Research Article

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IN VITRO ANTIBACTERIAL POTENTIAL OF SOLVENT FRACTIONS OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *VERNONIA COLORATA* AGAINST SELECTED HUMAN PATHOGENIC BACTERIA

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ABSTRACT
Vernonia colorata has been used in traditional medicine for the treatment of diseases related to certain bacteria. Crude leaf extracts of the plant have also been reported to contain antibacterial agents in previous *in vitro* studies. Fractionation of crude solvent extracts may lead to isolation and subsequent characterization of the active compound(s). In the current research, crude aqueous and ethanolic leaf extracts of *V. colorata* were evaluated for antibacterial activity against five human pathogenic bacteria. The crude extracts were further fractionated by solvent-solvent partitioning using petroleum ether, chloroform and diethyl ether. The various fractions were tested against selected bacteria using the agar-well diffusion method. Crude ethanolic extract showed MIC ranging between 5-6 mg/ml while aqueous extract showed MIC between 6-7.5 mg/ml. All fractions from the aqueous extract at 10 mg/ml did not show zone of inhibition against the bacteria tested. However, the chloroform fraction of the ethanolic extract showed activity only against *S. aureus* at 10 mg/ml with zone of inhibition of 15.00 ± 0.20 mm. The antibacterial activity of the chloroform fraction of the ethanolic extract was significant in comparison with control ($P < 0.05$). The results suggested that the crude ethanolic leaf extract of *V. colorata* possess superior antibacterial activity as compared with aqueous extract. The result further suggests that chloroform fraction of the ethanolic leaf extract possesses antibacterial activity hence turn out to be a good candidate for further isolation and characterization of antibacterial agents.
Keywords: Solvent fractions, antibacterial activity, *Vernonia colorata*, phytochemicals, agar well diffusion

INTRODUCTION

The increased emergence of illness caused by pathogens has lead to the curiosity of many researchers and microbiological scientists in the investigation of antibacterial activities of medicinal plants. Medicinal plants have been the source of medicine to many African and Asian cultures since time immemorial. Recently, nearly 30 % or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or Ayurvedic medicines[1-4]. As a result, most of the medicinal plants have been proved scientifically as sources of new antibacterial drugs. However, there is still an urgent need to continuously screen plants for potential microbial growth inhibition activity and subsequent discovery of new lead antimicrobial compounds. This is due to the increased incidence of multi-drug resistant pathogens as a result of indiscriminate use of commercial antimicrobial drugs in the treatment of infectious diseases thus making it a global growing-problem. *Vernonia colorata* is one of the several species of *Vernonia* (including *V. calvoana* and *V. amygdalina*) eaten as leaf vegetables. Common names in some West and Central African states are bitter leaf, ewuro, ndole and onugbu. The leaves have a bitter and sweet taste, and are a typical ingredient in the egusi soup. However, in Ghana, the plant is considered as wild and *V.amygdalina* is the only plant from the *Vernonia* species used as leaf vegetable. *V. colorata* is among the most traditionally used species to treat infectious diseases[5,6]. It is used traditionally for the treatment of diabetes, skin rashes and acute hepatitis. It has also been reported in the treatment of schistosomiasis, the epileptic seizures, fevers and acute hypertension[7,8]. This plant is also used in the traditional treatment of Buruli ulcer in Cote d' Ivoire[9]. Recent *in vitro* study has shown that crude extracts of this plant possess phytochemicals that have been reported to be active against certain bacteria[9-11]. In a previous investigation[11], it has also been shown that crude ethanolic leaf extract of *Vernonia colorata* showed inhibition on both *Pseudomonas aureginosa* and *Staphylococcus aureus* whereas the aqueous extract showed inhibition only against *Staphylococcus aureus*. The current research is therefore aimed to evaluate different solvent fractions of both ethanolic and aqueous extracts of Ghanaian *V. colorata* for bacterial growth inhibition. This may lead to possible isolation and characterization of the active principles.

MATERIALS AND METHODS

Reagents

Reagents used were chloroform, diethyl ether, petroleum ether, acetone, Dimethyl sulphoxide from BDH. All reagents used were of analytical grades and glass distilled water was used.

Equipment and Apparatus

Rotary evaporator (Eyela Japan), Freeze dryer (Eyela Japan), water bath, culture plates, sterilizer, sterile borer, incubator (J. P. selecta) and autoclave (J.P, selecta) were used to carry out this work.

Plant Material

Fresh leaves of *Vernonia colorata* were harvested from a piece of land at Mampong-Akuapim in the Eastern

Region of Ghana in the month of January 2012. The leaves were later taken to the herbarium department of the Centre for Scientific Research into Plant Medicine, Mampong-Akuapim, for botanical identification and authentication.

