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Research Article

Photochemical Analysis of the Extracts of Secondary Metabolites from Leaves of *Irvingia Gabonensis* (Bush Mango)

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Abstract: Microorganisms are important sources of bioactive natural products with enormous potential for the discovery of new molecules for drug discovery, industrial use and agricultural applications. In this study, photochemical analysis of the extracts of secondary metabolites produced by endophytic fungi from leaves of *Irvingia gabonensis* was carried out using recommended standards. Results revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids and glycosides. Extracts from all five cultures of the endophytic fungi gave a wide variety of phytochemicals, except saponin. From the results, the different natural products fractions are a potential in the provision of the basis for the synthesis of novel therapeutics that would aid in the fighting of life threatening diseases once resistance builds up. Therefore, the search for the secondary metabolites that could enable the synthesis of drugs should be encouraged. The potential value of investigating metabolite production by endophytic fungi from leaves of *Irvingia gabonesis* have been demonstrated from the result to be a source promising antimicrobial compounds against some human pathogenic microbes.
Keywords: Toxicology, pollution, diseases, endophytes, antimicrobial activity, plants, health.

INTRODUCTION

Environmental degradation, loss of biodiversity and spoilage of land and water also added to problems facing humanity. Endophytes, microorganism that reside in the tissue of living plants, are relatively understudied and potential source of novel natural products for exploitation in medicine, agriculture and industries [1]. The resistance of pathogenic microorganisms to drugs and antibiotics has become a major challenge in the health sector leading to reduction in drug effectiveness and economic wastage. This challenge has once again stirred up a need in scientific research and the discovery of new and more effective antimicrobial metabolites becoming a major research interest. According to some researchers [2], endophytic bacteria isolated from leaves of *Ocimum sanctum* screened in dual culture was found to be active against various phytopathogenic fungi such as; *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani, Alternaria solani, and Colletotrichum lindemuthianum.* The endophytic fungal extract from leaf of *Ocimum basilicum* was found to have antimicrobial [3] activity against *Bacillus cereus* and *Staphylococcus aureus*. Also, Kusari and Spiteller, [4] stated that the production of Hypericin ($C_{30}H_{16}O_8$), a naphthodianthrone derivative, and emodin ($C_{15}H_{10}O_5$) believed to be the main precursor of hypericin, by the endophytic fungus isolated from an Indian medicinal plant, exhibits an antimicrobial activity against several bacteria and fungi including *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enteric and Escherichia coli, and fungal organisms Aspergillus niger and Candida albicans*. Strobel [5] summarizes endophytes as chemical synthesizer inside plants which produce bioactive substances with low toxicity toward higher organisms. These microorganisms may produce a large number of novel natural products for medical, agriculture and industrial uses such as antibiotics, anticancer reagents, biological control agents and other useful bioactive compounds [6, 7]. An endophyte is and endosymbiont, often a bacterium or fungus that lives within a plant for at least a part of its life without causing apparent disease [8]. The interaction of endophytic fungi with host plants results in a compromise between mutualism and antagonism to create a harmonious symbiotic system. Plants can limit the growth of endophytes, and thus endophytes may use a wide range of mechanisms to survive. Endophytes not only decompose some plant metabolites with ectoenzymes in order to retrieve essential nutrients and energy to survive, but produce beneficial compounds and support or promote the

growth of host plants to achieve a balanced living environment [9]. The bioactive natural products from endophytes are promising resources for medicine, agriculture and industry [10]. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, synthesized *via* various metabolic pathways e.g. polyketide, isoprenoid, amino acid derivation [7]. The compounds are classified into several chemical structural groups such as alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavonoids [11]. The rational selection of host plant is crucial to increase the chances of isolation of novel microorganisms which may produce new bioactive compounds [9]. Therefore, it is necessary to understand the methods and rationale used to provide the best opportunities to isolate novel endophytic microorganisms as well as ones making novel bioactive products. Since the number of plant species in the world is so great, creative and imaginative strategies must be used to quickly narrow the search for endophytes displaying bioactivity [12]. Therefore, this research is aimed at the photochemical analysis of the extracted secondary metabolite produced by the endophytic fungi isolated from leaves of *Irvingia gabonensis*.

