusie aduane*, *Opaa*, *Dufufui*, *Boho*, *Krupi Atrodua* and *Akosobolo* with different myths surrounding the existence of the names [6]. The myth behind the Akan name" *Kusieadwe* or *Kusie aduane"* is that during famine and droughts the fruits and seeds of the plant were the staple food consumed by wild rats and porcupines for survival.

Allanblackia species are said to be versatile in nature because they have found many uses in the traditional herbal medicine, food industries, cosmetics, soap industries as well as wood industries in different countries in Africa. Literature and ethnomedicinal records of the genus Allanblackia reveals the presence of some plant secondary metabolites including, alkaloids, glycosides, tanins, flavonoids, triterpenes and anthocyanines [7] and hence its use in managing skin infections and other conditions such as smallpox, scrotal toothache, elephantiasis, measles, bronchial infections, asthma stomach ache, pains and cough. [8, 9]. In spite of the promising antimicrobial value of this plant as stated above, it remains underutilized and even neglected especially in Ghana. It is against this background the that this research was undertaken to assess and explore the presence of some phytochemicals together with the antimicrobial properties of *Allanblackia parviflora* specie in Ghana

MATERIALS AND METHODS

Materials

Materials for Analysis

The following drugs, reagents and chemicals of analytical grade were employed in this work; Meyer's reagent, Muller Hinton agar, nutrient broth, Dragendorf's reagent, tetrazolium salt, DMSO, Ciprofloxacin and common laboratory solvents of analytical grade including methanol and petroleum ether.

Methods

Sampling and Authentication

The stem bark of *Allanblackia parviflora* was sampled from several farmlands in Afosu (Eastern region of Ghana) and was authenticated by Mr. Osafo Asare at the herbarium in the Pharmacognosy Department of Kwame Nkrumah University of Science and Technology - The specie was assigned voucher specimen number KNUST/HMI/2015/LO10 for reference purposes and deposited at the department's herbarium.

Sample Preparation

The stem bark of *A. parviflora* were cut into small pieces, washed with water, air-dried under room temperature for 2 weeks and milled into fine particles. 200 g of the powdered sample was serially extracted with methanol, distilled water and petroleum ether using cold maceration for 72 hours. The extracts were filtered and concentrated using a Rotavapor (Type R-210 Buchi, Switzerland) to yield 7.3 %, 2.21 % and 6.52 %, w/w of methanol, aqueous and pet-ether crude extracts respectively. The crude extract was stored in a refrigerator until required.

Phytochemical Screening of Allanblackia Parviflora

Qualitative test for the screening and identification of bioactive constituents in the stem bark extracts of *Allanblackia parviflora* were investigated using standard procedures described by [Trease and Evans [10] and Sofowora[11] as employed by Ayensu and Quartey [12].

These included test for general alkaloids, anthraquinones, reducing sugars, flavonoids, glycosides, phytosterols, saponins, tannins, triterpenoids, cyanogenic glycosides and glycosides.

Antimicrobial assay of extracts

The stem bark extracts of Allanblackia parviflora were tested against 10 microbial strains obtained from the Kwame Nkrumah University of Science and Technology precisely the microbiology section at the Faculty of Pharmacy and Pharmaceutical Science. The microorganisms used in the assessment included nine (9) bacterial and one (1) fungal strains of which five were type cultures and others were clinical strains. The bacterial strains also included four Gram-positive bacteria (i.e. *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis ATCC 29212 Streptococcus paratyphi A* and *Bacillus subtilis* NCTC 10073), five Gram-negative bacteria (*Salmonella typhi, Neisseria gonorrhoeae, Escherichia coli ATCC 25922, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* ATCC 4853) and one fungal strain (*Candida albicans).* The microbial stains were sub-cultured 24 hours prior to experiment in a nutrient broth at 37 ^{o}C.

