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Research Article

Antibacterial Activities and Characterization of the Extract of *Pleurothecium recurvatum*

Adewole E*, Ojo A., Orisakeye O.T, Adewumi D.F., Peters O.A
Department of Chemical Sciences, Afe-Babalola University, Ado-Ekiti, Nigeria

***Corresponding author**
Adewole E
Email: adewolen50@yahoo.com

Abstract: *P. recurvatum* has been a very lead candidate for the discovery of novel antibiotics. The fungi was isolated from cocoa farm soil in Eri-pose, Ondo State Nigeria. Ethyl acetate extract was obtained after the culturing, extraction and purification of the cultured collected from a locally fabricated fermenter. The extract was fractionated through column chromatography using a mixture of solvent. The B_4 fraction was screened for its antimicrobial efficacy. When tested against *B. substilis*, the zone of inhibition after 24hrs of incubation was 13.00 mm, also, screened against *E. coli*, the zone of inhibition was 11.00mm. The antibacterial activity of fraction was better than that of streptomycin standard when screened against *B. substilis*, its zone of inhibition was 12.00mm and had the same zones of inhibition with the fraction when screened against *E. coli* The results of the GC-MS revealed presence of major novel compounds which includes Furan, 4,5-diethyl-2,3-dihydro-2,3-dimethyl, 5-ethyl-5-methyl having retention time 5.20 and % total of 0.96, 2-phenyl-2-oxazoline having retention time 5.71 and % total of 2.14, 1,3,5-triazine,2,4 (1H,3H)-dione,6 ethylamino) with retention time 11.50 and % total of 1.36, Hydrocinnamic acid-0-[1,2,3,4-tetrahydro-2- naphthyl) methyl] having retention time 7.44 and % total 1.24. These major bioactive compounds have been found very useful in the formulation of novel drugs as they possess various pharmacological activities.
Keywords: Isolation, Fractionation, Antibacterial, GC-Ms, Bioactive Compounds

INTRODUCTION

Bioactive compounds are chemical substances that can inhibit the growth of and even destroy harmful microorganisms. They are derived from special microorganisms and are produced on an industrial scale using fermentation process [1]. The principles of antibiotic action were not discovered until the twentieth century, the first known use of antibiotics was by the Chinese over 2,500 years ago [1]. In Africa, especially in Nigeria, bioactive organic compounds from soil microorganisms have not been subject of intensive investigation by chemist. The major emphasis has been on isolation and structure elucidation of natural products from medicinal plants and this necessitate this research work.

MATERIALS AND METHODS

Soil collection

Soil sample was collected from cocoa farm in July 2010 in Eri-pose, Nigeria. Random sampling method was used in collecting soil sample and the collection was done using soil auger at a depth of 0-10cm. The collected soil was put into polythene bag and stored inside refrigerator.

Isolation of the fungi

One gram of soil sample was transferred to a sterile Erlenmeyer flask containing 50ml sterile water. The flask was shaken on rotary shaker for 30 minutes for the detachment of the spore chains. The flask was kept aside for 30 minutes to settle down the particulate matter. The clear supernatant was diluted with sterile water (dilutions 10^{-1} – 10^{-3}) was used on inn ocular. 1 ml of each of these dilutions was pipetted out into the medium then plated into petri dishes 6 mm diameter and incubated at 28^0C for 3 weeks and potato dextrose agar was used [2].

Identification

Cultural observation

Using the natural eyes and microscope at low power magnification (x40), parameters such as, colony color, color change in the medium, characteristic of the submerged hyphae whether rhizoid, spiral or regular and characteristic shape of mature fruiting bodies is strictly observed [2].

Microscopic observation

A small piece of mycelium free of medium was transferred by using inoculating needle on to a glass slide that contains a drop of cotton blue in loctophenol and the mycelium was spread properly with

another needle. The preparation was covered with a cover slip and observed under medium power (x100) and later at high power (x400) magnifications. Details of spore coloration, shape, septation and surface marking were studied [2] and later confirmed to be *P. recurvatum*.

