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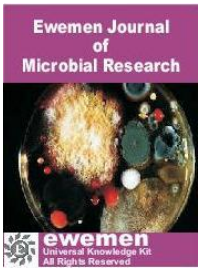

Original Research

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL SCREENING OF DRIED ROOT EXTRACTS OF *ALCHORNEA CORDIFOLIA*

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ABSTRACT

The use of plants as herbs for the treatment of human ailments is a common practice in all parts of the world. This has in no doubt contributed immensely towards the growth and rapid development of phytomedicines. *Alchornea cordifolia* has been a source of several novel and bioactive natural compounds. Traditionally, it is used for the treatment of several ailments. The dried roots were extracted with n-hexane, n-butanol and 95% ethanol. The crude extracts obtained were investigated for chemical and biological activities. Qualitative Phytochemical screening of the dried root extracts obtained by maceration confirmed the presence of various phytochemicals. The crude extracts were also investigated for biological activity and the results showed high concentration dependent activity against *S. aureus, B. subtilis, P. aeruginosa* and *C. albicans* with zone of inhibition ranging from 10-23 mm at concentrations ranging from 12.5 to 100 mg/mL. The 95% ethanolic extract demonstrated the highest activity against the test organisms. The aim of the present study is to evaluate the chemical and biological activities of the bioactive constituents of *A. cordifolia* dried root extracts. The present analysis suggests that *A. cordifolia* possesses remarkable Phytochemical and antibacterial property.

Keywords: *Alchornea cordifolia,* root extract, antimicrobial, phytochemicals

INTRODUCTION

The plant kingdom is highly essential for its biodiversity and has become an indispensable part of modern medicine because of the ability of plants to produce a host of bioactive molecules that are used by man for the treatment of diseases. These natural compounds found in all forms of plants and animals are the end products of metabolic processes and are called secondary metabolites (Cox and Ballick, 1994; Okwute and Egharevba, 2013). Some of the main bioactive constituents of medicinal plants are flavonoids, saponins, tannins, phenols, polyphenols, anthraquinones, alkaloids, cardiac glycosides,

anthocyanins, volatile oils and others. Their presence and quantity vary from one plant to another (Okwute and Egharevba, 2013). All across the globe, people and cultures have experimented with various plant parts to determine what effect they might have. Through regular practice, traditional healers have found some healing power. These represented the first crude drugs and the knowledge was passed down through the generations and systemized for example in traditional Chinese medicine and Ayurveda (Patrick, 2013). Many of these traditional medicines have real, beneficial effects and extracts of these crude drugs have led to the discovery of their active ingredients and eventually to the development of modern chemically pure drugs (Sneader, 2005).

A large number of currently prescribed drugs have been either directly derived from or inspired by natural products (Cutler and Cutler, 2000). Some of the oldest natural product based drugs are analgesics (Schr**ö**r, 2008). A significant number of antibiotics are based on natural products. The first antibiotic to be discovered, penicillin, was isolated from the mould penicillium (de la Bedoyere, 2005; Okwute and Egharevba, 2013).

A host of others are found to be effective as antimalarial, antitumor, anti-inflammatory, tannins and pesticidal agents (Okwute and Egharevba, 2013). *A. cordifolia* is a shrub distributed throughout tropical Africa. It is used in traditional African medicine for the treatment of variety of respiratory problems, genital-urinary problems, female sterility and intestinal problems including gastric ulcers, diarrheas, amoebic dysentery and worms. It is also taken as a blood purifier, as a tonic and to treat anaemia and epilepsy. The leaves are mostly used, but also the stem bark, stem pith, leafy stems, root bark, roots and fruits enter into local medicine. The leaves, root and stem-bark contain terpenoids, steroid glycosides, flavonoids, tannins, saponins, carbohydrates and imidazopyrimidine alkaloids alchorneine, alchornidine and several guanidine alkaloids. Different leaf, stem bark and root extracts have shown significant activities against a range of bacterial and fungal pathogen of humans with the root bark showing the strongest activity (Mavar-Manga *et al*, 2007). The aim of the present study is therefore to evaluate the chemical and biological activities of the bioactive constituents of *A. cordifolia* dried root extracts.

MATERIALS AND METHODS

Materials

Fresh roots and leaves of *A. cordifolia* were collected from Maji village, Suleja, Niger State, Nigeria in November, 2013. They were authenticated in the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. The roots were cut into small bits to facilitate drying. The pieces of the roots were air dried at room temperature for three weeks and then pulverized to fine particle size using Hammer mill machine. The powdered plant material was stored in a tightly closed polythene bag ready for use for the preparation of extracts.