Preparation of Media (Nutrient Agar)

28 g of Mueller-Hinton agar powder was weighed into a beaker and about 500 ml of distilled water was added to completely dissolve it. Enough distilled water was added to make up 1liter. 10 ml portions of the prepared agar were poured into test tubes and sealed firmly with the aid of cotton wool. The various portions were then sterilized in an autoclave (Type; Portable 18L high pressure steam sterilizer, model YX-280A) at a temperature of about 121 °C for 15 minutes.

Antimicrobial screening of various extracts

The preliminary antimicrobial assessment employed the Agar Well diffusion method employed by Osei-Djarbeng [13] and Amponsah [2]. The assay involved the inoculation of the microbial strains into the nutrient broth followed by incubation (incubator type Heraeus, Kendro B12) at 37 °C 24 for hours. 1 ml of the sub-cultured organisms was inoculated into a prepared Mueller-Hinton agar in a sterile petri dish and uniformly smeared on the agar with the aid of a sterile cotton swab. Various wells were then created in the inoculated agar with sterile cork borer (3mm in diameter) and appropriately labeled. 0.2 g of the methanolic and pet-ether extracts were reconstituted in 10 ml each of 2 % dimethyl sulfoxide (DMSO) and the same procedure was repeated for the aqueous extract using distilled water as the vehicle. 100 µL of 0.02 mg/ml of each extract were carefully dispensed into each well. The prepared petri dishes were allowed to stand for about 30 minutes to enable the extracts diffuse well into the media and were later incubated at 37 ^{o}C for 24 hours. Positive control used in the experiment was Ciprofloxacin (at the concentration of 0.0001 g/10ml) and the negative control was 2 % DMSO.

After the incubation, the effect of the various extracts on the test microbes were shown as clear zones of inhibition. These zones of inhibition were measured using a pair of divider and ruler and the results obtained were reordered. All the experiments were repeated three times to compute the mean zone of inhibition.

Broth Dilution Method (Minimum Inhibitory Concentration (MIC))

Broth dilution Assay was carried out to determine the MIC of the extracts that exhibited considerable antimicrobial activity in preliminary screening. The test microbes were prepared from a 24 hours nutrient broth culture which was then adjusted to obtain a suspension of 10^8 cfu/ml. 2 % concentration of the stem bark extracts were reconstituted in 2 % (DMSO) and serially diluted to obtain various concentrations ranging from 0.158 mg/ml to 20 mg/ml. Sterilized 96-well micro-plates were used in the assay with each well contained 100 µl of double strength nutrient broth, 80 µl of each prepared extract and 20 µl of the various cultures organisms. The prepared plates were subjected to Incubation for 24 hours at a temperature of 37^{o}C. The growth of the organisms was estimated by adding 20 µl of 5% tetrazolium salt solution and further incubated for about 15 minute. A dark coloured well infers the presence of microorganisms, since these organisms possess some dehydrogenase enzymes that react with the salt to give a dark colour. Hence the concentration of the dark coloured well represents MIC. Both ciprofloxacin and DMSO were used as positive and negative control respectively and all experiments were performed in triplicates.

RESULTS

Qualitative phytochemical screening

The results obtained from the qualitative phytochemical screening conducted on the various stem bark extracts (aqueous, methanolic and petroleum ether) and pulverized sample of *Allanblackia parviflora* species found in Ghana is summarized in Table 1.

The outcome revealed the presence and absence of alkaloids, tanins, flavonoid, reducing sugar, cardiac glycosides anthraquinones, phytosterols and triterpenoids in the various samples. However cynogenetic glycosides was absent in all the samples screened.

