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Research Article

ISOLATION OF TAXOL PRODUCING *FUSICOCCUM* SPECIES FROM CURRY LEAF AND ITS RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS

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ABSTRACT

Objective: The potential compound of taxol has been used treatment of cancer in the medical field. This study is focused on the extraction of taxol from *Fusicoccum* sp.

Methods: The pathogenic strain was grown in MID medium for the taxol production and extracted from the *Fusicoccum* culture filtrate and processed to the ultraviolet (UV)-spectroscopy analysis as well as infrared (IR) spectra analysis was done, and the sample was performed to the high-performance column chromatography and further processed to the random amplified polymorphic DNA (RAPD) analysis of specific primer is PGFO1, PGFO2 are performed.

Results: UV-visible spectral analysis showed maximum value for 273 nm. The result of IR-spectrum analysis to find out the functional groups present in sample and characteristic peak to conform the existences of OH group. The presence of taxol was confirmed using column chromatography. An analysis of taxol production was on the external standard of authentic taxol. The column chromatography process shows the amount of taxol production by *Fusicoccum* spp. 325 µg/L. RAPD analysis indicates the genetic relation among all isolates of fungus was analyzed by two random primers PGF01 and PGF02.

Conclusion: The present investigation revealed that the taxol production from biological source was a significant effect, and the presence of taxol amount is analysis in *Fusicoccum* sp. 325 µg/L. The RAPD analysis of genetic stability of *in vitro* grown fungus and genotypic with two different primers shows that five fungus species with corresponding primer efficiency was amplified.

Keywords: Taxol production, *Fusicoccum* sp., Ultraviolet-spectroscopy, Infrared, Spectrum analysis, Scanning electron microscopy.

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INTRODUCTION

Extraction of the bioactive natural compound to lead the effective rout of therapeutic purposes and synthesis of the useful drug in clinical area. The biological active properties are used to different type of disease like cancer as well as AIDS [1]. The taxol has a successful mode of action in pharmaceutical area and mainly used in the application of chemotherapy treatment of cancer [2]. The taxol compound chemical structure is classified as texane diterpenoids or taxoid. The taxol diterpene was isolated from the bark of pacific yew tree (*Taxus brevifolia*) has proved through anticancer activity [3]. A pathogenic fungus of *Fuciccocum* is a genus of anamorphic fungi in the family Batryosphaeriaceae produced taxol in culture [4-8]. The possibility that entophytes biosynthesis associated plant product was the first comprehended and published by [9].

Batryosphaeria is cosmopolitan and its species occur on a wide range of monocotyledonous and gymnosperm hosts endophytes fungi are well- known producers of secondary metabolite [10]. A literature survey covering more than 23,000 bioactive microbial secondary product are antifungal, antibacterial, antiviral, cytotoxin, and immunosuppressive agent shows that producing strain is mainly from the fungal kingdom species of Bateryoshareiaceue are the pathogen and entophytes associated with woody plant. The first report recorded on the isolation of taxol-producing fungi from *T. brevifolia* appeared in 1993 [11]. Mainly fungi and bacteria were commonly called endophytes which have been shown to have the nature potential for accumulation of various bio compound are may directly be used as the therapeutical agents [12]. A secondary metabolite is generally produced following active growth are many have unusual chemical structure some metabolites were found in a range of relate fungi. The random amplified polymorphic DNA (RAPD) markers were used to examine the degree of genetic variation within the putatively asexual basidiomycete's fungus. The filamentous fungi is identified the fungus cultures (e.g., shape, and size, of conidia, color,) and isolate 5 fungi were identified and using RAPD analysis of specific primer is PGF01, PGF02 and are performed. This work focused to continue the investigation of taxol production from biological sources and purification of taxol.

METHODS

Isolation of fungus

Infected curry leaf was collected and further process of technical method is carried out. The plant leaf was put into the surface sterilizing with help of the 70% ethanol and water mixing content. Infected curry leaf was incubating and the potato dextrose agar (PDA) medium of Petri plate. The fungus grows after 10 days was isolated. The pure culture maintained for further study. The fungus was grown with (PDA) medium in Petri plate for 10 days at room temperature. The mother culture was subcultured, and the fungus was identified based on the colony, morphological structure of conidia, and shape.

