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Phytochemical and Volatile Components Evaluation of Antimicrobial Root Extracts of *Dichrostachys cinerea* (Sickle Bush)(Fabaceae) (L) Wight & Arn

*** S. K.Okwute[1,2], B.M. Adeniyi.[1]**
[1]Department of Chemistry,
Faculty of Science,
University of Abuja,
P.M.B 117, Gwagwalada,
Abuja,
Nigeria.

[2]Department of Pure and Applied Chemistry,
Veritas University, Bwari,
Abuja,
Nigeria.

Email:profokwute@yahoo.com

Abstract

The crude ethanolic extract of the root of Dichrostachys cinerea (Fabaceae) was screened for phytochemicals and antimicrobial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aureginosa, Salmonella typhi, and Candida albicans using agar-well diffusion method. The phytochemical screening of the root extract results showed the presence of alkaloids, flavonoids, essential oils, phenols, terpenes, tannins, unsaturated sterols, and saponins. However, coumarins, and anthocyanins were absent. The crude extract was subjected to column chromatographic fractionation to give nine fractions of which two, DCR1 and DCR2, were subjected to gas chromatography-mass spectrometry (GC-MS) analysis. The crude extract and column chromatographic fractions DCR1 and DCR2 gave MIC of 1 mg/mL against Staphylococcus aureus, but were not active against the other test organisms. The gas chromatography-mass spectrometry analysis of the antibacterial fractions, DCR1 and DCR2, based on standard MS library data revealed the presence of caryophellene, phthalates, a phenol and fatty acids and their ester derivatives. These phytoconstituents could account for the biological properties of the extracts of the plant. The results of this work therefore support its use in ethno-medicinal preparations, particularly against infections.

Keywords: Antimicrobial activity; Dichrostachys cinerea; Volatile components

INTRODUCTION

Medicinal plants contain physiologically active ingredients that have been exploited in traditional practice for the treatment of various ailments (Ayensu, 1978, Adebanjo *et al*, 1983). At least 12,000 of such compounds have been isolated so far, a number estimated to be less than 10% of the expected total (Lai and Roy 2004). The use of plants as medicines predates written human history, and many of the pharmaceuticals currently available to physicians

Author for Correspondence

have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium (Swain and Tony, 1968). The annual global market for plant-based pharmaceutical drugs has been projected to increase from US$29.4 billion in 2017 to about US$39.6 billion in 2022 (bccresearch. com, 2017). In fact, according to the World Health Organization, among the active natural compounds obtained from higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Atnas *et al.,* 2021, Noureddine and Lahcen 2024). One resourceful plant in terms of traditional medicinal value is *Dichrostachys cinerea* also called Bell mimosa (Chinese) and lantern tree or Sickle Bush (English). It is native mostly to Africa, including Nigeria, and parts of Southeast Asia and Australia. In Nigeria, it is popularly called *Dundu* among the *Hausa*-speaking people of northern Nigeria and *Kora* among the *Yoruba*-speaking people of Southwestern Nigeria (Gill, 1992). *Dichrostachys cinerea* belongs to the family Fabaceae (Mimosaceae); a semi-deciduous to deciduous thorny shrub of small rounded crown, 3m wide and up to 7m high, found in tropical and sub-tropical conditions. The leaves are compound and pinnate, while the flowers have two sets of colours-pinkish white basally and yellow terminally (Bein e*t al.,* 1996; Mann *et al*., 2003).

The plant has received much attention in the literature, partly because strong claims abound of the usefulness of every part of the plant, and partly because of the variety of compounds that have been isolated from various species of the genus (Reham e*t al.,* 2017). Traditionally, the plant is used in the treatment of leprosy, syphilis, dysentery, headache, and toothache (Kirtikar *et al.,* 1998). Burkill (1997) also reported that parts of the plant are used for livestock feed and tanning. More recent investigations have shown it to possess insect repellent, anti-snake venom, anti-inflammatory, antimicrobial, analgesic, antimalarial, antioxidant and anti-sickling properties (Kambizi and Afolayan, 2001, Pawan,2008, Sreedevi *et al*., 2009, Renganayaji, 2013, Ribalta *et al*., 2015, Abdullahi, 2016, Okhuarobo *et al*., 2017, Fadipe *et al*., 2020, Raoof *et al*., 2020, Abdullahi and Yusha'u, 2021, Swadhini and Dhanalakashmi, 2021, Rhaakur, 2023).