Table 1: Phytochemicals constituents of the stem bark extracts and pulverized sample of *Allanblackia parviflora*

Test	Pulverized Sample	Methanolic Extract	Aqueous Extract	Petroleum Ether Extract
Tanins	+	+	+	+
Flavonoid	+	+	+	-
Reducing Sugar	+	+	+	+
Saponins	-	+	-	-
Triterpenoids	+	+	+	-
Phytosterols	-	+	-	-
Anthraquinones	+	+	+	-
General Alkaloids	+	-	+	-
Cardiac Glycosides	+	+	+	+
Cyanogenic Glycosides	-	-	-	-

(Key: + indicates present and – indicates absent)

Antimicrobial Activity

Table 2 provides the summary of results acquired from the preliminary Antimicrobial screening of the stem bark extracts (at the concentration of 0.002 mg/ml) of *Allanblackia parviflora* against 10 microbial strains.

These include four gram positive bacteria *(Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis ATCC 29212, Streptococcus paratypi A* and *Bacillus subtilis* NCTC 10073), five gram negative bacteria (*Salmonella typhi, Neisseria gonorrhoeae, Escherichia coli ATCC 2592 Klebsiella pneumoniae* and *Pseudomonas aeruginosa* ATCC 4853) and a fungus (*Candida albicans)* using ciprofloxacin (at the concentration of 0.0001 g/10ml) and 2% DMSO as positive and negative controls respectively. The results revealed considerable microbial effect (zone of inhibition) of the extracts notably methanolic and aqueous against the test organisms whereas petroleum ether extract was inactive against all the test organisms.

Table 3 also gives the summary of the Minimum Inhibitory Concentrations (MIC) of the extracts that showed considerable microbial effects against the test organisms in the preliminary assay**.** Ciprofloxacin recorded the lowest MIC values followed by methanol and aqueous extracts respectively**.**

Table 2: Antimicrobial activity of the stem bark extracts of *Allanblackia parviflora*

	Zones of inhibition in mm at a Conc. of 0.002 mg/ml ± SEM				
Microbial Strains	Methanolic Extract	Aqueous Extract	Pet-Ether Extract	Ciprofloxacin	2% DMSO
E. coli	13.00 ± 0.0	12.50 ± 0.5	NA	15.50 ± 0.5	NA
E. faecalis	20.00 ± 0.0	15.00 ± 0.0	NA	16.00 ± 0.0	NA
S. aureus	17.50 ± 0.5	15.00 ± 0.0	NA	21.50 ± 0.5	NA
C. albicans	17.00 ± 0.0	12.50 ± 0.5	NA	21.00 ± 1.0	Na
B. subtilis	15.00 ± 00	13.00 ± 0.0	NA	16.00 ± 0.0	NA
S. paratyphi A	10.00 ± 0.0	10.50 ± 0.5	NA	16.75 ± 0.2	NA
S. typhi	15.50 ± 0.5	14.00 ± 0.0	NA	19.50 ± 0.5	NA
N. gonorrhoeae	I0.50 ± 0.5	10.00 ± 0.5	NA	15.50 ± 0.5	NA
P. aeruginosa	14.50 ± 0.5	12.50 ± 0.5	NA	19.50 ± 0.5	NA
K. pneumoniae	13.50 ± 0.5	12.50 ± 0.5	NA	15.50 ± 0.5	NA

(Key NA- Not Active)

Table 3: Minimum Inhibitory Concentrations of the stem bark extracts of *Allanblackia parviflora*

Microbial strains	Methanolic extract (mg/ml)	Aqueous extract (mg/ml)	Ciprofloxacin (mg/ml)
E. coli	2.50	5.00	0.03
E. faecalis	2.50	5.00	0.05
B. subtilis	5.00	5.00	0.01
S. aureus	2.50	2.50	0.05
C. albicans	1.25	5.00	0.01
S. paratypi A	2.50	5.00	0.03
S. typhi	2.50	5.00	0.05
N. gonorrhoeae	2.50	5.00	0.05