Extraction of taxol

Extraction of taxol was processed after incubating the broth culture for 3-4 weeks; the culture filtrate was passed through four-layered cheesecloth. To avoid fatty acid contamination of taxol, 0.25 g of $NaCO_3$ was added to the filtrate. The culture solution was extracted with equal volumes of di chloral methane was added. Anorganic phase was evaporated.

Column chromatography

The column of silica gel was loaded and crude sample dissolved in di chloromeathne. The sample was processed in a stepwise procedure with solvent in 70 ml of 100% dichloromethane 20:1 v/v dichloromethane, ethyl acetate 10:1 v/v dichloromethane, and ethyl acetate. The separation processes are important to effectively isolated taxoids from

their natural sources. Subsequently, taxoids were extracted and purified from anorganic solvent by column chromatography. An authentic taxol was set to evaporate to dryness. The residue was fixing to column chromatography.

Ultraviolet (UV)-spectroscopic analysis
The fraction was dissolved in chloroform and absorption spectrum was recorded between 200 nm and 430 nm using Perkin Elmer spectrophotometer, with less molar absorbent, the λ max with 273 nm is absorbed in sample indicates the existences of a UV active chromospheres. In the sample, UV spectra give λ max value for 273 nm compared with authentic taxol [13].

Infrared (IR)-spectrum analysis
The functional groups present in camptothecin (CPT) were determined by IR-spectra. The spectra were measured using Perkin Elmer PF2 800 in KBr. Both the control and treated have characterstic peaks to conform the existences of OH group CH_3/CH_2 group as well as an aromatic group. The peak in the range 3402 refers to OH/NH group. The peak in the range 2954/2922/2851 refers to the existence of SP3 hybridized C-H stretching the peak at 1741/1742 refers to existences of the carbonyl group. The peak in the range 1659-1763 refers to existences of the aromatic group. The peak at 2174 the control shifted to 2336 in treated refers to the changes.

DNA extraction for RAPD
DNA extraction method is commonly used to polymerase chain reaction (PCR)-based technique. The RAPD method used to indicate the genetic variation and variety of different fungal species.

RAPD analysis
Amplification was carried out with a 50 µL reaction mixture containing primer (2 µM/µL) -8.0 µL, ×10 buffer - 5.0 µL, 2 mM dNTP mix - 5.0 µL, Taq DNA polymerase (5U/µL) - 0.5 µL, templete DNA (50 ng) - 2.0 µL, Sterile distilled water - 29.5 µL. Mainly two types PCR primer used PGF01-5′-GAA ACA GCG G-3′, PGFO2-5′-GGA GCC CAC-3′. PCR amplification condition was maintained through the process end. Amplification was carried out with Eppendrof Mastercycler® ep, 94°C for 5 minutes, 34 cycles of 94°C for 40 seconds, 36°C for 30 seconds, 72°C for 90 seconds, and a final extension at 72°C for 10 minutes. The amplified product was separated on a 2.0% agarose gel in ×1 TBE at 75 v for 3 hrs. The gel was stained with ethidium bromide and the amplified product was visualized under a UV transilluminator.

Scanning electron microscope (SEM) studies
The sample are collected from liquid broth and the sample are carried out by the primary fixation with glutaraldehyde for 4 hrs then washed with phosphate buffer in 3 times each 5 minutes gap. The spores are fixed post fixation osmiam detroxide, after washing with phosphate buffer 3 times each 5 minutes gap. Dehydration process was followed 20% acetone, 30, 40, 50, up to 100 again, 100% acetone. The sample was changed, then sample was sputter coating (gold coating), then the samples were observed and take photograph.

