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Isolation of *Neurospora Crassa* from Cocoa Farm Soil, Antibacterial and Identification of Bioactive Compounds in the Extract Using Gas Chromatography - Mass Spectrometer

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ABSTRACT

Soil has been a free gift of nature that the creator has giving mankind for exploration, there is a world-wide effort by the pharmaceutical chemists to continue to search for new antibiotics, anti-viral, antiparasitic and host of other novel drugs as a result of the grave consequences posed by the current diseases that have developed resistance to many of the known drugs. Neurospora crassa from Fifty years old cocoa farm soil in Ikota, Nigeria was isolated, cultured to yield maximum bioactive compounds using locally designed and fabricated fermenter and bioactive compounds were extracted in ethyl acetate and purified through column chromatography. The fraction X5 was screened for antibacterial activities using agar well diffusion; it has strong zones of inhibition (mm) against Staphylococcus aureus, Bacillus substillis, and Escherichia coli. The results were better than streptomycin standard. The strong antibacterial activities displayed may be connected to the presence of bioactive compounds revealed by the Gc-Ms and these are: 1H-indene,2,3-dihydro-4-methyl having retention time 5.485, height area 310344 and % total 8.267; 1H-Indene,2,3-dihydro-1,2-dimethyl with retention time 6.375, height area 99678 and % total 3.214 and 1H-indene,1-ethylidene having retention time 8.743, height area 134010 and % total 3.567, Naphthalene having retention time 6.191, % of total 18.907 and peak height 700240. From the research work, the cocoa farm soil is an eye opener for the pharmaceutical chemists where they can beam their search point into for the discovery of new and novel compounds that may have various biological activities.

Keyword: cocoa farm soil fermenter, antibacterial, 1H-indene, 2,3-dihydro-4-methyl, 1H-Indene,2, 3-dihydro-1, 2-dimethyl, 1H-indene,1-ethylidene and Naphthalene.

INTRODUCTION

Infectious diseases caused by bacteria, fungi and viruses are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance.

As a result of the continuous evolution of microbial pathogens towards antibiotic-resistance, there have been demands for the development of new and effective antimicrobial compounds. Fungi play a major role in soil ecosystems along with bacteria, protists, small invertebrates and plants, through complex trophic interactions. Most soil fungi are regarded as saprobes, decomposing organic matter and contributing to nutrient cycling, while several species form mycorrhizal associations with plants or are plant pathogens[1]. Also recognized as prolific secondary metabolite producers, fungi have provided several bioactive compounds and chemical models currently used as pharmaceuticals, and soils are traditionally the main source of fungal genetic resources for bioprospection programs [5]. Despite that, the biodiversity and biotechnological potential of the soil mycobiota in many tropical regions is still poorly studied. Due to their pharmaceutical potential secondary metabolites of fungi have been studied for more than 70 years.

Many medicinal chemists have been making efforts in discovery new antibacterial and antifungal dugs by researching into plants and various soils. To expand the search for new antibacterial and antifungal bioactive compounds, fifty years old cocoa farm soil in Ikota, Nigeria was examined.

MATERIALS AND METHOD

Soil sample collection

Soil sample collection was done in July 2010 in Ikota, Ondo State, Nigeria. Random sampling method was used in collecting soil sample directly and the collection was done using soil auger at a depth of 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 (EM) 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 innocular. One ml of each of these dilutions was pipette out into the medium, plated into Petri dishes 6mm diameter and incubated at 280C for 2-4 weeks and potato dextrose agar was used.

Identification

Cultural observation: using the natural eyes and microscope at low power magnification (x40), parameters such as, colony colour, colour change in the medium, characteristic of the submerged hyphae whether rhizoid, spiral or regular and characteristic shape of mature fruiting bodies are strictly observed.

Microscopic observation: A small piece of mycelium free of medium was transferred using inoculating needle on to a glass slide containing 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 colouration, shape, septation and surface marking were studied and Neurospora crassa was identified and confirmed by professor of microbiology in Microbiology laboratory.

Culturing the fungi

The fungi with strong antagonistic efficacy were culture in the laboratory for maximum yield of bioactive compounds using the fabricated fermenter, five ml of already cultured fungi was put into sterilized potato broth of 500ml and poured into the fermenter and allowed to excrete maximum yield for two weeks, the air pump supplied continuously sterilized air and the culture being mixed together using powered mixer.

EXTRACTION AND PURIFICATION

The fungi cultures were centrifuged and extraction of compounds from multiplied fungi was carried out in a separating funnel using ethyl acetate. The extract was concentrated using rotary evaporator. The fraction was eluted using mixture of 50% ethyl acetate and 50% hexane through column chromatography and the fractions was concentrated using rotary evaporator to obtained X5 extract.

