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Phytochemical Analysis of Endophytic Fungus *Fusarium sacchari* FC6BBR Isolated from *Aeglemarmelos*.

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Abstract

Fungal species expands an endophytic role inside the tissues of medicinal plants and are known to produce metabolites, phytochemicals and enzymes in a wide range with biological properties. In the present investigation, the most dominant and potent endophytic fungal strains of Aeglemarmelos, an ethno medicinal plant prevalent in the forest of Western Ghats, Tamil Nadu, India were screened for phytochemical analysis. The representative potent strain was *Fusariumsacchari*FC6bBR belonged to the same order Pleosporales. The growth of the endophytic fungus was similar with the highest mycelia formation in the respective media. This endophytic fungal strain produced different phytochemicals in a considerable range such as phytochemicals constituents' flavonoids and phenols, and this unveils that the strains have highest antioxidant properties. The production of enzymes and phytochemicals provided the insights into their origin and ecological role in the host plant. Hence, this study explains thephytochemicals in endophytic fungus.

Keywords

endophytic fungus, phytochemicals, Western Ghats.

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1. INTRODUCTION

The phytochemicals are the metabolites or secondary products produced by the plants and trees. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (1). Ecological function of secondary products may have some bearing on potential medicinal effects for humans. For example, secondary products involved in

plant defense through cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines in humans, if not too toxic (2). In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. This synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body (3). The phytochemicals include alkaloids, flavonoids, steroids, terpenoids, coumarins, glycosides, phenols, etc.

There has been an increasing interest in the research on flavonoids from plant and endophytic sources because of their versatile health benefits reported in various epidemiological studies. The bioavailability, metabolism, and biological activity of flavonoids depend upon the configuration, total number of hydroxyl groups, and substitution of functional groups in their nuclear structure. Most recent researches have focused on the health aspects of flavonoids for humans. Many flavonoids isolated from endophytic microorganisms are shown to have coronary heart disease prevention, free radical scavenging capacity, antioxidative activity, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids explore the potential antiviral activities. In plants, flavonoids help in combating oxidative stress and play a role as growth regulators (4) similarly the flavonoids from endophytic fungi acts as an antioxidant in oncology. For pharmaceutical purposes cost-effective bulk production of different types of flavonoids has been made possible with the help of microbial biotechnology. Furthermore, the endophytes are the economical sources in discovering the novel enzymes for medical purposes and this interest has been drawn after the discovery of taxol which is an anticancer drug used in medicinal fields. Interestingly, knowing the mineral wealth of Western Ghats regions (hotspot of India), we aimed our research to isolate endophytes from this unexplored area. The present study was undertaken to investigate the prevalence of endophytic fungi and to assess the ability of these fungi in the production of industrially relevant enzymes and phytochemicals.

2. MATERIALS AND METHODS:

2.1. Isolation and identification of endophytic fungi

The samples from *Aeglemarmelos* (Vilvam tree) collected in Western Ghats regions, Coimbatore, TN, India were processed and sterilized by modified method of Mani et al (5) and the sterile samples were placed on fungal isolation media to isolate the endophytic fungi. The potent strains were identified morphologically through SEM analysis and molecularly by amplification of ITS 1 and ITS 4 regions of rDNA sequencing. The growth characterization of the potent strain was measured spectrophotometrically at 450 nm in Sabouraud's Dextrose Broth (SDB) and Malt Extract Broth (MEB) for 21 days incubation period in shaking conditions.

2.2. Phytochemicals characterization

The qualitative and quantitative phytochemical analysis in potent endophytic fungal strain was screened by following the descriptions and protocols of Nirjanta Devi et al.(6) The phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, saponins, steroids and cardiac glycosides were analysed for the presence in different crude metabolite extracts of endophytic fungal isolate. The crude metabolite extracts were prepared in hexane, petroleum ether, ethyl acetate and methanol for both the endophytes and concentrated for characterization.

3. RESULTS AND DISCUSSION

Endophytes produce considerable variation in extracellular enzymes and in this study two potential endophytes produced variable amount of extracellular enzymes. The strain FC6bBR has been isolated from *Aeglemarmelos*at Vellingiri hills, Coimbatore, India and they have been identified as *Fusarium sacchari* belonging to the order Pleosporales. The growth pattern of this strain was shown in fig 1 by the optical density units in different days of incubation. The growth of FC6bBr growth was higher from 15th day of incubation reaching 3.72 OD units (Fig 1). Both the strains produced extracellular enzyme in a medium range.

These crude metabolites produced by the fungal strain FC6bBr was taken in different solvents for the determination of phytochemical constituents qualitatively and quantitatively. The hexane extract for the strain FC6bBr showed positive activity for flavonoids, phenols and tannins; petroleum ether extract exhibited activity for tannins and steroids; ethyl acetate extract showed positive activity for

flavonoids, tannins, cardiac glycosides and steroids whereas methanol extract showed activity only for tannins (Table 1). The strain exhibited the presence for flavonoids and phenols in hexane, petroleum ether and methanol extract. So, the extracts were assessed for quantification of these two phytochemicals flavonoids and phenols with the standards rutin and gallic acid respectively.

Table 1: Phytochemical constituents of FC6bBr

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

S.No.		Hexane	Pet. Ether	Ethyl acetate
1	Alkaloids			
2	Flavonoids	+		+
3	Phenols	+		
4	Tannins	+	+	+
5	Cardiac glycosides			+
6	Steroids		+	+
7	Saponins			

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

	Strain FC6bBr	Hexane		Ethyl acetate
1.	Phenols (µg gallic acid/ mg of the extract)	20.0	-	-
2.	Flavonoids (µg rutin/ mg of the extract)	22.0	-	89.5

'+' denotes presence of phytochemicals; '-' denotes absence of phytochemicals.

The strain exhibited highest value of 20.0 µg GAE/ mg of the extract. Phenolics are the well-known compounds, owing to the potent antioxidant activities and bioactivities, are also known to diffuse the free radicals. This unveiled the potency of antioxidant capacity was higher in FC6bBr and in several investigations the phenolic compounds are found to have an important role in stabilizing the lipid oxidation process and also association with the antioxidant assessment. However, the content of the phenolics were dependent on the solvents used for the extraction process. In a study of Liu et al.(7), higher amount of phenolics were present in the polar solvents but in our study there is a new finding of highest phenolics were present in non-polar solvent extraction; this is a controversial part to the research of Liu et al.(7). Report of Huang et al. (8) stated the analysis of 292 endophytes from Chinese traditional plants showed the antioxidant capacities which were significantly correlated with the total phenolic content. The total flavonoids were present in hexane and ethyl acetate extract of FC6bBr with the values of 22.2 and 89.5 µg rutin/ mg of the extract respectively. Hence, flavonoids are considered to be the strong scavengers of ROS. However, the phenol content and flavonoid content was highest in FC6bBr.

CONCLUSION

The endophytes are very useful to the industries by the production of enzymes and metabolites and, this can be also used as biocontrol agents. To the best of our knowledge this is the first report of endophytic fungal extracellular enzyme and phytochemicals production from ethno medicinal tree A. marmelos of Western Ghats (Nilgiris cluster) of Tamil Nadu state, India. The presence of phenols and flavonoids represents the strong antioxidant activities which can reduce the cell damage responses. Further studies will be carried out to purify the enzymes and produce through bioprocess and biotechnological process and applied for medicinal purposes.

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