DISCUSSION

Qualitative Phytochemical Screening

The results obtained from the qualitative phytochemical screening of the stem bark extracts of *Allanblackia parviflora* as shown in Table 1 above reveals the presence of major phytochemicals in the various extracts. Secondary metabolites including alkaloids, tanins, flavonoids, cardiac glycosides, reducing sugar, triterpenoids and anthraquinones were present in the pulverized plant material whereas saponins and phytosterols were absent. Methanolic and aqueous extracts showed the presence of almost all the phytochemicals mentioned above however, general alkaloids as well as phytosterols and saponins were absent in the methanol and aqueous extracts respectively. Petroleum-ether extracts on the other hand showed the presence of only tanins, reducing sugars and cardiac glycosides with the rest being absent. This effect exhibited by the pet- ether extracts may be attributed to the fact that most of the compounds embedded in the plant may be polar in nature hence, insoluble in non-polar solvent like petroleum ether. Also cyanogenic glycosides which accounts for approximately 90% of plant toxins called cyanogens were absent in all the plant samples Screened. This therefore suggests to some extent the safety of the *Allanblackia parviflora* plant in general. A number of these phytochemicals though not directly involved with major processes including growth, reproduction and metabolism, have shown pronounced pharmacological activities. For example most phenolic compounds from plants including flavonoids (Chrysin, Quercetin, and Rutin) and tannins (Ellagitannin) have been proven in scientific literature to have antimicrobial, anthelmintic and antidiarrheal activities. Some plant flavonoids have also shown anticancer, antioxidant, antiinflammatory as well as anti-allergic activities. Other phenolic derivatives such as Aspirin have demonstrated potent anti-inflammatory and analgesic properties [14].

Also phytochemicals including alkaloids have been associated with most anticancer (vincristine and vinblastine), antimalarial (quinine), analgesic (morphine and cocaine), antiinflammatory (colchicine), agents used in the health systems today. Other alkaloids such as Diterpenoid alkaloids and glycoalkaloid (s. solamargine) isolated from, Ranunculaceae, or buttercup family and *Solanum khasianum* are reported to possess antimicrobial properties and HIV inhibitory activities respectively. Terpenoids including Artemisin (Sesquiterpenoids) and Taxol have been used in managing malaria and some solid tumors respectively [12]. Based on the evidences stated above, it may be stipulated that the presence of these phytochemicals in the stem bark *Allanblackia parviflora* might partially or significantly account for its diverse application in traditional herbal medicine especially in areas where they are mostly prevalent.

Antimicrobial Activity

The study assessed the antimicrobial activities of the stem bark of *Allanblackia parviflora* species in Ghana against ten microbial strains comprising of one fungus, five gram negative bacteria and four gram positive bacteria. The assessment employed the Agar well diffusion method for the preliminary screening and broth micro-dilution method for MIC determination.

The outcome of the assay however revealed that all the test organisms (*S. typhi, E. coli, E. faecalis, S. paratypi A., C. albicans, B. subtilis, K. pneumoniae, N. gonorrhoeae, P. aeruginosa S. aureus)* showed considerable susceptibility to the ciprofloxacin, followed by methanolic and the aqueous extracts with zones of inhibitions ranging between 9.5-21.5 mm. However, the pet-ether extract showed virtually no activity against all the 10 microbial strains.

In terms of the individual crude extracts, methanol which comprises the polar constituent of the plant recorded the highest zones of inhibition at 20 mm followed by 17.5 mm against the, *E. faecalis and Staphylococcus aureus* respectively with the same Minimum Inhibitory Concentration of 2.50 mg/ml. This remarkable antimicrobial activity of the crude extract against *E. faecalis, Staphylococcus aureus and P. aeruginosa* therefore justifies the traditional use of the plant in managing tooth infections (*E. faecalis and P. aeruginosa)*, skin and bronchial infections(*S. aureus*) since these organisms are the common pathogens in most tooth infection, skin and respiratory related diseases respectively [15, 16]. The lowest antimicrobial activity was exhibited by *S. paratyphi A* at 10.00 mm and MIC of 2.50 mg/ml.

