International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Molecular characterization and evaluation of phytochemical constituents of endophytic fungi derived from *Mitracarpus scaber Zucc*. (Rubiaceae)

Ifeoma S. Asogwa [1, 2, *], Ikechukwu, A. Onah [3], Anthony C. Agbo [2], Emmanuel C. Ibezim [1] and Anthony A. Attama [1, 4]

[1] *Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.*
[2] *Department of Pharmaceutical Microbiology and Biotechnology, Bingham University, Karu, Abuja, Nigeria.*
[3] *Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.*
[4] *Institute for Drug-Herbal Medicine-Excipient Research and Development, University of Nigeria Nsukka 41001, Enugu state, Nigeria.*

International Journal of Science and Research Archive, 2024, 11(02), 267–278

Publication history: Received on 20 january 2024; revised on 03 March 2024; accepted on 05 March 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.11.2.0376

Abstract

In quest to ameliorate the issues of recurring incidences of multidrug-resistant organisms and over-exploration of plants, researchers have delved into researches involving endosymbiotic microorganisms, known as endophytes. These are microorganisms inhabiting the internal part of plants, which have been verified to possess great potentials of bioengineering novel products for therapeutic purposes. The aim of this study was to isolate and identify endophytic fungal species present in *Mitracarpus scaber*, molecularly characterize the pure isolates, and test for phytochemical compounds present. Freshly collected non-diseased leaves of *M. scaber* were subjected to a four-step surface sterilization. Thereafter, they were sliced into fragments of 1 cm-length, exposing the leaf blades and the midribs, and were aseptically inoculated on sterilized malt extract agar, containing chloramphenicol (500 mg/l), in Petri dishes. Hyphal tips of actively growing fungi from the plant material were harvested and further sub-cultured for purification and isolation. Segments were aseptically cut from the actively growing pure isolates, on malt extract agar and inoculated onto the fermentation medium (sterilized local rice), in 500 ml Erlenmeyer flasks, which were properly sealed with sterile cotton and kept at static condition at 25 ^{o}C for 21 days. Ethyl acetate was used to extract the metabolites from the end products of the fermentation processes. Four isolates obtained from the endophytic fungi (EDF), present in the leaf of *M. scaber*, were subjected to molecular identification. The DNA extraction was done using Zr fungal/bacterial DNA miniprep. The extracted DNA was amplified through PCR, and sequenced. The resultant sequences were compared with GenBank database, using Basic Local Alignment Search Tool. The result of phytochemical screening of the extracts revealed the presence of flavonoids, tannins and phenols, in large amounts; terpenoids, alkaloids and cardiac glycosides, in moderate amounts; steroids, hydrogen cyanide and saponins in very low quantities, while that of molecular characterization identified four organisms: *Penicillium sclerotiorum, Clasporium cladosporiodies, Cryptococcus nemorosus,* and *Phyllosticta capitalensis.*

Keywords: Endophytic fungi; Multidrug-resistance; *Mitrcarpus scaber*; Molecular characterization; Metabolites; Phytoconstituents.

1. Introduction

Nature is sufficiently equipped with varieties of plants in which virtually all their parts, including the leaves, stem, roots, fruits, seeds, barks as well as the flowers could be useful as both traditional and allopathic medicines (1). *Mitracarpus*

* Corresponding author: Ifeoma S Asogwa

scaber is one among these numerous plants that are of vast ethno medicinal use. It belongs to the plant family of Rubiaceae, which is abundant in the tropics and Orients. *M. scaber* grows on degraded soils in Africa and Asia (2), but it was also found in Latin America (3). In Togo and many tropical country of African *M. scaber* can be harvested from June to November. It is a yearly herbaceous plant with erect stems of about 53 cm high and branches that are about 8 cm long. The plant has lanceolate leaves that are simple, alternate, oblong and about 4.5 cm long with an upper scabrous surface. Its inflorescence consists of clusters of small whitish flowers arising above the leaf axils which turn brownish yellow as the plant matures. At maturity, this plant makes white flowers at the level of each armpit of the leaves. Traditionally, it is commonly used in West Africa in the treatment of diseases, such as, infectious dermatitis, eczema, ringworm, toothaches, amenorhoea, dyspepsin, hepatic diseases, scabies, leprosy, etc (4, 5, 6). In the southern part of Nigeria (Delta State), the freshly harvested leaves of M*. scaber* are macerated and mixed with palm kernel oil to be used for skin diseases and tinea infections, and also a good relief for itching and lice infestation (7). The young leaves are squeezed and rubbed on a fresh cut, wounds and ulcers. It has also been reported (8) that the plant can be used for infantile toothache, anti-parasitic and venereal infections. In Togolese traditional medicine, the plant was used to treat infected wounds and skin (9). Furthermore, phytochemical analysis conducted (10, 11) revealed the presence of tannins, saponins, and anthraquinones. However, on further investigation, alkaloids and cardiac glycosides were found to be present, also (11). Qualitative phytochemical analysis of *Mitracarpus scaber* leaf equally showed that there are tannins, flavonoids, alkaloids, saponins, glycosides, cardiac, glycosides, anthraquinone and steroids, present (12). On that same study, the quantitative analysis was carried out and the results obtained showed a reasonable amount of total flavonoids and saponins compounds - 4.32 g% for saponins and 4.51 g% for flavonoids respectively there is no report yet on molecular characterization of endophytic isolates isolated as well as, the phytochemicals present in the secondary metabolites derived from leaf part of *M. scaber*, thus this research aimed at evaluating the molecules and phytochemicals present in ethyl acetate metabolic extract of endophytic isolates from the leaf part of the *M. scaber.*