Previous phytochemical investigations on the various parts of the plant revealed the presence of important phytoconstituents, including alkaloids, steroids, tannins, saponins, flavonoids, phenols, carbohydrates, terpenes, and essential oils, with the composition depending on the part of the plant and the geographical location (Sreedevi *et al.,* 2009, Aworet-Samseny *et al.,* 2011,Neondo *et al.,* 2012, Viajayalakshmi, 2013, Renganayagi, 2013, Thakur, 2013, Syed *et al.,* 2018, Fadipe *et al*., 2020).In our continued investigation of Nigerian medicinal plants for potential drug leads, we wish to report on the phytochemical and antimicrobial characteristics of the root extracts of D. cinerea. While some researchers have reported on the biological properties of some parts of the plant, particularly the leaves and barks, the antimicrobial property and the volatile chemical constituents of the root extracts of the Nigerian species have not been investigated. The investigation of the volatile components is very significant as the volatiles are very important in the traditional management of malarial fever and inflammations.

MATERIALS AND METHODS

Collection and preparation of sample

The root sample of *Dichrostachys cinerea* was collected from a plantation at the Idu Green Area of Abuja and was identified at the Herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. The dry root was then pulverized to give a coarse powder.

Laboratory equipment and consumables

Solvents used for extraction and chromatography were purified by re-distillation. Silica gel for column was a powder of 239-400 mesh size. Thin-layer chromatographic plates were aluminium sheets pre-coated with silica gel 60F254.The gas chromatography-mass spectroscopy machine was Hewlett-Packard 6890 linked to the Hewlett-Packard 5973 mass-selective detector. The carrier gas was helium. The media for *in-vitr*o antimicrobial screening were Mueller Hinton agar and broth. The organisms were clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Salmonella typhi*, and *Candida albicans* and were tested for credibility and viability.

Laboratory Procedures

Extraction of plant material

The crude ethanolic extract of the root of was obtained according to the method described by Okogun (2000). The powdered root sample (500 g) was transferred into a large beaker and 1000 ml of 95% ethanol was added at ambient temperature (28 ± 2 ^{0}C). Extraction was allowed to proceed for 48 hrs with occasional shaking after which it was filtered through a sterile filter paper (Whatmaan No.4). The filtrate was concentrated to dryness using a rotary evaporator to give the crude extract (5.00g) which was kept at 4 ^{0}C until further use.

Phytochemical screening of crude extract of *Dichrostachyscinerea* root

Phytochemical tests were carried out based on standard procedures described by Banu and Cathrine (2015) for alkaloids, phenols, flavonoids, saponins, coumarins, terpenes, tannins, steroids, anthocyanins, glycosides and essential oils.

Column chromatography of crude extract of *Dichrostachys cinerea* root

The dried crude ethanolic extract (4.65g) was subjected to fractionation using column chromatography on silica gel and eluting with mixtures of n-hexane and ethyl acetate, and then mixtures of ethyl acetate and methanol. Sixty-nine (69) fractions of 25 ml each were collected and evaporated to dryness. Thin Layer Chromatography was used to monitor the column fractions and to also ascertain the purity of fractions collected using a mixture of n-hexane and ethyl acetate (9:2) as solvent. Those with the same Rf values were combined to give 9 combined fractions DCR1-DCR9.

Antimicrobial screening of crude extract and column fraction

The antimicrobial screening was done using standard methods(Lopez *et al*., 2003, Fabry *et al*., 1996). Chloramphenicol (500 mg, Carlo Eardo) was used as the standard antibiotic while the solvent, DMSO, was used as the blank/negative control. Serial dilutions of chloramphenicol (0.08, 0.04, 0.02 and 0.01 mg/ml) were prepared. Inhibition zones of both were determined after incubation at 37 ^{0}C for 24 hrs

The bacteria were grown in Mueller Hinton agar and Mueller Hinton broth. The turbidity of the homogeneous suspensions was adjusted to approximately 0.5 Mac Farland standards for antimicrobial activity assay (Lopez *et al*., 2003).

Testing extract for antimicrobial activity

The crude extract was screened for antimicrobial potential using the agar diffusion method (Lopez *et al.,*2003; Fabry *et al*.,1996). The agar plates were inoculated with each of the test organisms and wells of 10 mm diameter were made in the agar using a sterile cork-borer. Concentrations(1.0 ml each) of the extract in DMSO (6.0, 4.0, 2.0, and 1.0 mg/ml) were poured into the wells in each plate. The control experiment consisted of 0.1 ml sterile DMSO. The

plates were allowed to stand for 1 hr at room temperature and then incubated at 37 ^{0}C for 24 hrs for the bacteria and 48 hrs for the fungus, *Candida albicans*.

The column fractions, DCR3, DCR4, DCR6, DCR7, and DCR8 were obtained in very minute quantities and were therefore not screened. However, 1mg/mL concentrations of the remaining four fractions, DCR1, DCR2, DCR5, and DCR9, were prepared in DMSO and screened. Each extract (0.5 ml) was then added to the molten agar (19.5 ml) in a petridish and allowed to set. The plates were then inoculated with the test organisms and incubated at 37^{0}C for 24 hrs for bacteria and 48 hrs. for the fungus, *C. albicans*.