RESULTS

The taxol was extracted by di chloromeathne and the solvent separated which evaporated for further process, and programed in UV-visible spectrum analysis (Fig. 1) and performed to the IR-spectrum analysis (Fig. 2). UV-visible absorption spectrum was recorded between 200 nm and 430 nm using Perkin Elmer spectrophotometer. In the sample, UV-spectrum gives maximum value for 273 nm. The result of IR-spectrum analysis to find out the functional groups present in CPT was determined by IR-spectrum. The treated have characteristic peaks to conform the existences of OH group CH_3/CH_2 group as well as aromatic group. The peak in the range 3402 refers to OH/NH group. After the purification of taxol was confirmed by UV absorption dissolved in 100% chloroform. Two extracts showed an absorption peak ranging from 235 λ max to 273 λ max. The presence of taxol was confirmed using column chromatography. The crude extract of *Fusiccocum* spp. in column chromatography was filtered through 0.2 µm membrane. The analysis of taxol production was based on an external standard of authentic taxol. The column chromatography process showed the amount of taxol production by *Fusicoccum* spp. 325 µg/L. Isolation of fungus is morphologically analysis by SEM (Plate 1). These are cylindrical-like structure in microscopic view. The RAPD analysis of genetic stability of the *in vitro* grown fungus and genotypic with two different primers showed that five fungus culture isolates obtained from two sites [14]. The five fungus species with corresponding primer efficiency was amplified. The high efficiency of primer is indicating of a larger area of the genome that compliments and allows base pairing between the primer and the genomic DNA (Plate 2) [15]. As the primer, efficiency depends on the total number of bands amplified by the primer and this could include a number of common monomorphic one. This study revealed that the characterization assessment of their genetic diversity and determine efficiency of primer.