MATERIALS AND METHODS

The plants (*Irvingia gabonensis)* were collected with tweezers, hand gloves, plastic bags and containers from a field outing and survey plan. The plant collection was followed by identification by a taxonomist in the department of botany University of Nigeria Nsukka, Enugu state, Nigeria. The convenient sampling technique used was simple random selection method. This was used in order to give the different plant leaves an opportunity to represent therapeutic values of the plant vegetation within the geographical locations of the study area. In preparation for nutrient broth, the laboratory bench was cleaned with cotton wool soaked in ethanol, this is done to avoid contamination and enhance aseptic conditions. 0.637g of the potato nutrient broth powder was weighed into a 200ml conical flask and then dissolved with 50ml of distilled water. The media was homogenized by agitating and then dispensed into different test tubes, sterilized by autoclaving at 121 0C for 15 minutes. Also, sabouraud dextrose agar was prepared by the laboratory bench being cleaned with cotton wool soaked in ethanol, this is done to avoid contamination and enhance aseptic conditions. 33.35g of sabouraud dextrose agar powder was weighed into a 500ml conical flask and then dissolved with 500ml of distilled. The media was homogenized by agitating and then sterilized by autoclaving at 121 0C for 15 minutes, after which it was aseptically poured into sterile Petri dishes and allowed to gel. Finally, Mueller hinton agar was prepared in the laboratory by first cleaning the bench with cotton wool soaked in ethanol, this is done to avoid contamination and enhance aseptic conditions. 38g of Mueller hinton agar powder was weighed into a 1000ml conical flask and then dissolved with 1000ml of distilled. The media was homogenized by agitating and then sterilized by autoclaving at 121 0C for 15 minutes, after which it was aseptically poured into sterile Petri dishes and allowed to gel. The following were also carried out; isolation of endophytic fungi and fermentation and extraction of secondary metabolites. Antimicrobial assay for the test organisms was carried out in that the test organisms used were obtained from the microbiology laboratory section and mycology laboratory section of the University of Nigeria Teaching Hospital Ituku- ozala Enugu State, Nigeria. The test organism used were six and they include two gram positive bacteria (Staphylococcus *aureus* and *Bacillus subtilis*), two gram negative bacteria (*Pseudomonas aeruginosa and Escherichia coli)* and two fungi *Aspergillus and Penicillium*. From the pure culture in a bijou bottle, a loopful of each test organism was transferred into different test tubes containing an already sterilized nutrient broth. After the inoculation, the test tubes were put in an incubator at a temperature of 35-37°C for the bacteria and 25-27°C for the fungi. The bacteria culture was incubated for eighteen (18) hours while the fungi was incubated for about twenty-four (24) hours. The agar well diffusion method described by Subbulakshmi, *et al.* [13] with modification was used to evaluate the antimicrobial and antifungal activity against the test microorganisms. The test organisms were spread aseptically using a cotton swab on the surface of the already prepared Mueller Hinton agar for the bacteria and the fungi was aseptically inoculated on the already prepared Sabouraud dextrose agar. All culture plates were allowed to dry for about five (5) minutes and four wells were made on the agar using a 6mm sterile cork borer. Two wells were filled with 200µl of 100mg/ml concentration of the extract, other well was filled with 200µl of 50mg/ml concentration of the positive control and the last well was filled with 200µl of the negative control. The plates were kept on the work bench to allow the agents diffuse into the agar and incubated accordingly. Ciprofloxacin and fluconazole were used as the positive controls for the antibacterial and antifungal evaluation respectively while distilled water was used as the negative control.The Mueller Hinton agar plates were incubated at 37°C for twenty-four (24) hours and the Sabouraud dextrose agar plates were incubated at 27°C for two (2) days. The antimicrobial activities were evaluated by measuring the diameter of the inhibition zones in millimeters and the readings were recorded. The experiment was replicated twice and an average of two independent reading for each microorganism was used. The phytochemical analysis (qualitative test) of the crude metabolite was carried out on all the extracted secondary metabolites. The test for Alkaloids was done by taking I ml of 1% hydrochloric acid which was added to 1g of the extract in a test tube. The mixture was heated for 20 minutes in a water bath and shook continuously while heating. It was allowed to cool and then filtered. 0.5ml of Meyer's reagent was added to 1ml of the filterate from above. It was