The experiments were done in triplicate. The zones of inhibition were then noted as positive(+)(growth) and negative (-)(no growth).

Determination of Minimum Inhibitory Concentration(MIC) of crude extract and column fractions

There was no special arrangement for the determination of MICs for the crude extract and the column fractions due to limited quantities of the extracts. The least concentration of each extract that did not permit any visible growth of the inoculated test organism, *Staphylococuss aureus*, against which they were active was regarded as the minimum inhibitory concentration.

Gas chromatography-mass spectrometry(GC-MS) of fractions DCR1 and DCR2

The two combined fractions, DCR1 and DCR2, from n-hexane-ethyl acetate column fractions 3-7 and 8-14 respectively, which were found to possess antibacterial activity against *Staphylococcus aureus* were then subjected to GC-MS analysis to investigate their volatile components.

RESULTS AND DISCUSSION

Phytochemical screening of crude extract of *Dichrostachys cinerea* root

The results of the phytochemical screening of ethanolic extract of the root of *Dichrostachys cinerea* are shown in Table 1.

Table 1: Phytochemical screening of crude extract of *Dichrostachys cinerea* root

Phytochemicals	Results
Essential oils	+++
Phenols	+
Terpenes	++
Tannins	+
Alkaloids	+
Flavonoids	+
Unsaturated sterols	+
Coumarins	-
Saponins	+
Anthocyanins	-
Glycosides	+

Key: (+++) = very high, (++): high, (+): average, (-): not present.

The results showed the presence of tannins, flavonoids, alkaloids, steroids, glycosides, triterpenoids and saponins, while coumarins and anthocyanins were detected in trace amounts in the plant extract. Previous reports on the phytochemical constituents of

Dichrostachys cinerea showed varying results depending on the part of the plant investigated (Sreedevi *et al*., 2009, Aworet-Samseny *et al*., 2011, Neondo *et al*., 2012, Viajayalakshmi, 2013, Renganayagi,2013, Syed *et al*., 2018, Thaakur *et al*., 2023). However, compared to previous reports the root has been shown to contain greater accumulation of tannins, saponins, alkaloids, flavonoids, carbohydrates, terpenoids and steroids in this work.

The presence of these components in this species is an indication that it may have some medicinal potentials. This is due to the fact that each of the components identified has one therapeutic usage or another. The antibacterial activities of alkaloids, essential oils, terpenoids and flavonoids have been reported by various researchers (Hassan *et al*., 2005).

Antimicrobial screenings of crude extract and fractions of *Dichrostachys cinerea*

The crude extract and column fractions DCR1 and DCR2 were screened for antimicrobial activities. The results are shown in Tables 2 & 3.

Table.2: Antimicrobial screening of crude ethanolic extract of root of *Dichrostachys cinerea*

Test organism	DMSO	Activity in mg/ml of different concentrations of crude extract			
		6.0	4.0	2.0	1.0
Sa	+	-	-	-	-
EC	+	+	+	+	+
Pa	+	+	+	+	+
Ca	+	+	+	+	+
St	+	+	+	+	+

Key: (-): No growth, (+): Growth

Table.3: Antimicrobial screening of column fractions of root of *Dichrostachys cinerea*

Fractions	Pa 1mg/mg	Sa 1mg/ml	St 1 mg/mg	Ec 1mg/ml	Ca 1mg/ml
DCR1	+	-	+	+	+
DCR2	+	-	+	+	+
DCR5	+	+	+	+	+
DCR9	+	+	+	+	+
DMSO	+	+	+	+	+
CP(500 mg)	+	-	+	+	+

Key: CP=Chloramphenicol; (+) = growth, (-) = No growth

The crude root extract of *Dichrostachys cinerea* and column chromatographic fractions were tested against four bacterial strains and one fungal strain using chloramphenicol as a standard antibiotic and DMSO the solvent as blank. From the results (Tables 2 and 3), the crude extract was active against only *Staphylococcus aureus* (Gram-positive) just as only fractions DCR1 and DCR2 were active against only *Staphylococcus aureus*. The selective activity of the extracts may be attributable to the presence of phenolic constituents such as flavonoids and phenols, as well as the high lipophilicity of some components such as terpenoids, steroids and essential oils in the extracts (Cheverria *et al*., 2017, Shamsudin *et al*., 2022). Thus, both the crude extract and the two fractions showed minimum inhibitory concentration (MIC) at 1 mg/mL (Tables 2 and 3).

. It should be noted that while in this work the crude ethanolic extract and some fractions were active against only *Staphylococcus aureus*, some workers had reported that the methanolic root extract showed activity against *S. aureus, C. albicans, P. aeruginosa, B. substillis,* and *C. coli*

(Neondo *et al.,* 2012). This difference in activity may be associated with geographical location, which also affects the chemical composition of plants (Kumar *et al.,* 2017).