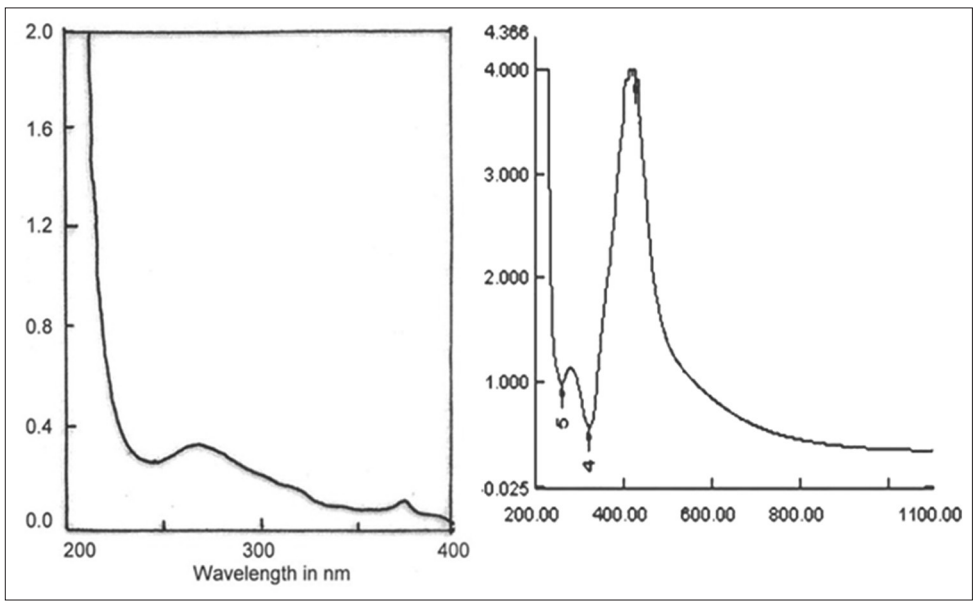

Fig. 1. Ultra violet spectrum of extracts of *Fucicoccum*. sp

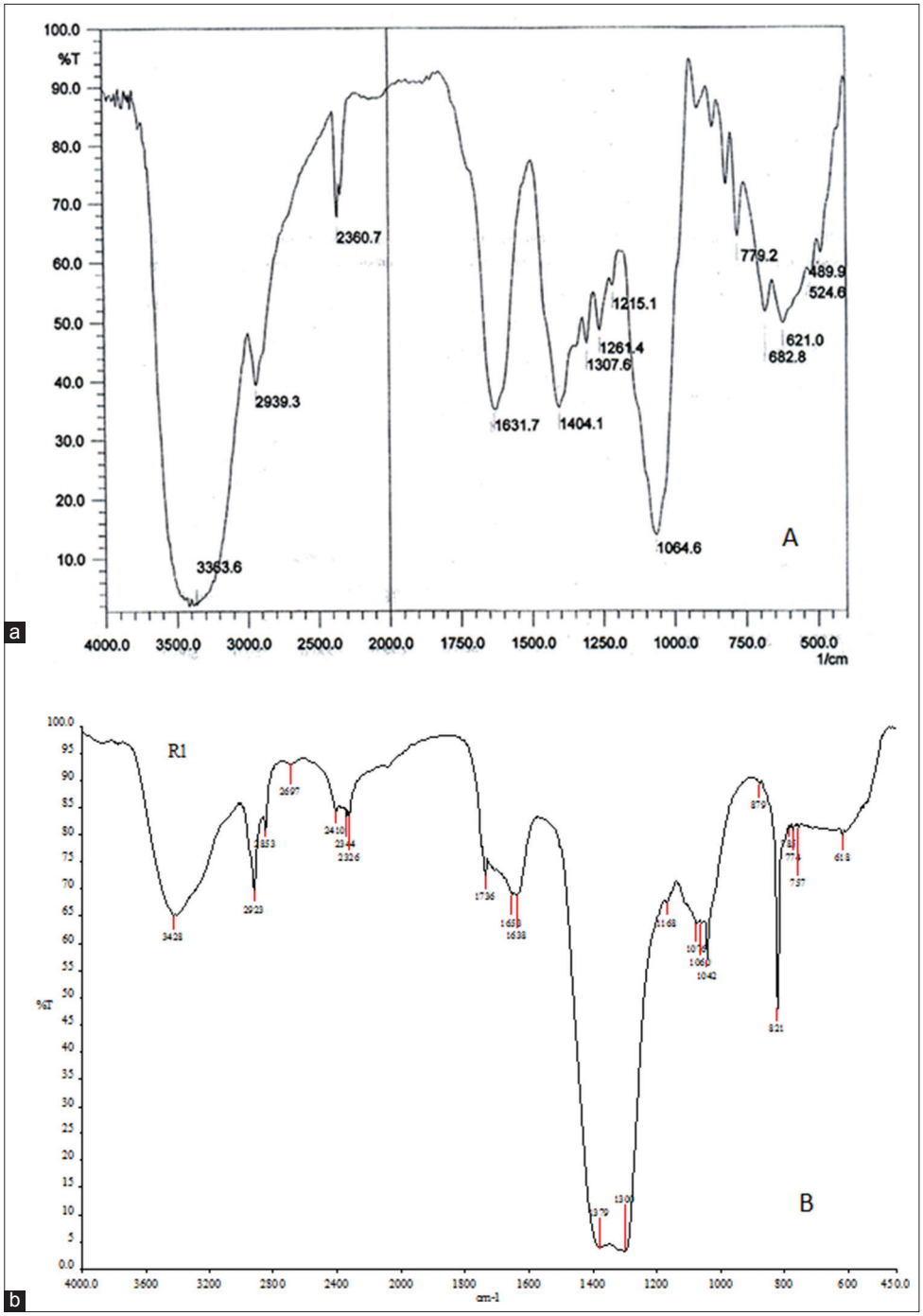

Fig. 2. Infrared-spectrum of taxol extracted sample from *Fucicoccum*. Sp. (a) Authentic taxol and, (b) extracted sample from *Fusicoccum.* sp.

