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Original Research Article

Antibacterial Activity of Chromatography Fractions from *Jateorhiza macrantha* (Hook F.) Exell Mendonça (Menispermaceae) Leaves

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Abstract

Jateorhiza macrantha is a known medicinal plant used in ethno medicine for the treatment of diarrhea, cough, boils and ulcer. This study is aimed at evaluating the antibacterial activity of chromatography fractions from the aqueous ethanol extract of the leaves of *Jateorhiza macrantha* as a preliminary step towards isolation and characterization of its anti-bacterial constituent(s). The aqueous ethanol extract (AEE) was obtained by exhaustive cold maceration in 70% aqueous ethanol and further fractionated using vacuum liquid chromatography (VLC) techniques with a mobile phase gradient of n-hexane:chloroform:methanol (4:0:0, 3:1:0, 2:2:0, 1:3:0, 0:4:0, 0:3:1, 0:2:2, 0:1:3, and 0:0:4 v/v/v) into three fractions: non-polar (NPF1, eluted with n-hexane:chloroform:methanol 4:0:0, 3:1:0, 2:2:0 v/v/v), moderately polar (NPF2, eluted with n-hexane:chloroform:methanol 1:3:0, 0:4:0, 0:3:1, 0:2:2, 0:1:3 v/v/v) and polar (NPF3, eluted with n-hexane:chloroform:methanol 0:0:4 v/v/v). Antibacterial activity was evaluated using the agar well diffusion method against clinical isolates of pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pnuemoniae*, and *Salmonella typhi* and the Minimum Inhibition concentration (MIC) extrapolated following the graphical approach. Functional group investigation was done using infra red spectroscopy. Three chromatography fractions NPF-1 –NPF3 obtained had MIC range of 5.0 mg/ml > MIC <0.26 mg/ml. NPF3 was relatively pure showing a single spot on TLC and its IR spectra are evidence for OH, N-H, C-O, C=C, and C-H stretching and deformation vibration frequencies typical of amino derivative of a lactone glycoside. The observed antibacterial activity justifies the ethno medicinal uses of *Jateorhiza macrantha*.

Keywords: *Jateorhiza macrantha*, antibacterial agents, chromatography fractions, amino lactone glycoside.

INTRODUCTION

Complications and deaths due to Pathogenic bacterial infections are a global challenge which is increasingly becoming worrisome with the high incidence of drug resistant strains of pathogenic bacteria [1]. Poor sanitation, poor access to healthcare, human migration, new disease agents, poverty and antibiotic resistance among others, contribute to the uncontrolled epidemic of bacteria diseases [1-3]. In the global drive to discover and develop novel antibacterial agents, recourse to botanicals has continued to be viable sources of antibacterial lead compounds [4]. *Jateorhiza macrantha* is one of the two species of medicinal plant in the genus *Jateorhiza* of the Menispermaceae family. It is widely used in ethnomedicine. Its leaf saps is used as pain-killer, stem hairs for subcutaneous parasitic infections, bark as an anti-abortifacient and as antidote for venomous stings and bites [5-10]. It is also use in the treatment of hypertension [11], and dysmenorrheal [12], poor sexual libido, dysentery, wound and ulcer [9, 10]. Several compounds like the diterpenoid: columbin, isocolumbin, plamarin, isojateorin, chasmanthin and palmatosides A have been characterized from its closely related species *Jateorhiza palmate* [13] with scarce literature on similar attempt for *Jateorhiza macrantha.* This study is aimed at evaluating the antibacterial activity of chromatography fractions from the aqueous ethanol extract of the leaves of *Jateorhiza macrantha* as a preliminary step towards isolation and characterization of its anti-bacterial constituent(s).

MATERIALS AND METHODS

MATERIALS

Unless otherwise indicated all reagents and chemicals (of analytical grade) used were obtained from BDH and/ or Sigma Aldrich. Stock cultures of clinical strains of the bacteria (Gram positive: *Staphylococcus aureus*, *Bacillus subtilis*, and gram negatives:

Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonae) were obtained from the Department of Microbiology, Faculty of Sciences University of Port Harcourt. The fresh leaves of *Jateorhiza macrantha* were sourced from Ijebu forests in Ore Ondo State, South Western Nigeria and authenticated by a Taxonomist Dr. Chukwuka S. Kanayo of the Department of Botany University of Ibadan with Voucher specimen number UIH 22610 deposited in the herbarium of same University. The leaves were air dried at room temperature, ground to fine powder and stored in an air tight container until further use.