Gas chromatography-Mass spectroscopy analysis of column chromatographic fractions DCR1 and DCR2

The combined fractions DCR1 and DCR2 from column chromatography of the crude extract of *D. cinerea* root were subjected to GC-MS analysis to identify some of the volatile constituents. The analysis data are presented in Tables 4 and 5, respectively. The components of the fractions were identified by comparing their relative retention times and mass spectra with those of Mass Spectra Data and literature citations (Adam, 2007, Lea et al., 2017)

Table. 4: GC-MS analysis of column fraction DCR1 of *Dichrostachys cinerea* root

Retention Time(Mins)	Name of Constituent	Peak Area %	% Quality
20.015	**Ethyl tetracosanoate**	**3.83**	**81**
24.118	**Hexadecanoic acid, ethyl ester**	**6.43**	**72**
24.158	**Octadecanoic acid, ethyl ester**	**2.57**	**74**
32.100	**Octadecanoic acid,17-methyl ester**	**12.38**	**50**
33.222	**Bis(2.ethylhexyl)phthalate**	**14.17**	**91**
33.691	**Hexacosanoic acid, methyl ester**	**8.39**	**90**
37.010	**Nonadecanoic acid, ethyl ester**	**52.24**	**80**

Table.5: GC-MS analysis of column fraction DCR2 of *Dichrostachys cinerea* root

Retention Time(Mins)	Name of Constituent	Peak Area %	% Quality
10.305	Caryophellene	1.11	99
12.686	Phenol-2,4-bis(1,1-dimethylethyl)	3.06	91
19.953	Isopropyl myristate	1.31	94
20.811	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl)ester	1.58	86
23.248	n-Hexadecanoic acid	7.03	93
33.466	Hexadecanoic acid, ethyl ester	9.14	97
24.049	Isopropyl palmitate	2.52	87
25.468	9-Octadecanoic acid(z)-methyl ester	1.09	95
26.653	Linoleic acid ethyl ester	5.29	99
26.773	9-Oxabicyclo[6.1.0]nonane,cis-	5.41	87
27.265	Octadecanoic acid, ethyl ester	2.79	95
30. 790	Hexanedioc acid, bis(2-ethylhexyl)ester	1.50	91
33.331	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	30.05	90
34.137	Nonadecanoic acid, ethyl ester	1.59	91

The constituents are arranged in order of GC elution on Ublon HR-1 column (Tables **4** and **5**). The significant volatile components(>50% quality) of the fractions included the sesquiterpenoid, β-caryophellene, the bicyclic alicyclic compound, 9-oxabicyclo[6.1.0]nonane,cis-, phthalates, phenol,-2,4-bis(1,1-dimethylethyl) and a large number of fatty acid esters, including isopropyl myristate. Caryophellene, a sesquiterpene, is a component of some plants including cannabis, clove, rosemary, black pepper and lavender. It has been reported to exhibit sedative, anticancer and anti-inflammatory effects (Andr`ea *et al.,* 2022). The bicyclic alicyclic, 9-oxabicyclo[6.1.0]nonane,cis- has previously been identified as one of the volatile components of the leaves and stem bark of the anti-malarial plant, *Mangifera indica* using GC-MS(Asanga *et al.,* 2023). Phenol,2,4-bis(1,1-dimethylethyl) is anti-

phytopathogenic fungi compound and has been previously identified in the extract of *Pseudomonas fluorescens* TL-1. It is reported to be a precursor to many complex compounds and widely as anti-oxidant and also has antimicrobial activity(Jianguo *et al.,* 2019). Isopropyl myristate is the isopropyl ester of the naturally-occurring myristic acid. It is generally considered to be a synthetic compound, but few plants including the leaves and fruits of *Mangifera indica* var. coquinho (Anarcadiaceae), a plant used to manage backache and bronchitis, have yielded it though in small quantities (<0.1%) from the immature fruit by headspace solid phase microextraction (HS-SPME) and by hydro-distillation (HD). The compound is used as a safe flavouring agent in many industrial foods and a base oil for hair sprays and topical skin treatment creams (Samya *et al.,* 2011)). Fatty acids and derivatives have been found to possess a number of bioactivities, including antimicrobial properties (Yoon *et al.*,2018).

CONCLUSION

In this research it has been shown that the crude ethanolic root extract of *Dichostrachys cinerea* has antimicrobial activity and contains important classes of natural products such as essential oils, phenols, terpenes, sterols and glycosides. Also, the volatile components of the root include terpenoids, phenols, phthalates and fatty acids and their esters. These are classes of natural compounds that have been known to possess various biological activities. The results obtained from this study could account for the rational usage of this plant in ethno-medicinal remedies in Nigeria.

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