The Minimum Inhibitory Concentrations recorded for the methanolic extracts also ranged between 1.25 and 5.0 mg/ml with *C. albicans* and *P. aeruginosa* recording the lowest at 1.25 mg/ml and B. subtilis recording the highest at 5.0 mg/ml. Hence all the MIC values were greater than 1000µg/ml. Substantial antibacterial and antifungal activities was again demonstrated by the aqueous extract with the highest zone of inhibition at 15.00 mm against *Staphylococcus aureus* and *E. faecalis* and the same MIC of 5.00 mg/ml. ***N.*** *gonorrhoeae* again recorded the lowest zone of inhibition value for the aqueous extract at 10.00 mm and MIC of 5.00 mg/ml. Though the activities recorded for this extract was quite considerable, methanolic extracts was much more effective against the microbial strains

Petroleum ether extract which covers the non-polar constituents of plant showed no antimicrobial activity against all the microbial strain. This effect may be a result of the inability of pet-ether to extract active phytochemical compounds that are responsible for this activity. More to this point, several phytochemical studies indicates that

antimicrobial activity are mostly associated with flavonoids and these were absent in the pet-ether extract as shown in Table 3.1 above hence the inactive effect.

Therefore, comparing the antimicrobial activities of the three (3) stem bark extracts of *Allanblackia parviflora*, both methanolic and aqueous extracts gave a broad spectrum activity (i.e. against bacterial and fungal strains,) but methanolic extracts generally inhibited microbial growth more than aqueous and pet-ether in the respective strains.

Ciprofloxacin which was used as the standard drug generally displayed the highest antibacterial and antifungal activities and the lowest mic values. The results however exerted some form of variability for the various microbial strains as seen in that of the extracts. The zones of inhibition ranged between 15.00 and 21.50 mm with *Staphylococcus aureus* demonstrating the highest antimicrobial activity and N. gonorrhoeae showing the lowest activity. The MIC values also ranged between 0.0125mg/ml and 0.05mg/l with C. albicans and B. subtilis recording the lowest values thus, exhibiting the highest activity. Ciprofloxacin was preferable to other antibiotics because it has activity against a broad spectrum of microorganisms. It was clearly seen that ciprofloxacin exhibited higher activities and lower MIC values than the extracts because the drug constitute a single compound whiles the crude extracts contains innumerable chemical substances which may interfere with the activity. Nevertheless, further isolation and purification of compounds responsible for such activities may give even better activities and MIC values for the active extracts.

Also considering the types of microbial strains, the active extracts generally showed prominent activities with gram positive bacteria than gram negative bacteria. This effect may be as a results of the difference in the structure of their cell walls. Generally, gram positive bacteria exert higher susceptibility against chemicals since their cell wall is characterized by its thickness comprising of peptidoglycan which is bonded to different cellular units whiles gram negative bacteria contain some hydrolytic enzymes as well as proteins (porin). These enzymes act to degenerate various substance that are introduced into the cell from the outer environment and the protein also acts as barrier that prevents polar substance across the outer layer which explains the observed effect[13]. The significant results obtained from this assay, therefore suggests the possible use of the stem bark of the plant for managing bacterial infections especially skin related ones and even fungal infections since *C. albicans* also demonstrated significant activity.

CONCLUSION

This study has shown from the results that the stem bark extracts of *Allanblackia parviflora* contain major phytochemicals including alkaloids, tanins, flavonoids, cardiac glycosides, reducing sugar, saponins, phytosterols triterpenoids and anthraquinones which work synergistically to produce marked antimicrobial activities in the various extracts. The microbial studies also revealed antibacterial and antifungal activities of the various extracts inferring broad spectrum antimicrobial activity of the plant (stem-bark). Further studies to consider other pharmacological activities (such as anticancer, analgesic, antidiarrheal) and toxicological studies of the plant to ascertain the safety profile of the plant is underway.

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