METHODS

Extraction and Column Chromatography Fractionation

The aqueous ethanol extract (AEE) was obtained by exhaustive cold maceration of the powdered *Jateorhiza macrantha* leaves (100 g) at room temperature in 1 L of 70% ethanol for 72 hours. The extraction solvent was replaced with fresh solvent at 24 hours interval during the 72 hours period of extraction. The filtrates obtained were pooled and concentrated using a rotary evaporator and further dried over the water bath at < 45^{o}C to afford the crude 70 % aqueous ethanol extract (AEE). Using a mobile phase gradient (500 ml each) of : n-hexane:chloroform:methanol (4:0:0, 3:1:0, 2:2:0, 1:3:0, 0:4:0, 0:3:1, 0:2:2, 0:1:3, and 0:0:4 v/v/v) determined from a prior thin layer chromatography(TLC) analysis, the vacuum liquid chromatography (VLC) separation was carried on a column packed with normal phase silica gel (mesh 200-400). Fractions were collected at 100 ml intervals. Fraction having similar TLC fingerprint based on retardation factors and colour reaction after exposure to chromogenic detection methods were pooled into three fractions: non-polar (NPF1, eluted with n-hexane:chloroform:methanol 4:0:0, 3:1:0, 2:2:0 v/v/v), moderately polar (NPF2, eluted with n-hexane:chloroform:methanol 1:3:0, 0:4:0, 0:3:1, 0:2:2, 0:1:3 v/v/v) and polar (NPF3, eluted with n-hexane:chloroform:methanol 0:0:4 v/v/v).

Phtochemical Screening

This was done using standard phytochemical screening reagents as reported [14, 15]

Functional Group Analysis

This was done using infra red (IR) spectroscopic technique on a KBr disc.

Antibacterial Activity Assay

The agar Well diffusion method was used [16]. Overnight culture broth was used to obtain 0.5 McFarland bacteria standard for seeding sterile molten Mueller-Hinton agar medium, maintained at 45^{o}c. Using a sterile cork borer, holes of 5mm size were made on each of the solidified, segmented, labeled, seeded agar plates. With the aid of sterile syringe, the wells were loaded with 0.1 ml of different concentrations of the test samples (AEE -10 mg/ml; and its chromatographic fractions- 10 and 5 mg/ml, and ciprofloxacin - 2µg/ml). Thereafter the preparations were allowed to stand for 30 minutes to ensure pre-diffusion and then incubated at 37^{o}c for 24hr. The inhibition zone diameter (IZD) of each concentration against the test organisms were determined and recorded. A sterility control test was done with each of the extract/fractions without the organisms. Following a four-fold serial dilution approach from the 5 mg/ml concentration, the minimum inhibition concentration (MIC) determination of the chromatography fractions, NPF1 – NPF3 from the crude 70 % aqueous ethanol extract (AEE) of *Jateorhiza macrantha* leaves were similarly done using the agar diffusion method and their respective MICs determined following the graphical approach from the intercept on the x- axis from a plot of square of the IZD against Logarithm of concentration [16].