Microorganism

Five different microbes of standard strains were purchased at Centre for Scientific Research into Plant Medicine Mampong-Akuapim, Ghana with standard codes. The microbes were; *S. typhi* (ATCC 19430), *S. aureus* (ATCC 25923), *K. pneumonia* (ATCC 33495), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853)

Extraction of Plant Material

Vernonia colorata leaves were air dried and pulverized (course grinded). About 300 g portion of the pulverized sample was weighed and soaked in 2.5 L of 70 % ethanol for 24 hours. The suspension was then filtered. The filtered recovery volume measured was 1.78 L. The filtrate was concentrated using rotary evaporator at a reduced pressure with temperature between 40-50^{o}C into slurry and then freeze dried. Another 300 g of the powder was soaked in 3.5 L distilled water for 24 hours. The suspension was then filtered and concentrated. The slurry obtained from the concentration was then freeze dried.

Fractionation of Crude Plant Extracts

The crude extracts were fractionated using three different solvents (petroleum ether, chloroform and diethyl ether) according to the procedure of Pollack *et al*.[12].

Obtaining Petroleum Ether Fraction from Extract (Fraction 1)

About 200 ml of the sample of crude aqueous extract was poured into a separation funnel and 200 ml of petroleum ether was added and covered by the stopper. The separatory funnel was then removed from the Iron ring and the stopper was held tightly. The funnel was inverted slowly and the stopcock was opened to release the pressure toward the back of the hood. The stopcock was closed and the funnel was shook gently. It was vent again and this step was repeated until no more gas escaped. The separatory funnel was placed back in the Iron ring stand and was allowed for the layers to separate. The stopper was then removed and the bottom layer, which is the aqueous phase or layer, was drained into a clean container and the petroleum ether fraction or layer was also drain into another clean container since the aqueous phase was denser than the petroleum ether phase. The petroleum ether fraction was then concentrated using the rotary evaporator and then freeze dried. The freeze-dried sample was labeled (PFA) and (PFE) for aqueous and ethanolic crude extracts respectively.

Obtaining Chloroform Fraction from Extract (Fraction 2)

The aqueous phase obtained from Fraction 1 was poured back to the separation funnel and 200 ml of chloroform was added and covered by the stopper. The steps in Fraction 1 were then repeated and the chloroform layer was drained into a clean container. The chloroform fraction was then concentrated using the rotary evaporator and then freeze dried. The freeze-dried sample was labeled (CFA) and (CFE) for aqueous and ethanolic crude extracts respectively.

Obtaining Diethyl Ether Fraction from Extract (Fraction 3)

The aqueous phase obtained from Fraction 2 was poured into a separation funnel and 200 ml of diethyl ether was added and covered by the stopper. Steps in Fraction 2 were then repeated to obtain the diethyl ether fraction. The diethyl ether fraction or layer was also drain into another clean container since the aqueous phase was denser than the diethyl ether phase. The diethyl ether fraction was then concentrated using the rotary evaporator and then freeze dried. The freeze-dried sample was labeled (DFA) and (DFE) for aqueous and ethanolic crude extracts respectively.

Antimicrobial Susceptibility Test for Crude Extracts and their Solvent Fractions

The spreading method of Cruickshank *et al*.[13] and dose (agar) diffusion method were used. Twenty-four hours old cultures of the organisms to be tested were taken. A loop full of the cultures was uniformly spread over the surface of sterile Muller Hinton Agar (MHA) for *P. aeroginosa, S. aureus, E. coli, K. pneumonia* and *S. typhi* with a sterile bent rod. 10 mg/ml of the freeze-dried samples of the various fractions were each prepared in 20 % dimethyl sulphoxide (DMSO). Chloramphenicol was also used as test control on the microbes. About 100 µl of the prepared fraction extracts were used to fill holes bored by 5 mm cork borer in the inoculated agar. The plates were made in triplicate. All plates were incubated at 37^{o}C for 24 hours. Diameters of the zones of inhibition in the triplicate plates were measured by calculating the difference between cork borer (5 mm) and the zone of inhibition[14,15].

Determination of Minimum Inhibitory Concentration (MIC) of Crude Aqueous and Ethanolic Leaf Extract

Various concentrations of both aqueous and ethanolic extracts ranging between 10.0 and 4.0 mg/ml were introduced into different test tubes; each tube was inoculated with an overnight culture of *S. aureus, P. aeruginosa, E. coli* and *K. pneumonia* diluted to give a final concentration of 10^6 cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration (MIC) in each case[16].

RESULTS AND DISCUSSION

Both crude aqueous and ethanolic leaf extracts showed broad-spectrum activity against the test organisms. The MIC of the ethanolic extract ranged from 5 mg/ml to 6 mg/ml. The MIC of the aqueous extract ranged from 6 mg/ml to 7.5 mg/ml (Table 1). The antibacterial activity demonstrated by both crude extracts were consistent with previous investigations[9,10].