2. Materials and methods

The materials used for the study include: weighing balance, disposable syringe and gavage needle, endophytic fungi extract, dimethyl sulfoxide (DMSO) and ethyl acetate (Sigma Aldrich, U.K), Sodium hypochlorite, 70% alcohol, ethidium bromide (Bio-Rad Laboratories, USA), Bashing Bead, Buffer (Zymo Research, USA), sterile distilled water (Lion's Table Water, UNN) and normal saline (Juhel Pharmaceutical, Awka).Other reagents were of analytical grade and were used without further purification.

2.1. Sample collection and authentication

The plant of *Mitracarpus scaber* is predominantly found in Obukpa in Nsukka Local Government Area of Enugu State Nigeria. The leaves part of this healthy and fully matured plant (5 kg) were collected and duly identified by a taxonomist, Mr. Alfred Ozioko, of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. It was further authenticated, by a botanist in the Department of Plant Science and Biotechnology and was assigned a voucher number UNH NO 12a and deposited at the herbarium collection center of the Department.

2.2. Sterilization of sample materials

The whole processes of sterilization, cultivation, isolation and purification followed procedure described by Okezie *et al* (13) with some modifications. Firstly the samples were subjected to pre-treatment step which involved washing the leaves separately under the tap water followed by sterile distilled water. This helped to remove adhering soil particles at the surfaces of the leaves. Afterwards, the second step involved the surface sterilization of the plant material to eliminate surface epiphytes. To achieve this, the samples were put through four step surface sterilization which entailed soaking the freshly collected plant parts in slow running tap water for 15 min, after which they were further immersed in 70% ethanol for 3 min and washed twice with distilled water. In addition, the already washed samples were again immersed in sodium hypochlorite solution (4%) for 5 min and washed thoroughly, thrice in distilled water and then rinsed in 70% ethanol for 3 min, before a final rinse in sterilized distilled water.

2.3. Cultivation and isolation of endophytes (fungi)

The washed samples were dried in the laminar flow cabinet on a sterile filter paper. To expose the tissues in the mid-ribs and the leave vein, a sterile knife was used to cut the samples to approximately 1 cm in length exposing the endophytes more. Having a total of 30 segments, three to six segments were planted per Petri dishes containing malt extract agar incorporated with chloramphenicol (500 mg/L) which were previously sterilized. The Petri dishes were properly sealed with parafilms, then incubated at 25 ^{o}C and were checked on alternate days. After 7 days, hyphal tips of the actively growing fungi from the plant material were inoculated onto a sterile malt extract agar (MEA) plates for sub culturing and were incubated for another 7 days, to enable the organism grow and mature fully to expected optimal

purity of the endophytes. This was repeated at an interval of two weeks to maintain purity, and the maximum growths of the fungi were also observed on MEA. For identification, the morphological characteristics were observed visually such as the colony color, elevation and texture (14). The process of optimization was followed for the production of metabolites, and the starting materials were taken from freshly sub cultured plates.

2.4. Purification of endophytic fungi isolates

The hyphal tips of a stock culture were sub-cultured by picking the hyphal tip, placing on a fresh MEA and incubating at 25 ^{o}C for 7 days. This was done to get rid of contaminates present in old culture. All transfers were done aseptically, to maintain pure isolates. Upon incubation, it was discovered that the mid-rid, gave two isolates as well as the leave blade making a total of four fungi, isolated from the culture.