ANTIBACTERIAL ANALYSIS

The antibacterial analysis was carried out using agar well diffusion method, the micro-organisms used for these test were Bacillus substilis, Staphylococcus aureus, Escherichia coli and streptomycin as standard. 2ml 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 450C. 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 370C for 24hours and the zones of inhibitions were observed around each well after 24hours of incubation. The results were quoted as the radii (mm) of the zone of inhibition.

Gas chromatography- mass spectrophotometer (GC-MS) analysis:

Analysis was conducted using an HP (Hewlett Packard, 5890 series И GC hyphenated with 5989 Mass Spectrometer). MS conditions were as follows: Detector mass spectrometer voltage 70eV and its source temperature was 300oC. The injector temperature was 240oC and the split less mode 0.5µL injection. The HP 55% dimethyl-95% diphenylpolysiloxane non-polar column was performed with length 30 cm x 0.25 mm, coating thickness film 0.25 µm. The oven was adjusted at 100°C for 1 min and initial time 1.5 min with 40oC which ended by a final temperature of 300oC and 4 min hold time where the total run time was 45 min. 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 AND DISCUSSION

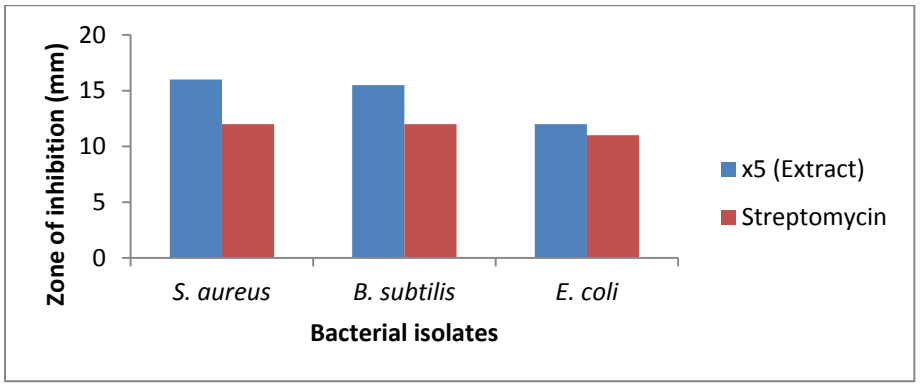

Figure 1 showing antibacterial analysis of extract X5

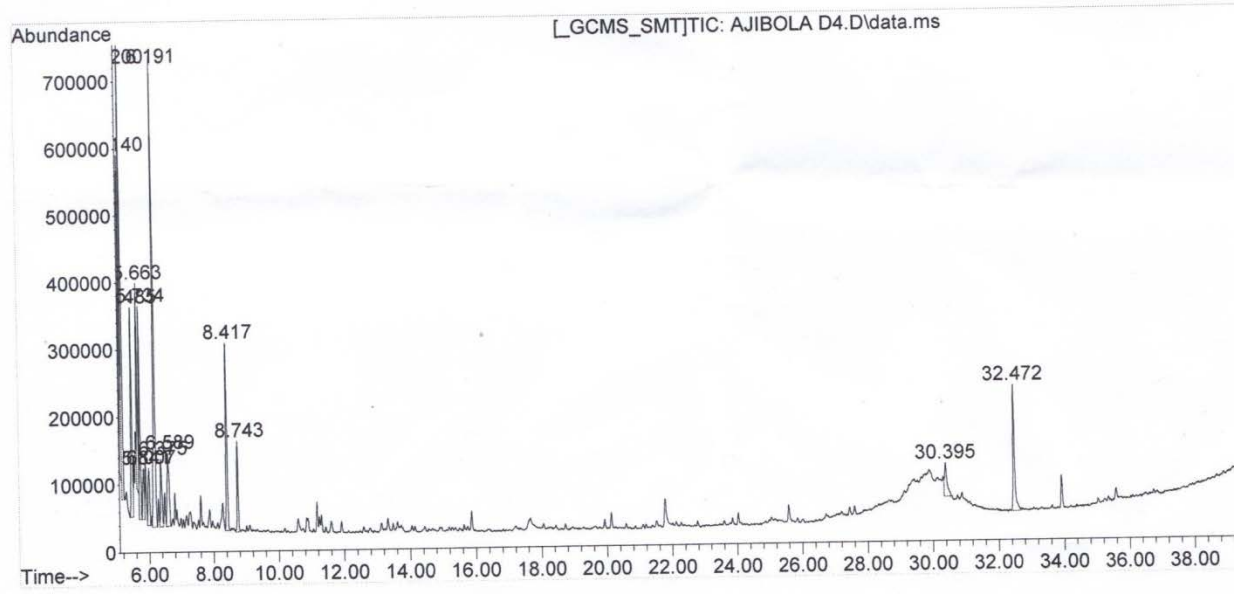

Figure 2. Chromatogram of X5 extract of Neurospora crassa

Table1. Results of gas chromatography-mass spectrophotometer

COMPOUND NAME	RETENTION TIME	% OF TOTAL	PEAK HEIGHT
Benzene,1,2,4,5-tetramethyl	5.140	9.819	472953
1H-indene,2,3-dihydro-4-methyl	5.485	8.267	310344
Benzenebutanal	5.841	2.464	74674
Benzene,1-ethyl-2,3-dimethyl	5.734	6.923	315214
Benzaldehyde,4-(1-methyl ethyl)-	6.007	2.857	84648
Naphthalene	6.191	18.907	700240
Benzene,1-ethyl-2,4,5-trimethyl	6.589	4.660	108080
Naphthalene,1-methyl	8.417	7.167	277367
1H-indene,1-ethylidene	8.743	3.567	134010
Dodecanoic acid, 1,2,3-propanetriyl ester	30.395	2.692	49828

DISCUSSION

From the results of antibacterial analysis (figure 1), the extract displayed strong antibacterial activities against the tested isolates; it has zones of inhibition (mm) against Aspergillus Aureus 16.00, against Bacillus substilis (15.50), and Escherichia coli (12.00) and the results were better than that recorded for streptomycin used as standard. When the streptomycin was screened against Bacillus substilis, the zone of inhibition was

(12.00mm), against Escherichia coli, the zone of inhibition was (11.00mm) and against Bacillus substilis the zone of inhibition was (12.00mm). The strong antibacterial activities displayed by the ethyl acetate extract may be connected to the presence of bioactive compounds revealed by the gas chromatography-mass spectrophotometer in table 1.