Culturing

A starter culture from a sample of previously isolated, cold stored organism was put into 1litre capacity of sterilized potato dextrose broth and later transferred into the fabricated fermenter for two weeks.

Extraction and purification

The fungi cultures were centrifuged and extraction of active compounds from multiplied fungi was carried out in a separating funnel using ethyl acetate. The extract was concentrated using rotary evaporator [2].

Column Chromatography

The fraction was eluted using mixture of 50% ethyl acetate and 50% methanol using column chromatography and purified. The fraction was again concentrated using rotary evaporator [2] and designated as B_4

Antibacterial Analysis

The micro-organisms used for these test were *Bacillus substili*, *Escherichia coli* and *Streptomycin* as standard. Agar well dilution method was used. Two ml of the test organisms (24hrs old culture) was aseptically injected into the sterilized plate. Twenty ml of sterilized nutrient agar was poured on top of the test organisms aseptically after it has been cooled to 45^0C. The medium was swirled gently for even distribution of inoculums and allowed to solidify. Sterile cork borer of 1mm diameter was used to make 4 wells on the solidified agar into which 0.5ml extracts were injected into the well with the use of sterilized clinical syringe separately. The plates were incubated at 37^0C for 24hours and the zones of inhibitions were observed around each well after 24hours. The results were quoted as the radii (mm) of the zone of inhibition.

Gas chromatography-Mass Spectrophotometer

GC-MS analysis of this extract was performed using a Perkin Elmer GC Claurus 500 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (30 m x 1µ Mdf. Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used .Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 2µl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C /min, to 200°C ,then 5°C/ min. to 280°C, ending with a 9min. isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and the running time was 45 minutes. The components were identified by comparing their retention times with those of authentic samples, as well as by comparing their mass spectra with those of (NIST)

RESULTS

Table 1: Antibacterial activities of B_4 fraction showing zones of inhibition (mm)

	Test organisms	
	B. substilis	***E. coli***
B_1 fraction	13.00	11.00
Streptomycin Standard	12.00	11.00