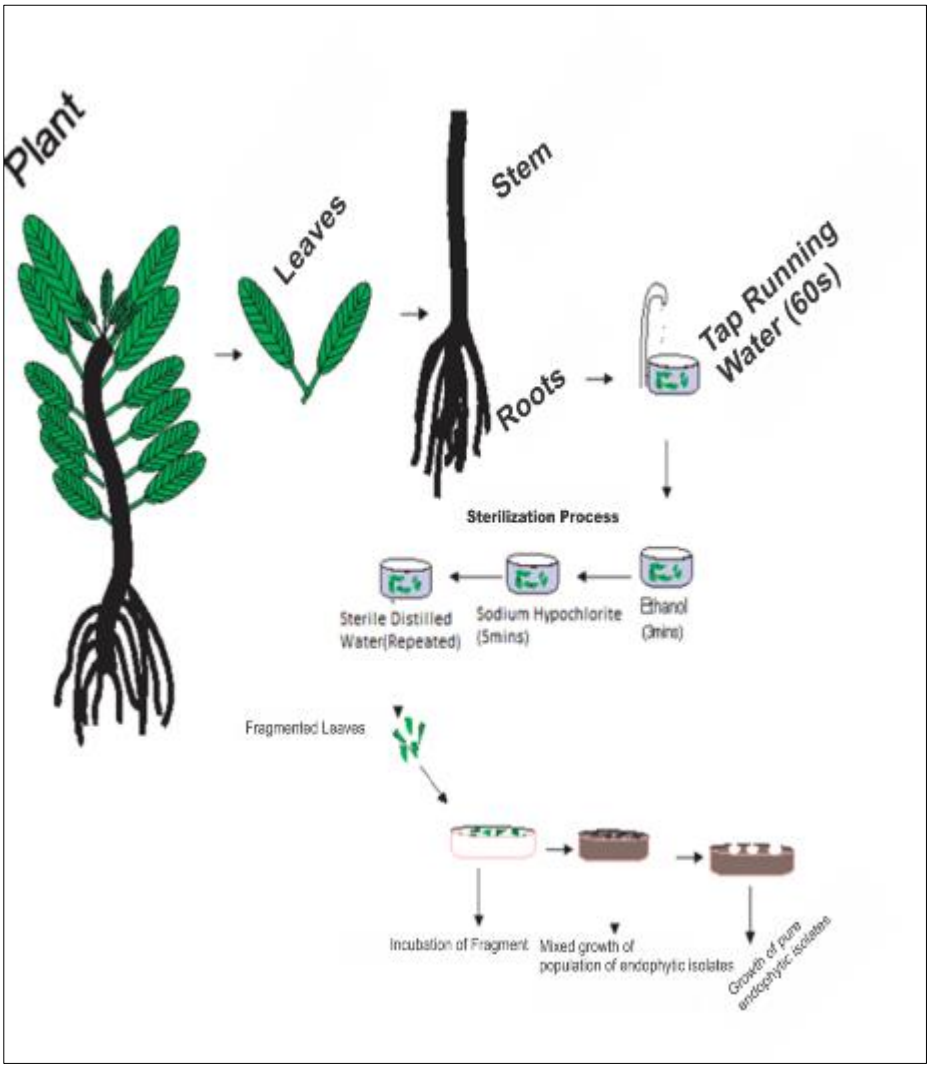

Figure 1 Step-by-step process of sterilization, cultivation and isolation of pure isolates of endophyic fungi

2.5. DNA extraction

The fungal DNA was extracted using QUICK-DNA™ fungal/bacterial mini prep kit (USA), according to the manufacturer's instruction.

Briefly, 50-100 mg (wet weight) of fungal cells that have been re-suspended in up to 200 µl of sterile water was added to a ZR Bashing Bead™ Lysis Tube. Again, 750 µl of Lysis Solution was added to the tube. The tube was secured in a bead beater fitted with a 2 ml tube holder assembly (Disruptor Genie™) and processed at maximum speed for 5 min. The ZR Bashing Bead™ Lysis Tube was centrifuged in a micro centrifuge at 10,000 x *g* for 1 min. Further, up to 400 µl of the supernatant was transferred to a Zymo-Spin™ IV Spin Filter (orange top) in a Collection Tube and centrifuged at 7,000 rpm (~7,000 x *g*) for 1 min. The base of the Zymo-Spin IV™ Spin Filter was snapped off prior to use. Again, 1,200 µl of

Fungal DNA Binding Buffer was added to the filtrate in the Collection Tube above, after which, 800 µl of the mixture from collection tube was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 x *g* for 1 min. The flow through from the Collection Tube was discarded and the addition of 800 µl of the mixture was repeated. A volume of 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at 10,000 x *g* for 1 min.

In addition, 500 µl Fungal DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at 10,000 x *g* for 1 min. The Zymo-Spin™ IIC Column was added to a clean 1.5 ml micro centrifuge tube and 100 µl (25 µl minimum) DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 x *g* for 30 seconds to elute the DNA. The obtained ultra-pure DNA prepared was now ready for the next step.

2.6. Amplification

The ribosomal RNA (rRNA) gene of fungi was amplified according to the method of Marksun (15). A pair of primer NS1: 5'GTAGTCATATGCTTGTCTC3'and reverse primer NS4: 5'CTTCCGTCAATTCCTTTAAG3'were used to amplify the highly specific nucleic acid for endophytic fungi, targeting the gene encoding for 18S rRNA. PCR was carried out in a programmable thermal controller (MJ Mini Biorad). PCR reaction mixture (25 µl) consisted of 10 µl template DNA, 12.5 µl PCR Master Mix (PCR Buffer, 4 mM $MgCl_2$, 0, 4 mM of each dNTP, 0,05 U/µl Taq polymerase; Fermentas), 1 µM of each primer (NS1 and NS4) and double distilled water to make up the volume. The PCR cycling conditions were: denaturation at 94 ^{o}C for 1 min, primer annealing at 45 ^{o}C for 2 min, and extension at 72 ^{o}C for 2 min for a total of 30 cycles and a final extension step at 72 °C for 10 min. PCR amplicons were electrophoresed in a 2 % agarose gel.