The culture media used for the biological assay were Mueller Hinton Agar (MHA), Potato Dextrose Agar (PDA), Nutrient Agar (NA) and Mueller Hinton Broth (MHB). The media were used for Sensitivity, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination. All the media were prepared according to manufacturer's instructions against *Staphylococcus aureus (Sa), Bacillus subtilis (Bs), Escherichia coli (Ec), Pseudomonas aeruginosa (Pa)* and *Candida albicans (Ca).* The test organisms which include *Staphylococcus aureus (Gram-Positive Bacteria), Bacillus subtilis (Gram-Positive Bacteria), Escherichia coli (Gram-Negative Bacteria), Pseudomonas aeruginosa (Gram-Negative Bacteria), Candida albicans (Fungi)* were clinical isolates obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. The organisms (isolates) include:

Extraction of Plant Material

The dried pulverized roots (500 g) were taken in a clean, flat bottomed glass container and soaked in 2.5 L of n-hexane. The container was covered. It was regularly swirled and allowed to stand for 72 hours and filtered using sterile Whatmann No. 1 filter paper. The extract was evaporated to dryness on a water bath and stored in a dry sample bottle. The procedure was repeated using n-butanol and 95% ethanol.

Preliminary Phytochemical Screening

The crude extracts were subjected to preliminary phytochemical screening to verify the presence of secondary metabolites such as saponins (Frothing test), terpenoids (Salkowski's test), glycosides,

cardiac glycoside, proteins, tannins, phlobatannins, flavonoids, steroids, carbohydrates (Molisch's test), and alkaloids (Dragendroff's test).

Test for saponins

5 mL of each extract was agitated with 20 mL of distilled water in a graduated cylinder for 15 minutes. The solutions were observed for the formation of a layer of foam (Kumar *et al*, 2009).

Test for terpenoids

2 mL of chloroform and 3 mL of concentrated H_2SO_4 were added to 5 mL of each extract in a test tube. A monolayer of reddish brown colouration of the interface was observed (Ayoola *et al.*, 2008).

Test for glycosides

10 mL of 50% H_2SO_4 was added to 1 mL of each extract in a test tube. The mixtures were heated in a boiling water bath for 15 minutes. 10 mL of Fehling solution was added to the mixture and further boiled. A brick-red precipitate was observed in the extract indicating the presence of glycosides.

Test for cardiac glycoside

5 mL of each extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 mL of concentrated H_2SO_4. The solutions were observed for a formation of brown ring at the interface (Ayoola *et al.*, 2008).

Test for proteins

To 2 mL of each extract, 1 mL of 40% NaOH solution and 2 drops of 1% $CuSO_4$ solution was added. A violet colour was observed (Deb, 1998a,b).

Test for phlobatannins

A portion of each extract was boiled with 1% aqueous HCl. The solutions were observed for a red deposit of precipitate taken as evidence for the presence of phlobatannins (Edeoga *et al.*, 2005).

Test for Flavonoids

1 mL of 10% NaOH was added to 3 mL of each extract and observed. A yellow coloration was observed (Ayoola *et al.*, 2008).

Test for alkaloids

1% HCl and 6 drops of Dragendroff's reagent were added to each of the extracts. An organic precipitate indicating the presence of alkaloids was observed (Kumar *et al*, 2009).

Test for steroids

5 drops of concentrated H_2SO_4 was added to 1 mL of each extract in a test tube. The solutions were observed for a red colouration indicating the presence of steroids in the extracts (Edeoga *et al*, 2005).

Test for carbohydrates

2 drops of Molisch's reagent was added to 2 mL of each of the extracts and shaken vigorously. 2 mL of concentrated H_2SO_4 was added on the side of the test tube. A reddish violet ring appeared immediately at the junction of the two layers of n-butanol extract (Deb, 1998a,b; 2002).

Test for tannins

2 drops of 5% $FeCl_3$ was added to 1 mL of each extract in a test tube. A greenish precipitate was observed in each of the extracts (Edeoga *et al*, 2005).

Antibacterial screening

Preparation of varying concentrations of the extracts

Various concentrations of the extracts were prepared ranging from 12.5 to 100 mg/mL; this was obtained by measuring 1 mg of the extract and dissolved in 10 mL dimethyl sulphur oxide (DMSO), a solvent that dissolved the extract (100 mg/mL). A seriel dilution of the dissolved extract (100 mg/mL) was carried out into three different bottles containing DMSO to obtain concentrations of 50, 25 and 12.5 mg/mL respectively.

Sensitivity test of the crude extract using Agar Well Diffusion Method

The organisms used were standardized using Mc-Farland turbidity standard scale l, to obtain a bacterial cell density of 10^6 colony forming unit per millilitre (cfu/mL). The standardized inoculate were uniformly streaked (swabbed) into freshly prepared Mueller Hinton agar and potato dextrose agar plates respectively for the bacterial and fungal growth. Four wells were punched on the inoculated plates with a

cork borer (8 mm in diameter). The wells were properly labeled according to different number of the concentrations prepared. The wells were then filled up with the extracts about 0.2 mL per well. The plates were allowed to stay on the bench for 1 hour for the extract to diffuse on the agar. The Mueller Hinton agar plates for bacterial were incubated at 37°C for three days while the potato dextrose agar plates for fungi were incubated at room temperature (drawer) for three days.