The strong antibacterial activities may be connected to the presence of Naphthalene derivatives having higher percent total of 18.907% and 7.167% in the extract, also, Benzene,1,2,4, 5-tetramethyl having % of total 9.819, 1H-indene,2, 3-dihydro-4-methyl having % of total 8.267, Benzene,1-ethyl-2, 3-dimethyl with % of total 6.923, Benzene,1-ethyl-2,4, 5-trimethyl, % 9f total 4.660, 1H-indene,1-ethylidene % of total 3.567, Benzaldehyde,4-(1-methyl ethyl)- % of total 2.857. Naphthalene compounds have been found to have antibacterial activities and many Naphthalene derivatives have been synthesized and found to have antibacterial properties.[6], have tested azo-2-naphthol and 2-napthol naphthalene derivatives against five representative human pathogenic microorganisms i.e. Staphylococcus faecalis, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and pseudomonas aeruginosa. Both the azo-2-napthol and 2-napthol were found equally effective against all the organisms tested. Also,[4], synthesized 2-Aryl-2,3-dihydronaphthol[2,1-b] furo [3,2-b]pyridine-4 (1H)-ones and found effective against human pathogenic gram positive and gram negative bacteria.

The presence of indene derivatives in the extract has proven that if further researched, these compounds if isolated using advanced separation techniques, the compounds can be used medicinally because of the various biological activities the indene compounds possess. Because, Indene and their derivatives have attracted continuing interest over the years because of their varied biological activities [3] and found useful in the pharmaceutical industry.

CONCLUSION

From the results of the antibacterial and the gas chromatography mass spectrometer, it has been established that there are presence of bioactive compounds in the extract that if isolated using advanced separation techniques and characterized using various spectroscopic techniques and tested, the compounds may be useful in producing antibiotics particularly the indene derivatives that are known for their various biological activities [2].

REFERENCES:

1. ADRIO, Jose L. and DEMAIN, Arnold L. Fungal biotechnology. International Microbiology, September 2003, vol. 6, no. 3, p. 191-199.
2. Barbera J, Rakitin OA, Ros MB, Torroba T. Angew Chem, Int Ed. 1998;37:296–299.
3. Huffman, J. W.; Padgett, L. W. Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenes. Curr. Med. Chem. 2005, 12, 1395-1411.
4. K. Nagaraja, G.Prakash, M. Kumaraswamy, V. Vaidya, and K. Mahadevanb, Arkivoc, (xv) ,142-152 (2006).
5. PFENNING, Ludwig H. and ABREU, Lucas M. Diversity of microfungi in tropical soils. In: MOREIRA, F.M.S; SIQUEIRA, J.O. and BRUSSARD, L. eds. Soil Biodiversity in Amazonian and Other Brazilian Ecosystems. Wallingford, Oxford shire,
6. UK, CABI Publishing, 2006, vol. 1, p. 184-205.
7. V. Mkpenie, G. Ebong, I. B. Obot, B. Abasiekong, E. Journal of chemistry, 5,431-434(2008).

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