2.6.1. Electrophoresis of Polymerase Chain Reaction (PCR) products

About 2 g of agarose (Laboratories Conda, Madrid, Spain) was weighed into a 250 ml flask and a 100 ml of 1× Tris Acetate EDTA (TAE) buffer added, giving a 2% gel. The mixture was heated in microwave oven until it was completely dissolved (solution clear and transparent). The suspension was allowed to cool to about 60°C. A 2 µl volume of ethidium bromide was added and gently swirled to avoid air bubbles. It was then poured into a clean-levelled casting tray with combs already inserted and allowed to solidify at room temperature for at least 30 min. The combs were carefully removed to create wells and still in the casting tray, the gel was introduced into the electrophoresis chamber filled with sufficient amount of running buffer (1×TAE) just slightly above the gel surface. With a micropipette adjusted to 7 µl, the PCR product was gently mixed with 1 µl of loading buffer (Bromophenol blue and xylene cyanol) and gently loaded into sample wells. A 100 bp DNA ladder (NEB) was also loaded in the first wells at the same time to run simultaneously. Once the lid was closed, the power leads were connected to the electrophoresis apparatus at 100 volts and allowed to run for 10 to 30 min. The gel was visualized under UV trans illuminator and photographs taken with a digital camera (16).

2.6.2. Sequencing

The sequencing was done using the Big Dye Terminator v3.1 Cycle Sequencing Kit according to manufacturer's protocol. The reaction mixture for the cycle sequencing contained 1 µl forward primer NS1: 5'GTAGTCATATGCTTGTCTC3' and 1 µl reverse primer NS4: 5'CTTCCGTCAATTCCTTTAAG3' (Inqaba-Biotech, South Africa), 4 µl ready reaction mix (2.5X), 2 µl Big Dye Terminator Sequencing Buffer (5X), 2 µl PCR amplicon and 10 µl of Ionized water making a total reaction volume of 20 µl. The reaction mix was amplified using Gene Amp PCR System 9700 commenced with a pre-denaturation at 96 °C for 1 min, followed by 25 cycles of denaturation at 96 °C for 10 sec, annealing at 50 °C for 5 sec and extension at 60 °C for 4 min. The nucleotide sequences were read after the PCR cycling sequencing.

2.6.3. Sequence data analysis

The sequencing results generated were uploaded in http://blast.ncbi.nlm.nih.gov/Blast.cgi which is an intuitive interface for analyzing DNA sequences. The assembled sequences were end-trimmed, paired in their respective forward and reverse sequences to build consensus sequences. Sequence alignment and percentage similarity searches were compared with Genbank database using NCBI web-based site, BLAST.

2.6.4. Evolutionary relationships of taxa (phylogenetic study)

The evolutionary history was inferred using the Neighbor-Joining method (17). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 6 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair

(pairwise deletion option). There was a total of 832 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

2.7. Fermentation processes of the isolates

From previous research, it has been documented that fungi endophyte thrives more in local rice medium than when it another substrate (18). Hence, Local rice was used as the fermentation medium. The local rice (100 g) was weighed into a sterile conical flask, 200 mL of sterile water added onto it, and the content sterilized appropriately at 121 ^{o}C for 30 min, and then allowed to cool properly. Thereafter, segments were aseptically cut from the actively growing pure isolates on MEA and inoculated into the already sterilized local rice fermentation medium contained in a 500-mL Erlenmeyer flask. This was properly sealed with sterile cotton and kept on the shelf. The fermentation process was allowed for 21 days at 30 ^{o}C under static conditions.

2.8. Extraction of Fungal metabolites

The fermentation process was terminated by the addition of the extraction solvent (ethyl acetate) and each of the fermented medium in the sterile Erlenmeyer flasks was made homogeneous. Here, fungal biomass including the medium were cut into small lumps using a sterile glass rod and the mixture was homogenized with 500 mL of ethyl acetate in one liter Erlenmeyer flasks, and shook vigorously intermittently for 2 days and then filtered using Whatman filter paper (size: 188 mm). The filtrate was concentrated at 50 ^{o}C under reduced pressure using a rotary evaporator. The concentrated extract was further left to evaporate to dryness in a desiccator containing sodium hydroxide. The corresponding extracts were weighed and their respective percentage yields recorded in milligram, before proceeding for the phytochemical screening, using standard methods.