observed that a reddish-brown colour change indicates the presence of alkaloid. Also, test for Flavonoid was carried out by 1ml of the extract added in a test tube containing 10ml of distilled water and the mixture was shaken. After shaking, 1ml of 10% NaOH was also added. It was observed that a yellow colouration indicates the presence of flavonoid. For the test for Saponin, 3ml of the extract was put into a test tube. Then, 2ml of distilled water was added and shaken. Observation revealed a persistent frothing movement indicates the presence of saponin. The test for Tannin followed the addition of 2ml of the sample which was put into a test tube, boiled for 2 minutes and allowed to cool. Later, 3 drops of 10% ferric chloride solution was added to it. The occurrence of a bluish-green colouration or green precipitate indicated the presence of tannin. The test for Steroids involved the addition of 2ml of the extract was put in a test tube and 5 drops of conc. H_2SO_4 was also added. The appearance of a red coloration indicated the presence of steroids. Finally, the test for Glycosides was carried out by the addition of 1ml of the extract added into a test tube and 10ml of 50% H_2SO_4 was added to the solution. The test tube was heated for 15 minutes and allowed to cool. 10ml of Fehling's solution was added and the test tube was heated again. It was observed that a brick red precipitate indicates the presence of glycosides.

RESULTS

The weight of the secondary metabolites produced and extracted from the endophytic fungi after the 21 days of fermentation is shown in table 1. From the table, it can be observed that the endophytic fungi in extract EDF4 produced more secondary metabolites than others while the endophytic fungi in extract EDF3 produced the least amount of secondary metabolite as shown below (Table 1).

Table 1: Weight of Secondary Metabolite extracted

Endophytic fungal isolates	Initial weights of flasks (gram)	Final weight of flasks (gram)	Weight of secondary metabolite (gram)
EDF1	157.260	158.673	1.413
EDF2	191.240	192.500	1.260
EDF3	166.101	167.331	1.230
EDF4	162.910	164.850	1.940
EDF5	161.162	162.682	1.520

Key: EDF= Endophytic fungi

From Table 2, the Phytochemical analysis (qualitative test) of ethyl acetate solvent extract revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids and glycosides. Extracts from all five cultures of the endophytic fungi gave a wide variety of phytochemicals. Extract two showed the presence of all the phytochemicals aforementioned. Other extracts showed the presence of all phytochemicals except saponin.

Table 2: Qualitative phytochemical Test of the Crude Ethyl Acetate Extracts

Extracts	Alkaloids	Flavonoids	Saponins	Tannins	Steroids	Glycosides
1	+	+	-	+	+	+
2	+	+	+	+	+	+
3	+	+	-	+	+	+
4	+	+	-	+	+	+
5	+	+	-	+	+	+

Keys :+ = Present, **-** = Absent

DISCUSSION

Irvingia gabonensis is a species of African trees in the genus *Irvingia*. Recently, the plant has been shown to possess medicinal properties including antimicrobial effects against *Escherichia coli* and *Staphylococcus aureus* [14]. From this study, it was discovered that these five endophytic fungal isolates exhibited at least five of the test microorganisms used including a filamentous fungi *Penicillium chrysogenum* which is interesting because according to a research by Ananda *et al.*, [15] they reported the all the six endophytic fungal isolates from Tulsi inhibited all the test organisms except *Penicillium chrysogenum*. The preliminary phytochemical analysis (qualitative test) carried out on all endophytic fungal crude extracts showed the presence of alkaloids, flavonoids, saponins, tannins, steroids and glycosides. This result is in concurrence to the reports of various researchers that endophytes has shown the presence of different phytochemicals, saponins [16] and cardiac glycosides [17]. The presence of phytochemicals in endophytes is an indicator that they can be potential source of precursors in [18, 19] the development of synthetic drugs. According to Huang *et al.*, [20] phytochemical analysis is carried out in plant species but only few reports are available in endophytes. The present study leads to the need of further in depth studies on these isolated bioactive endophytic fungal isolates. Many are able to produce quite a good amount of antimicrobial compounds tested in preliminary test. Furthermore, the

best proved active isolates should be identified using available methods to place these fungi in the fungal kingdom. It is recommended that the secondary metabolites extracted from the endophytic fungi should be tested for further pharmacological activities like anticancer, antioxidant, antidiabetic, enzyme activity etc.

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