DISCUSSION

The early reports of taxol production from fungi was only from entophytic organism in taxus [9-11]. In the result of the investigation, the production of taxol from non-endophytic fungi especially pathogenic which was assumed that the taxol produced have role in biodegradation in the case of saprotrophs mechanism of the pathogen. The taxol production is too low to be exploited commercially at present and developed culturing technique. Commercial exploitation is the only solution of considered for better taxol production. The taxol production is very low in the present investigation. Quantification of column chromatography indicates that 325 µg/L txol was produced per liter of culture. The optimum culture condition for taxol production is processed to the further study. The genetic relatedness among all isolates of fungus was analyzed by two random primers PGF01, PGF02, to generate reproducible polymorphism. All amplified product and the primer had shown polymeric and the distinguishable banding pattern was indicate the genetic diversity [16]. Basically, information obtained from agarose gel electrophoresis were to a two discrete character matrix (0 and 1 for absence and presence of RAPD marker). RAPD patterns were scored by visually comparing RAPD amplification profile (Plate 2) and scoring the presence or absence of each band in each profile [17], and RAPD analysis is measuring genetic variation within fungal species. RAPD marker had been useful to the other investigations of genetic variation among geographically distant population of fungi. RAPD bands from all primer between each pair were isolates. Primer product consists banding pattern were chosen to analyze. The entire collection isolates bands sharing the pair of isolates were calculated as the number of identical bands shared by both isolates divided by the

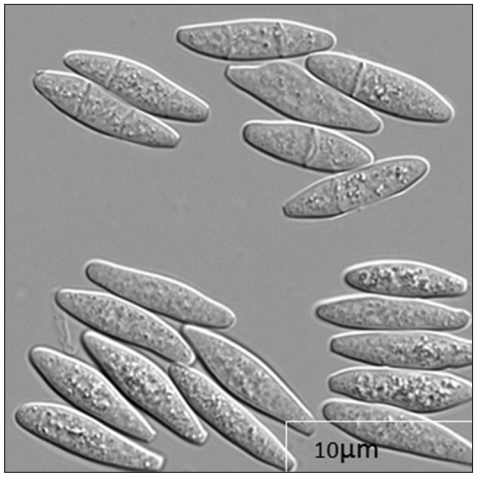

Plate 1. Scanning electron microscopic structure of *Fusicoccum* Conidia. *Fusicoccum* conidia were hyaline, thin-walled, non-septate; smooth fusiform, Conidia. Conidia are ascospores are multinucleate of genetically similar nuclei. This are cylindrical like structure in microscopic

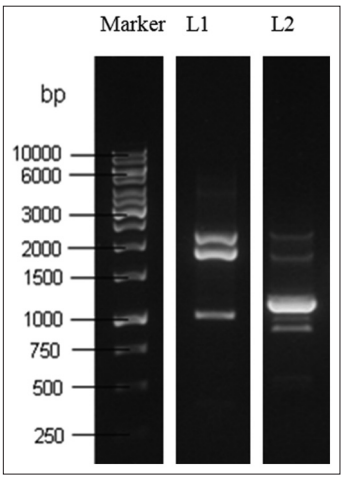

Plate 2. Random amplified polymorphic DNA Analyses L1. L2. Amplified product of *Fusicoccum* sp. Genomic DNA

total number of distinct band location presence in either two isolates. Selecting fungal PCR primer and DNA bar coding the environment sample and coverage of primers across fungal DNA bar coding primer had amplified sequencing of broad fungal taxa and accurately described. RAPD markers are used to examine the degree of genetic variation. Phylogenetic relationship between species these have been combined with morphology character [18]. RAPD analysis is screening for inter- and intra-species of genetic diversity with the *Fusicoccum* species. The RAPD pattern is tested in *Fusicoccum* species was satisfactory quality and described the optimized reaction.

CONCLUSION

The result of this study was the taxol extracted by dichloromeathne solvent, separated and evaporated for further process, then programmed in RAPD analysis. The presence of taxol was confirmed using column chromatography. The column chromatography process shows the amount of taxol production by *Fussicoccum* sp. 325 µg/L. The RAPD analysis of genetic stability of *in vitro* grown fungus and genotypic with two different primers showed that five fungus species with corresponding primer efficiency was amplified. As the primer, efficiency depends on the total number of bands amplified by the primer and this could include a number of common monomorphic one. Taxol production from biological sources was significant effect and presence of taxol amount is analysis in *Fusicoccum* sp.

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