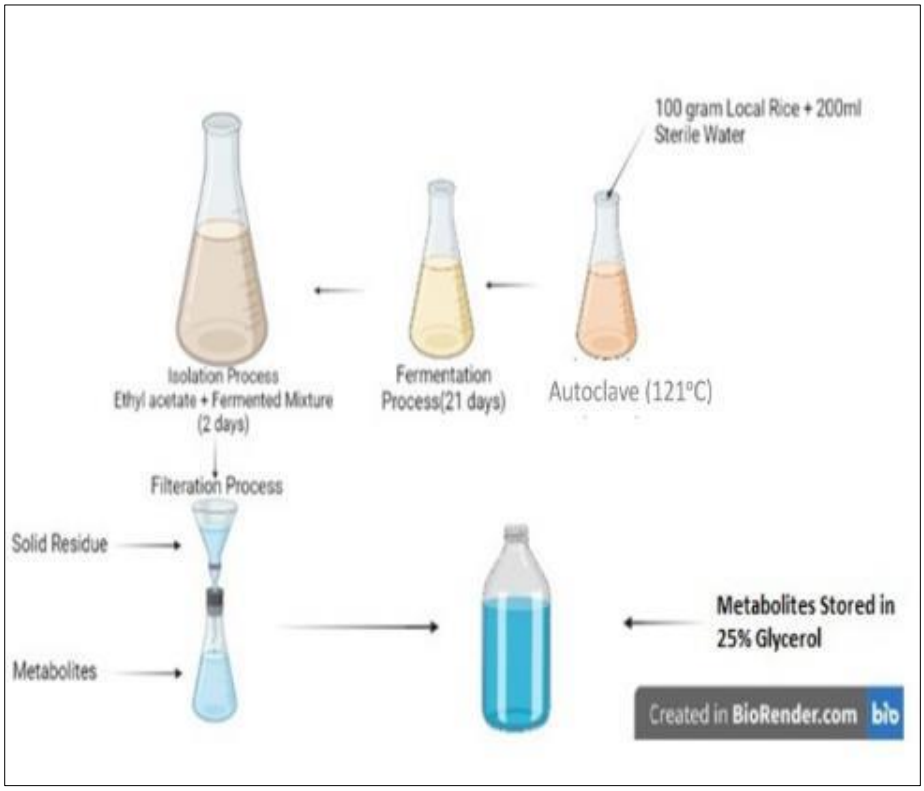

Figure 2 A summary of step-by-step procedure of fermentation and extraction of endophytic metabolites

3. Results and discussion

3.1. Molecular characterization of the pure isolates.

Figures 3 shows images of extracted high molecular weight DNA from the endophytic isolates. These are indicated in bands with their corresponding numbers 1-4 while M indicates the DNA ladder marker. Figure 3 depicts the electrophoregram of the overall PCR results. This shows the amplification of ITS region of the fungal isolates at about

550bp – 650bp with their distinctive bands of the different fungal isolates. The DNA of all the four fungal isolates were shown in distinctive bands with their unique codes (CC4a, CC4aa, CCaW, CC10B). The approximate molecular size of the amplicon generated from the PCR was 1350 base pair which was specific for the fungal isolates. The molecular weight marker used was 1kb plus DNA ladder marker. Hence the appearance of the various fungal DNA bands above 550bp is an indication of successful molecular characterization of the endophytic fungal isolates.