RESULTS AND DISCUSSION

The yield of the 70% aqueous ethanol extract of *Jateorhiza macrantha* leaves was 3.94 g (3.94 % w/w). Although at variance when compared to that reported for the hot water (4.33% w/w) and hot ethanol (2.45% w/w) extracts [10], this could be attributed to the use of cold extraction technique in this study. The choice of cold extraction was adopted because hot extraction method could pre-dispose the heat labile metabolite to decomposition [14]. Whereas secondary plant metabolites like: saponins, triterpenoids, cyanogenic glycosides, cardiac glycosides, and anthraquinones were absent, phenolic constituents like tannins and flavonoids, and carbohydrate derivatives in form of free and combined reducing sugars are evidently present from the phytochemical screening of the powdered leaves of *J. macrantha* (see Table-1). Alkaloids were also detected using Dragendorff's reagent. The observed trend in the phytochemical constituents is in agreement with that reported in the literature [10]. Tannins and flavonoids are phenolic compounds occurring naturally in plants with ecological function. Whereas tannins are of high molecular weight containing hydroxyl and carbonyl groups in sufficient amount and capable of forming strong complexes with protein and alkaloids [17], flavonoids like tannins, are also polyphenolic compounds but of lower molecular weight having three basic rings A(C6), B(C6) and C(C3) carbon skeleton [18] commonly referred to as the C6-C3-C6 system playing ecological role as well. Flavonoids are found occurring in nature in various plant parts such as flowers, seeds and barks [18]. Because, physiologically tannins have astringent property, binding and precipitating proteins they impart harsh taste in the mouths when fruits, seeds, leaves with high levels of tannins are consumed thus scaring herbivores and man

[17]. Pharmacological actions of tannins include antiseptics and as blood coagulant in the management of burns and wounds. In the gastrointestinal tract, its astringent property hinders activity of proteolytic enzymes because of the insoluble complexes formed with proteins [17]. Tannins have antioxidant and antimicrobial activities [19, 20]. In terms of biological activities flavonoids are reported to have anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function [18]. The presence of these constituents as seen in Table 1 could offer the rationale for the justification of the use of *Jateorhiza macrantha* in ethnomedicine for the treatment of bacterial infection and other associated opportunistic infections diseases. Of the three chromatography fractions NPF1, NPF2, and NPF3 (see Table-2) obtained, the polar NPF3 inhibited all the test pathogenic bacteria at 10 and 5 mg/ml while non-polar NPF1 and moderately polar NPF2 fractions were not active against *B. subtilis* though active against the other pathogenic bacteria (see Table-2). The MIC of the fractions NPF1 - NPF3 are as given in Table 3. The trend in the MIC showed that the NPF3 was the most active having the least MIC values except for the pathogenic bacteria *E.coli, K. pneumonia,* and *S. typhi.* The NPF3 was relatively pure showing a single spot on TLC. Infra-red spectroscopic analysis of the NPF3 as rationalized in Table-4, showed sharp vibrational bands at 3686 cm^{-1} for O-H stretching vibration that could come from an alcohol or even a sugar molecule. The symmetric (3226 cm^{-1}) and asymmetric (3348 cm^{-1}) bands typical of N-H stretching of a primary amine functional group and not a secondary amine which should show one absorption band nor a tertiary amine which should have none. The sharp peak at 1864 cm^{-1} could be attributed to C=O stretching of a carbonyl functional group probably due to a five or lower member lactone ring. The sharp band seen at 1624 cm^{-1} is for a C=C stretching indicating that the constituents have an olefinic moiety. The weak bands showing at 3015 and 2890 cm^{-1} are indicative of presence of olefinic =C-H and saturated C-H aliphatic stretching vibrations. The C-O vibration stretching at the region 1041 cm^{-1} is a confirmation of glycosidic or alcohol C-O bond. In view of these spectra data the compounds in the bioactive fractions NPF3 could be a primary amino derivative of a lactone glycoside with an unsaturated C=C moiety. The diterpene furanoid lactone glycoside, palmatosides A-G and the diterpene furanoid lactone chasnmantine [13] with similar functional groups as seen in NPF3 have been reported as bioactive constituents in *Jateorhiza palmata* the second of the two known species of the genus *Jateorhiza*. *Jateorhiza macrantha* is a recognized medicinal plant for the treatment of infectious diseases such as boils, cough and urinary tracts infections among others. Result of antibacterial assay of both the AEE and its chromatographic fractions against the selected six common pathogenic bacteria has confirmed its effectiveness when compared with the standard antibiotic Ciprofloxacin HCl.

