Antimicrobial and antioxidant activity of the endophytic fungus *Phomopsis* sp. GJJM07 isolated from *Mesua ferrea*

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Abstract

*Phomopsis* sp. GJJM07 was isolated as an endophyte from the medicinal plant, *Mesua ferrea*. The crude ethyl acetate extract of the fungus was evaluated for antimicrobial and free radical scavenging (DPPH) activity. Hence this endophytic fungus were grown in different media and were tested for its potent antimicrobial activity against the test pathogens, gram positive bacteria viz., *Bacillus subtilis, Micrococcus luteus*; gram negative bacteria viz., *Escherichia coli, Klebsiella pneumoniae* and yeast, *Candida albicans*. Among the different media, M1D medium showed good growth 1.57 g MDW/100 ml and broad spectrum of antimicrobial activity by exhibiting prominent zone of inhibition against the test pathogens such as *E. coli* (16±0.14), *K. pneumoniae* (16±0.19), *B. subtilis* (18±0.13), *M. luteus* (12±0.18) and *C. albicans* (12±0.20). *Phomopsis* sp. GJJM07 was also examined for the in vitro antioxidant activity by DPPH radical scavenging assay. The ethyl acetate extract of the fungus showed potent antioxidant activity with IC50 value of 31.25 µg/ml compared to the IC50 value of standard ascorbic acid, 11.11 µg/ml.

Keywords: *Mesua ferrea, Phomopsis* sp, antimicrobial activity, antioxidant activity.

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Introduction

Fungal endophytes have also been recognized as a repository of novel secondary metabolites, some of which have beneficial biological activities (Bills and Polishook, 1991). Endophytic fungi embraces affluent sources of bioactive compounds (Strobel et al., 2004) and recently numerous novel bioactive substances have been isolated from these microorganisms (Wagenaar et al., 2001; Li and Strobel et al., 2001; Brady, 2001; Shrestha et al., 2001; Kongsaaeree et al., 2003). The study on endophytic fungi from medicinal plants has received much attention in recent years as they are believed to be an excellent source of biologically active compounds. *Mesua ferrea* belonging to family Clusiaceae commonly called Ceylon ironwood, Indian rose chestnut, Cobra's saffron or Nagkesar. The seed oil of this tree found to be astringent, digestant, anti-poisonous, antimicrobial, antioxidant, anti-inflammatory, antipyretic and antihelminthic in many cases. In India, it is known for its use in fever, itching, nausea, leprosy, skin disorders, erysipelas, bleeding piles, metorrhagea, menorrhagea, excessive thirst, and sweating (Garg et al., 2009). The genus *Phomopsis* is a rich source of biologically active secondary metabolites including antimicrotubule phomopсидin (Kobayashi et al., 2003), antimalarial and antitubercular phomoxanthones (Isaka et al., 2001), antifungal phomoxanthone A (Elsaesser et al., 2005) and phomodiol (Horn et al., 1994). The compounds isolated and characterized from endophytes have potential for use in modern medicine, agriculture and industry. Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvement of free radicals in the pathogenesis of a large number of diseases is well documented. A potent scavenger of free radicals may serve as a possible preventive
intervention for the diseases (Gyamfi et al., 1999). Antioxidants may protect the body against ROS toxicity either by averting the formation of ROS by bringing disruption in ROS attack, by converting them to less reactive molecules or by scavenging the reactive metabolites (Sen, 1995; Hegde and Joshi, 2009). The natural antioxidants were characterized from the fungal compounds (Sun et al., 2004). Therefore the uses of antioxidants, both natural and synthetic are gaining broad significance in prevention of diseases. To date, many kinds of bioactive compounds have been isolated from various Phomopsis sp. (Prachya et al., 2007; Vatcharin et al., 2008). During the course of a bioactive survey of the endophytes from Mesua ferrea, the fungus Phomopsis sp. GJJM07 was isolated, identified and selected for further investigation. Hence the current study was designed to investigate the antimicrobial and antioxidant potential of endophytic fungus Phomopsis sp. GJJM07 isolated from a medicinal plant, Mesua ferrea.

Materials and methods

The endophytic coelomycetous fungi was isolated (Dobranic et al., 1995; Schulz et al., 1998) from the medicinal plant Mesua ferrea L., collected from Azhiyar (10.4739˚N, 76.9728˚E, located in Anamalai foothill of the Western ghats), Pollachi Taluk, Coimbatore district, Tamil Nadu, India.