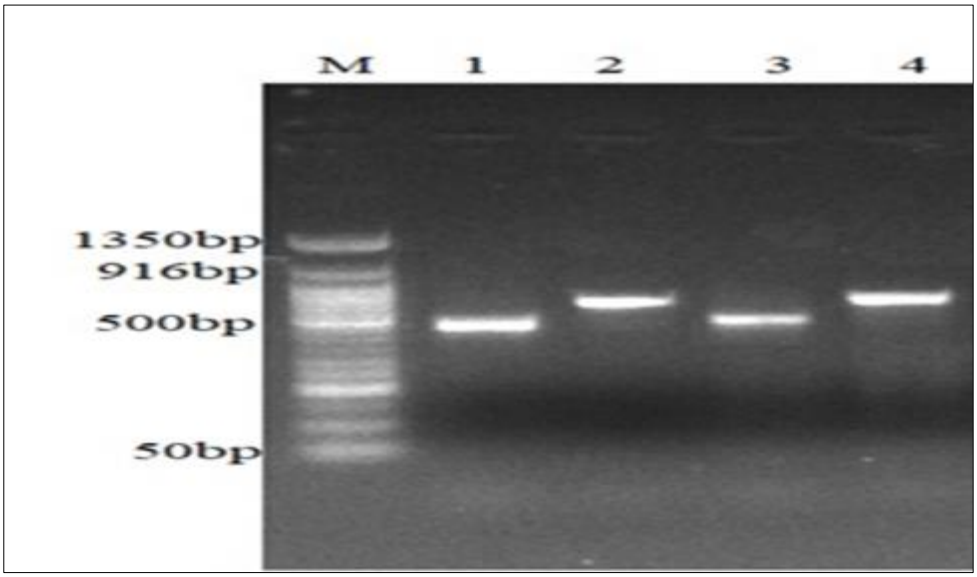

Figure 3 Images of Amplified ITS region of the isolates PCR

3.2. Sequencing and matching of the identified isolates with the GenBank data

The result of the DNA sequence analysis following blasting with genbank data base such as http://blast.ncbi.nlm.nih.gov/ Blast.cgi is presented in Table 1 followed by Phylogenetic tree (Fig 4) below. Several researchers (19-21) here reported molecular characterization techniques as the most accurate way of identifying the isolates of microorganisms. This technique was used by Deepthi (22) to authenticate and identify eight endophytic isolates from *Elaeocarpus sphaericus*. It was the opinion of Pecoraroa's (23) that the method of molecular characterization was the most acceptable tool of isolation and identification of endophytes. In the present study, sequenced amplicon from the endophytic DNA extract was matched with Genbank database to identify four isolates, which are CC4a, CC4aa, CCaW and CC10B, after which they were matched with their percentage similarities with four known organisms.

3.2.1. Gene sequence percentage similarity of CC4a

CC4a has 100.00% pairwise similarity with *Penicilliums clerotiorum* strain YP-H82, which has NCBI accession number as KY437712. The e value is 0 isolate sequence is as below:

ATTGGCGGGGGGGTACGGCATCGTCGCTTATTGAATGCTCCTCCTTGTAGTGGGGTCGGCAAGCAGCCGGCCGGGCCTACAGAG
CGGGTGACAAAGCCCCATACGCTCGAGGATCGGACGGTGCCGCCACTGCCTTTCGGGCCTGTCCCCCGGGGGGGACAAGACCCAA
CACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAG
ACTCGATGATTCACTGAATTCTGCAATTCACATTAATTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCC
GTTGTTGAAAGTTTTAACTAATTTTGCTTAATCGCTCAGACTGCATTCTTCAGACAGCGTTCAAGATTGTCTCCGGCAGGCGCG
GGCCCGGGGGCAGAAGCCCCCCGGCGGCCGTGAGGCGGGCCTGCCGAAGCAACAAGGTACAATAAACACGGGTGGGAGGTTGGA
CCCAGAGGGCCCTCACTCAGTAATGATCCTTCCGCAGGTCACCCCTACGGAAACTGAGTGAGGGCCCTCTGGGTCAACCTCCCCC
GGGTTTTGGTACCTGTGGTTCGGGGGGCCGCTCTAGCCGGGGGGGGTTTT

3.2.2. Gene sequence percentage similarity of CCaa

CC4aa has 80.00% pairwise similarity with *Cryptococcus nemorosus* isolate Y16H8a15, which has NCBI accession number as KP714272.

The BLAST hit retuned different fungi, but we picked *Cryptococcus nemorosus* and Uncultured *Sordariomycetes* clone 25A_B2_F4, because of their pairwise identity and frequency of occurrence. The e value is 0 and isolate sequence is as below:

GAAAATAGCGCCATAGGATCGTCGCTTATTGATATGCTCCTCCGCTTATTCACCTGCGGAACCGTCCGCTTATTGACATGCTCC
TCCGCTTATTGATATGCTCCTCCGCAGGTTCATTTACGGAATCCTCCGCTTATTGATATGCTCCTCCGCTTATTGATATGCGCAA
TCGTCGGTTTATTGACATGATCCTCCGCTTATTGACATACTCAACCGCATGTTCATTG

3.2.3. Gene sequence percentage similarity of CCAW

CCAW has 80.00% pairwise similarity with *Cryptococcus nemorosus* isolate Y16H8a15 which has NCBI accession number as KP714272.

Please note that this isolates has identity with more than one fungi. The BLAST hit retuned different fungi but we picked *Cryptococcus nemorosus* and Uncultured *Sordariomycetes* clone 25A_B2_F4 because of their pairwise identity and frequency of occurrence. The e value is 0 and isolate sequence is as below:

Also note that this isolate has similar identity with CC4aa

CAAAAAACGCATAGGATCTCGCTTATTGATATGCTCCTCCGCAGTATTGACATGCGGAACCGTCCGCTTATTGACATGATCCTC
CGCTTATTGATATGCTCCTCCGCAGGTTCATTTACGGAATCCTCCGCTTATTGACATGCTCCTCCGCAGATTCATATACGCAACC
GTCGGTTTATTGACATGATCCTCCGCTTATTGACATGCTCCTCCGCCGGTTGATAGA

3.2.4. Gene sequence percentage similarity of CC10B

CC10B has 98.50% pairwise similarity with *Phyllosticta capitalensis* strain Ae-26, which has NCBI accession number as KU663502. The e value is 0 and isolate sequence is as below:

TTTGAATATGCTCCTCCGCTTATTCACCTGCGGGACCGGCGGCTGACGGACAGCACTCCCAGCGAGAGTTTTACTACTACGCTCG
AGGCTAGGACGCCGTCGCCGAGGTCTTCAAGGCACGTCCGGCAGCGGACGTTGCCCAATACCAAGCAGAGCTTGAGGGTTGAAA
TGACGCTCGAACAGGCATGCCCTCCGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAAT
TCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAATCAATTAAAT
GATATATCAGGACTTCACAAAATGAATTCTTGAGTTTTGTATACTGGCGGGCACTTAGCCGGGCGTCCTGGCCAGTTAAGGCTG
GGGGCGCCGGCCGCCTGGGTCGGAACCAGGTCGACCCGCCAAAGCAACATAGTGAGTACACAAGGGTGAGAAGGTCATTTCGGC
GTTGTAGCGCCTACTCTGGAACCTTTCAATAGAAGTTATTACATTTCAGTAATGATCCTTCCGCAGGC

Table 1 Identified fungal species from leaf parts of *Mitracarpus scaber* with their pairwise percentage similarities

S/no	Isolate code	Probable organism(s)	Gene sequence % similarities	Ncbi accession number
1	CC4A	*Penicillium sclerotiorum*	100.00	KY437712
2	CC4AA	*Clasporium cladosporiodies*	80.00	KP714272
3	CCAW	*Cryptococcus nemorosus*	80.00	KP714272
4	CC10B	*Phyllosticta capitalensis*	98.50	KU663502

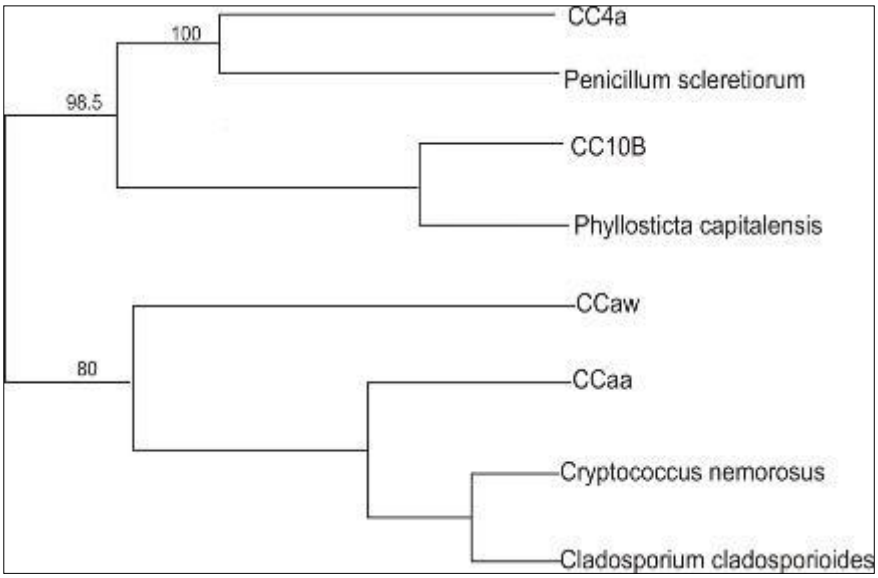

Figure 4 Evolutionary relationship of the recovered fungal isolates and their closest Gen Bank relative

3.3. Phytochemical screening

Tables 2 and fig. 5 show the qualitative and quantitative screening outcomes, respectively. Table 4 showed the diversities of bioactive compounds present in secondary metabolites produced by isolates from *Mitracarpus scaber* whereas quantitative assay (figure 14) gave an idea of the relative amount of those metabolites from the test sample.