Table-1: Phytochemical screening result of the leaves of *Jateorhiza macrantha*

Screened phytochemicals test	Results
Saponins:	
Frothing test	-
Emulsion test	-
Phenolics	
Tannins:$FeCl_3$ test	+
Phlobantannin test	-
Flavonoids:	
Shinoda test	+
$AlCl_3$ test	+
Carbohydrates:	
Molisch test	+
Free sugar(fehlings)	+
Combined sugar(fehlings)	+
Alkaloids:	
Dragendorff test	+
Mayer"s test	-
Hager"s test	-
Wagner" test	-
Test for triterpenoids:	
Liebermann burchard test	-
Salkowski test	-
Anthraquinone:	
Bontragers test for free anthraquinones	-
Borntragers test for combined anthraquinones	-
Cyanogenic glycosides	-
Cardiac Glycosides:	
Kedde's test:	-
Keller killiani test	-

Key: + means present, - means absent

Table-2: Results of antibacterial assay showing the inhibition zones diameter (IZD) of crude extract of *Jateorhiza macrantha* and its fractions

Test sample	conc.mg/ml		Inhibition Zone diameter (mm) against the selected pathogenic bacteria				
		Bacillus subtilis	***S.aureus***	***P. aeruginosa***	***K. pneumonia***	***E.coli***	***S.typhi***
AEE	10	15± 0.05*	10± 0.11	6.5±0.05	7±0.05	9± 0.05	7.5±0.20
NPF1	10.00	-	10±0.10	10±0.10	11±0.32	12±0.06*	10±0.06
	5.00	-	10±0.10	6±0.12	7±0.00	8±0.10	9±0.12
NPF2	10.00	0±0.00	9±0.11	8±0.20	10±0.00	8±0.10	9±0.20
	5..00	0±0.00	7±0.15	6±0.15	5±0.01	8±0.10	8±0.35
NPF3	10.00	9±0.10	8±0.06	9±0.00	9±0.17	9±0.20	9±0.20
	5.00	6±0.10	7±0.05	7±0.06	0±0.00	7±0.29	8±0.00
Ciprofloxacin HCl.	2 µg/ml	14	13	12	13	9	14

Key*: *significantly higher activity (p< 0.05) compared to the reference antibacterial drug ciprofloxacin HCl , AEE= Crude 70 % aqueous ethanol leaves extract of Jateorhiza macrantha; NPF1- (non-polar fraction eluted with n-hexane:chloroform:methanol 4:0:0, 3:1:0, 2:2:0 v/v/v), NPF2-(moderately polar fraction eluted with n-hexane:chloroform:methanol 1:3:0, 0:4:0, 0:3:1, 0:2:2, 0:1:3 v/v/v) and NPF3- (polar fraction eluted with n-hexane:chloroform:methanol 0:0:4 v/v/v).*

Table-3: Minimum inhibition concentration of the Chromatography fractions NPF1- NPF3 from the Crude 70 % aqueous ethanol leaves of *Jateorhiza macrantha* against the selected pathogenic bacteria

Test sample	Minimum inhibition concentration (mg/ml) against the selected pathogenic bacteria					
	Bacillus subtilis	***S.aureus***	***P.aeruginosa***	***K.pneumoniae***	***S.typhi***	***E.coli***
NPF1	nd	1.25	3.39	3.12	0.26	2.87
NPF2	nd	1.73	2.05	3.97	0.37	1.25
NPF3	2.87	0.52	1.01	5.00	0.73	1.73

Key: nd: not determined; NPF1 – NPF3 are chromatographic fractions from the Crude 70 % aqueous ethanol leaves extract of *Jateorhiza macrantha*

Table-4: Diagnostic infra red absorption bands for NPF3 Fraction

Absorption bands v,cm^{-1}	Description of bands
3686	O-H stretching
3226 3348	N-H asymmetric stretching and N-H symmetric stretching Indicative of primary amino group
3015	=CH stretching of olefinic bond
2890	C-H Stretching $CH_{3\ and}\ CH_2$ stretching
1864	C-O stretching of a lactone
1624	N-H bending of primary amines
1349	=CH deformation/bending
1246	C-N stretching of primary amine
1041 1175	C-O stretching of esters, alcohols and glycosidic bonds
835	N-H wagging of primary amine

CONCLUSION

The observed trend in the antibacterial activity of *Jateorhiza macrantha* leaves in this report has justified scientifically, the use of this plant in ethnomedicine for the treatment of bacterial infections. Further work is on-going to obtain in a pure form the bioactive constituents and elucidate their chemical structure using nuclear magnetic resonance and mass spectroscopic techniques.

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