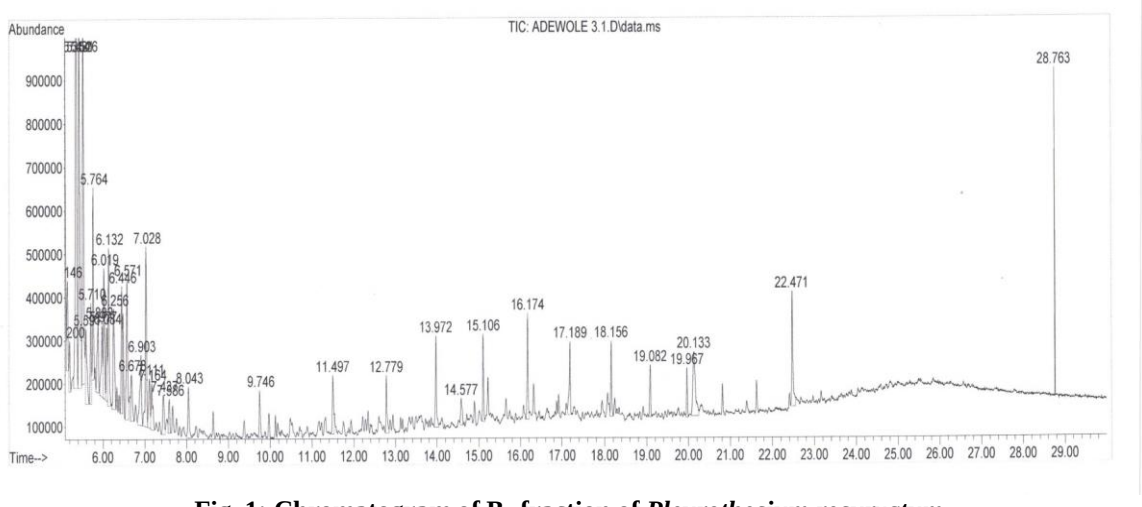

Fig. 1: Chromatogram of B_4 fraction of *Pleurothecium recurvatum*

Table 2: Showing major bioactive compounds identified in the fraction B_4

Compound names	Retention time	% total
Furan,4,5-diethyl-2,3-dihydro-2,3-dimethyl	5.20	0.96
Cis-p-mentha-2,8-diene-1-ol	5.59	2.13
5-Ethyl-5-methyl-2-phenyl-2-oxazoline	5.71	2.14
Oxirane,tetradecyl	5.76	3.53
Hydrocinnamic acid,o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]	7.44	1.24
Trans-p-mentha-1(7),8-diene-2-ol	6.45	2.78
Cis-p-mentha-1(7),8-diene-2-ol	6.68	1.32
1,3,5-triazine-2,4(1H,3H)-dione,6-(ethylamino	11.50	1.36
p-cymene	6.57	3.70

DISCUSSION

From the results of antibacterial analysis (Table 1), the extract displayed strong antibacterial activities against the tested isolates; it has zones of inhibition (mm) against *Bacillus substilis* (13.00mm), and *Escherichia coli* (11.00 mm). The result was better than that recorded for streptomycin used as standard, when the streptomycin was screened against *Bacillus substilis*, the zone of inhibition was (12.00mm) and against Escherichia coli, the zone of inhibition were the same (11.00mm). The strong antibacterial activities displayed by the fraction B_4 may be connected to the presence of major bioactive compounds identified by the use of Gas Chromatography Mass Spectrometer in table 2. Furan, 4, 5-dimethyl-2,3-dihydro-2,3-dimethyl- was identified, having retention time 5.20 and percentage total of 0.96 %. It is a derivative of furan compounds. The broad spectrum of biological activity of furan derivatives continues to attract the attention of synthetic chemists Furans, consisting of a five-membered aromatic ring with one oxygen atom, are an important class of heterocyclic compounds that possess important biological properties.

5-Ethyl-5-methyl-2-phenyl-2-oxazoline was also identified in fraction B_4. There are few of the natural products having oxazoline skeleton and this includes; Bistratamide, acinetobactin, ascidiacyclamide, *trans*, *trans*- ceratospongamide, agrobactin, westiellamide [3]. Oxazoline derivatives exhibit several pharmaceutical activities such as antidiabetic, antihypertensive, antidepressive, anticancer, anti HIV-1, antitumor and antialzheimer activities to mention a few [5].

In another development, oxirane, tetradecyl having retention time 5.76 and percentage total of 3.53 % was found in fraction B_4. Many of the oxirane derivatives have been found to possess antifungal and antibacterial activities, these includes New bile acid-based amino sterols that gave good yields from C-3beta-oxiranes as key intermediates and were found to have antifungal and antibacterial activities [4].

Moreover, Cis-p-mentha-2,8-diene having retention time 5.59, percentage total of 2.13 %, Hydrocinnamic acid,o-[(1,2,3,4-tetrahydro-2-naphthyl) methyl] having retention time 7.44, percentage of total 1.24 %, trans-p-mentha-1(7),8-diene-2-ol having retention time 6.45, percentage of total 2.78 %, cis-p-mentha-1 (7), 8-diene-2-ol retention time 6.68, percentage of total 1.32 % and 1,3,5-triazine-2,4 (1H, 3H)-dione,6-(ethylamino) having retention time 11.50 and percentage of total 1.36 % and p-cymene were all identified in the fraction B_4.

These compounds possess various biological activities such as antibacterial and antifungal properties and they are highly found useful in the formulation of antibiotic [6].

CONCLUSION

This research work has significantly revealed the rich potential of cocoa farms soil in the discovery of novel antibacterial and antifungal compounds, if these compounds could be isolated and characterized using various spectroscopic techniques, novel and lead candidates compounds may be discovered.

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