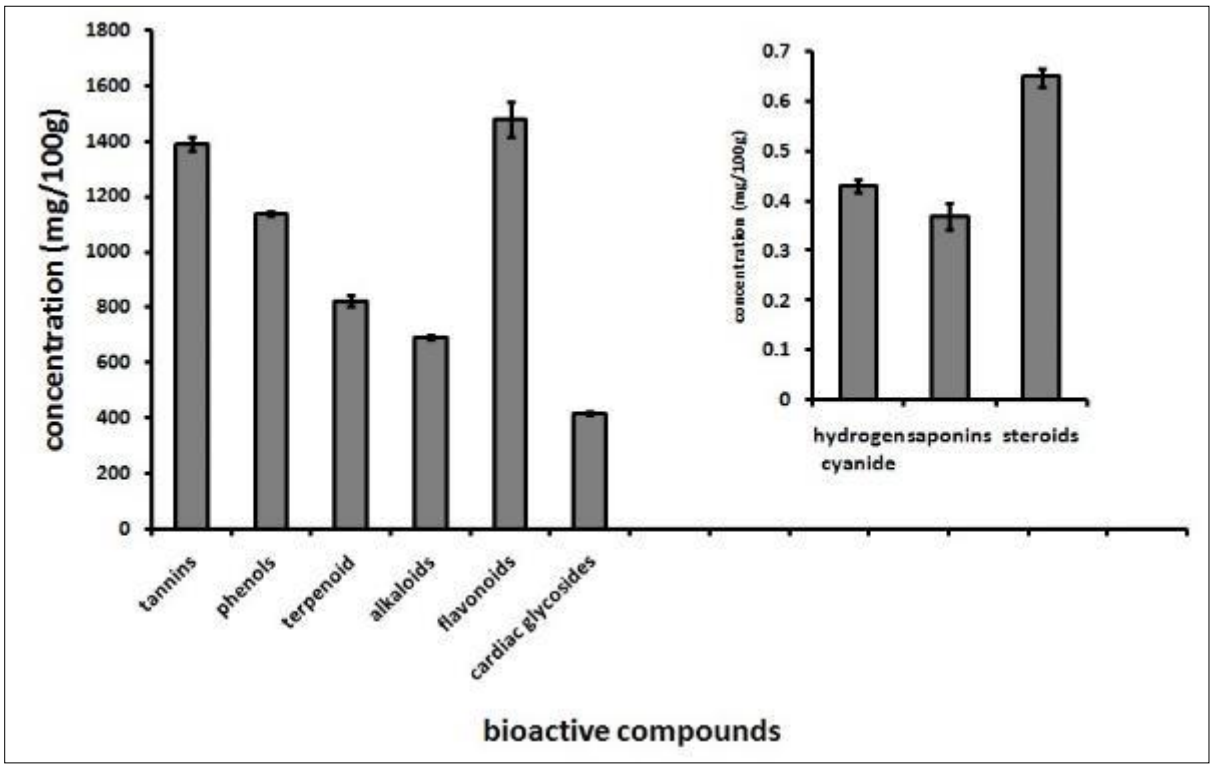

Figure 5 Quantitative Phytochemical screening

Table 2 Qualitative Phytochemical screening

Bioactive compounds	Present (+) / Absent (-)
Tannins	+ +++
Saponins	++
Steroids	+
Alkaloids	+++
Flavonoids	++++
Phenol	++++
Hydrogen cyanide	+
Cardiac glycosides	++
Terpenoid	+++

The results above show that the endophytic fungal isolates in symbiosis with *Mitracarpus scaber* leaves were able to produce bioactive compounds such as flavonoids, tannins, phenols, terpenoids, alkaloids, cardiac glycosides, steroids, hydrogen cyanides and saponins. This is a pointer to the possible pharmacological potentials of this extract. It has been deduced from literature that phenolic compounds have good antioxidant properties; especially the flavoniods which occured in highest concentration. Other than being anti-oxidants, flavoniods have anticancer, antimalarial and antifungal activities. (24-27). Similarly, other phenolic compounds such as tannins have been observed also to be the principal phytoconstituents produced by plants with good radical scavenging activities (27, 28). Alkaloids and terpenoids which occurred in moderate amount have their unique biological activities, as well. Alkaloids, biologically, exhibit some pharmacological actions as anticancer, antiviral, antimalarial as well as antibacterial and antifungal agents (28-31). Terpenoids, on the other hand, are utilized in drug, food, perfumery industries and possess insecticidal, anti-inflammatory cytotoxic, analgesic and anthelminthic activities (32-35). Furthermore, saponins and cardiac glycosides are present in smaller amounts compared to the ones mentioned above but had a greater preponderance than steroids, hydrogen cyanide which are the least in occurrence. In addition, comparing the present work with previous work done with *Mitracarpus scaber* leaf extracts, both researches have shown the presence of similar secondary metabolites with variation in percentage concentrations of some compounds such as saponins. These works on the qualitative phytochemical analysis of *M. scaber* leaf, revealed the presence of tannins, flavonoids, alkaloids, saponins, glycosides, cardiac glycosides, anthraquinone and Steroids (10-12). In that same study, the quantitative analysis was carried out and the results obtained showed high contents of total saponins and flavonoids compounds. This validates the work by Strobel and Daisy (36), which showed that endophytes residing in the tissues of plants have the potentials to produce bioactive compounds similar to or even the same as their host plants. This is thought to occur due to genetic transfers from host plants to endophytic microbes. This genetic transfer occurs as host plants and endophytes have the same pathway to synthesize secondary metabolites through certain enzymes in which endophytes and their hosts experience direct contact.

4. Conclusion

The molecular characterization identified four endophytic fungi, from pure isolates, in association with the leaf part of *Mitracarpus scaber.* These isolates show high level of similarities with some known microorganisms after blasting and upon fermentation gave rise to metabolic extracts. Phytochemical evaluation of these metabolic extracts of the endophytic fungi isolated from *Mitracarpus scaber* presented fascinating bioactive moieties such as flavonoids, tannins and phenol and more other, which when harnessed could serve as key sources of pharmaceutical active ingredient in formulation of drugs that could treat some of the emerging infectious diseases that are of much concern in healthcare system.

Compliance with ethical standards

Acknowledgments

The authors would like to say special thanks of gratitude to the management of Bingham University E-Library for access to materials and information provided for this Research work.

Disclosure of conflict of interest

All the authors of this article have no conflict of interest to declare.

Funding Statement:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for- profit sectors.

Consent for Publication

All the authors gave their consents.

Statement of ethical approval

This article does not contain any studies with human participants or animal performed by any of the authors.

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