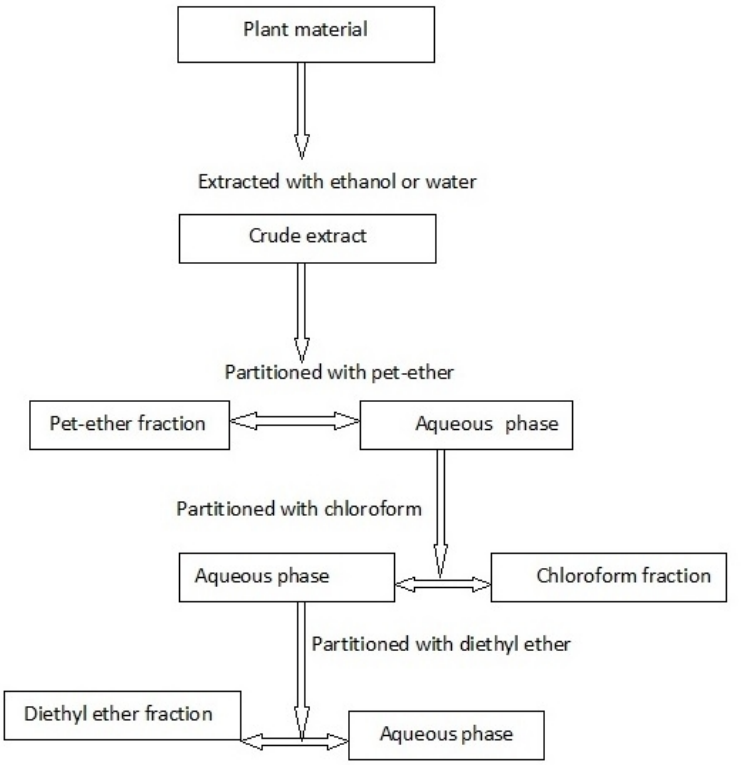

Figure 1: Scheme of extraction of plant material and fractionation with different solvents

Table 1: Minimum Inhibitory Concentrations of Crude extracts on organisms

Organism	MIC (aqueous extract) mg/ml	MIC (ethanolic extract) mg/ml
S. aureus	6.00	5.00
P. aerugenosa	6.50	5.00
K. pneumonia	6.00	ND
E. coli	7.50	6.00
S. typhi	ND	ND

ND: Not determined because of inactivity on test organism

Table 2: Mean diameters of Zone of inhibition (mm) of 10 mg/ml concentration of the various fractions of the ethanolic leaf extract of *Vernonia colorata* against test organisms

Organisms	PFE	CFE	DFE	DMSO	Chloramphenicol
P. aeruginosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S. aureus	0.00 ± 0.00	15.00 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	27.00 ± 0.00
E. coli	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.00
K. pneumonia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S. typhi	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

PFE: Petroleum ether fraction of the ethanolic extract; CFE: Chloroform fraction of the ethanolic extract; DFE: Diethyl ether fraction of the ethanolic extract

Table 3: Mean diameters of Zones of Inhibition (mm) of 10 mg/ml concentration of the various fractions of the aqueous leaf extract of *Vernonia colorata* against test organisms

Organisms	PFA	CFA	DFA	DMSO	Chloramphenicol
P. aeruginosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	26.00 ± 0.33
S. aureus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
E. coli	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.00 ± 0.33
K. pneumonia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
S. typhi	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

PFE: Petroleum ether fraction of the aqueous extract; CFE: Chloroform fraction of the aqueous extract; DFE: Diethyl ether fraction of the aqueous extract

The results indicate that the standard strains of *E. coli, S. aureus, K. pneumonia, S. typhi* and *P. aeruginosa* were resistant to all the three solvent fractions of the aqueous extract of *V. colorata*. Fractionation of the extract across different solvent phases may have caused the breakdown of possible active components. Reports indicate that fractionation of crude extract with several solvents may spread the active compound across the fractions resulting in low concentrations[17]. Seasonal variations may also contribute to the low antibacterial activity of the aqueous extract as evidenced in the MIC result. Plant material processing, inadequate fractionation process, degradation of active constituents during fractionation are all possible contributors to the results in Table 3. The solvents used for the fractionation are volatile hence part of bioactive compounds may possibly escape with the solvents. The mean diameters of zone of inhibition of the various fractions of the ethanolic extract of *V. colorata* are shown in Table 2. Of the three solvent fractions at concentrations of 10 mg/ml, only the chloroform fraction showed an intermediate inhibition on *S. aureus* with mean diameter of inhibition zone of 15.00 ± 0.20 mm. The active constituents responsible for inhibition in *Vernonia colorata* extracts might have spread through the various solvents due to different solubilities in the solvents. The active constituents might be more soluble in chloroform compared with the other solvents. Diethyl ether is known for its ability to mask active components in ethanolic extracts[18]. This could contribute to non-activity of the diethyl ether fraction. Sequence of fractionation may also lead to loss of the active compounds.

CONCLUSION

We conclude that crude aqueous and ethanolic leaf extracts of *V. colorata* possess broad-spectrum antibacterial activity. The ethanolic extract was found to be stronger in antibacterial activity compared with aqueous extract. Chloroform fraction of the crude ethanolic extract contains more active constituents compared with pet-ether and diethyl ether fractions and thus presents a good candidate for further isolation and identification of active antibacterial agents from leaf extracts of *V. colorata*.

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