At the end of incubation period, all plates were observed for any evidence of inhibition, which will appear as clear zones that were completely devoid of growth around the wells (zone of inhibition). The diameters of the zones were measured with a transparent ruler calibrated in millimetre (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Seriel dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB). Each tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism was used as a control. All tubes were then incubated at 37°C for 24 hours. After the incubation period, the tubes were examined for the presence or absence of growth using turbidity as a criterion.

The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The results from the Minimum Inhibitory Concentration (MIC) were used to determine the Minimum Bactericidal Concentration (MBC). A sterile wire loop was dipped into the tubes that did not show turbidity in the MIC test, it was then streaked unto a freshly prepared sterile nutrient agar plates. The plates were incubated at 37°C for 24 hours. After the incubation period the plates were then examined for the presence or absence of growth. This was done to determine if the antimicrobial effect of the extract was Bactericidal or Bacteriostatic.

RESULTS AND DISCUSSION

The results of the phytochemical study of the dried powdered root extracts of *A. cordifolia* are shown in Table 1. The results revealed that the roots were very rich in phytochemicals. The n-hexane crude extract of *A. cordifolia* was found to contain terpenoids, glycosides, proteins, tannins, flavonoids, steroids and alkaloid. The n-butanol crude extract of the plant showed the presence of saponins, terpenoids, glycosides, proteins, tannins, flavonoids, alkaloid and carbohydrate. Terpenoids, glycosides, cardiac glycoside, proteins, tannins, flavonoids, steroids, alkaloid and carbohydrates were richly present in the ethanolic extract of *A. cordifolia.*

Table 1: Results of phytochemical screening of *A. cordifolia* root extracts

Secondary Metabolites	Plant Extracts		
	n-hexane	n-butanol	95% Ethanol
Saponins	-	+	-
Terpenoids	+	+	+
Glycosides	+	+	+
Cardiac Glycosides	-	-	+
Proteins	+	+	+
Tannins	+	+	+
Phlobatannins	-	-	-
Flavonoids	+	+	+
Steroids	+	-	+
Alkaloids	+	+	+
Carbohydrates	-	+	+

Key: + = Present; - = Absent.

Terpenoids are known for their anti-viral, antibacterial, antimalarial, anti-inflammatory activity and for the inhibition of cholesterol synthesis and anti-cancer activity (Mahato and Sen, 1997). Flavonoids have several proven medicinal properties, such as anti-inflammatory, antioxidant, anti-cancer, anti-bacterial and anti-viral properties (Valsaraj *et al,* 1997). Alkaloids are also known for their analgesic, stimulant and antimalarial properties (Heinrich *et al,* 2004). Quinine, a notable alkaloid isolated from the bark of *Cinchona* species (Rubiaceae) in 1820, is one of the oldest and most important antimalarial drugs and is still used today. *A. cordifolia* is highly nutritious and as a result, it is widely used as goat feed in different parts of Nigeria because it is rich in proteins and carbohydrates.

The results of the antibacterial screening of the different crude extracts of root *A. cordifolia* against selected organisms are shown in Tables 2 and 3. The crude extracts demonstrated high activity against four out of the five organisms screened. It was however not active against *E. coli*. The activity was highest in the n-hexane fraction against *S. aureus* where the highest zone of inhibition was recorded at 23 mm, and least against *C. albicans* whose zone of inhibition was 11mm at the concentration of 12.5 mg/mL.

Table 2: Results of sensitivity of test organisms to various concentrations of n-hexane, n-butanol and ethanolic extracts of *A. cordifolia* dried roots.

T O	Zones of Inhibition (mm)											
	HE (mg/mL)				**BE (mg/mL)**				**EE (mg/mL)**			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
Sa	23	20	18	14	22	20	17	15	20	18	16	14
Bs	20	17	14	12	20	18	16	15	21	20	17	16
Ec	-	-	-	-	-	-	-	-	-	-	-	-
Pa	22	18	17	14	16	14	12	10	17	15	12	10
Ca	18	15	14	11	17	14	13	11	17	15	12	-

Key: TO = Test Organisms, HE = n-Hexane Extract, BE = n-Butanol Extract, EE = 95% Ethanol Extract.

Table 3: Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Extracts on the Test Organisms.

	MIC (mg/mL)			MBC (mg/mL)		
TO	HE	BE	EE	HE	BE	EE
Sa	25	6.25	3.125	50	12.5	6.25
Bs	25	25	25	50	50	50
Ec	-	-	-	-	-	-
Pa	25	3.125	3.125	50	6.25	6.25
Ca	25	25	12.5	50	50	25

Key: TO = Test Organisms, HE = n-Hexane Extract, BE = n-Butanol Extract, EE = 95% Ethanol Extract.

CONCLUSION

The findings in this study show that *A. cordifolia* root is rich in secondary metabolites. The presence of these phytochemicals could be responsible for the antimicrobial activity of *A. cordifolia*. However, further studies are needed to be conducted on the root extracts of *A. cordifolia* to isolate the active components responsible for the observed activities.

CONFLICT OF INTEREST

None declared.

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