Isolation of endophytic fungi

The healthy plant were washed in running tap water and the samples were surface sterilized by following the protocol described by modified method of Dobranic et al. (1995). The leaf segments were cut into 2 mm² segments and were surface sterilized by sequentially plunging into 70% ethanol for 5 seconds, followed by 4% sodium hypochlorite for 90 seconds and then rinsed with sterile water for 10 seconds. The excess moisture was blotted in a sterile filter paper. The efficacy of the sterilization procedure was ascertained with the method of Schulz et al. (1998). The segments were transferred to the petriplate containing PDA (Potato Dextrose Agar) medium amended with chloramphenicol. The petridishes were sealed using parafilm™ and incubated at 23 ±2°C in a light chamber with 12 hrs light followed by 12 hrs of dark cycles. The Petri dishes were observed at regular intervals starting from the second day onwards for the fungal growth. The emerging fungal propagules were isolated, purified and maintained by subsequent subculturing (Bills, 1996). The identifications of the endophytic fungi were based on their morphology and the mechanism of spore production using standard manuals (Sutton, 1980).

Test pathogens

Four different bacterial strains and yeast were employed for the successful completion of the study viz., Bacillus subtilis (MTCC 441), Micrococcus luteus (MTCC 1541), Escherichia coli (MTCC 443), Klebsiella pneumoniae (MTCC 109) and Candida albicans (MTCC 227) obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India were maintained on nutrient agar slants in refrigerated condition.

Mueller Hinton Agar (MHA)

The commercially available MHA purchased from HiMedia Pvt. Ltd, Mumbai, India was used in this study.

Phomopsis sp. GJJM07 in different media

To prepare inocula for fermentation studies, mycelial discs (9 mm) of 7 days old culture of Phomopsis sp. GJJM07 were removed and transferred aseptically into each 250 ml Erlenmeyer flask containing 100 ml of PDB, PDYEB, M-1DB, MEB and CDB medium. The seed flasks were incubated at 23 ± 2°C for 28 days under static condition. After incubation, the growth of fungi was measured by means of mycelial dry weight.

Extraction and antimicrobial activity

After 28 days of incubation period, the cultures were filtered through four layers of cheese cloth to remove mycelia. Then the culture filtrate was extracted with two equal volumes of solvent ethyl acetate. The organic phase was collected and the solvent was then removed by evaporation under
reduced pressure using rotary vacuum evaporator. The dry solid residue was re-dissolved in 10% DMSO for antimicrobial activity.

Mueller Hinton agar (MHA) medium plates were seeded separately with 10⁵ active growths of test organisms viz., B. subtilis, M. luteus, E. coli, K. pneumonia and C. albicans was distributed by a sterile cotton swab on the surface of the NA medium and left for 5 min in a laminar air flow cabinet to dry. Yeast pathogen was grown on SDA medium for antimicrobial activity. After drying, a well was created using sterile cork borer (9 mm) and the crude extract of Phomopsis sp. GJJM07 grown in different media was transferred to the well. The well that received similar volume of 10% DMSO was served as control. The plates were incubated for 24 hrs at 37°C. The development of inhibition zone around the well was measured and recorded.

Results and discussion

The fungus used in this study is the endophyte isolated from the mature leaves of the medicinal plant, Mesua ferrea. The endophytic fungus, Phomopsis sp. GJJM07 were isolated, purified and maintained by subsequent subculturing (Bills, 1996). Fungal endophytes have also been recognized as a warehouse of novel secondary metabolites, some of which have valuable biological activities (Bills and Polishook, 1991). The endophytic fungus Phomopsis sp. is known to be an affluent source of bioactive secondary metabolites with diverse structures (Rukachaisirikul et al., 2008). As a way to estimate the significance of this fungus Phomopsis sp. GJJM07 for industrial screening programmes, it was grown in different medium and evaluated for the production of antimicrobial activities against a panel of target microorganisms, including gram positive, gram negative bacteria and yeast. Among the different media, M1DB showed excellent growth (1.57 g MDW/100 ml) (Fig. 1) and antimicrobial activity against the test pathogens such as E. coli (16±0.14), K. pneumoniae (16±0.19), B. subtilis (18±0.13), M. luteus (12±0.18) and C. albicans (12±0.20) followed by PDB which exhibited significant activity against E. coli (14±0.17), K. pneumoniae (15±0.24),
B. subtilis (12±0.22), M. luteus (10±0.14) and C. albicans (11±0.16). CDB, PDYEB and MEB showed slight inhibitory effects on the test pathogens (Fig. 2). The data clearly revealed the pronounced activity of ethyl acetate extract in M1D medium. Thus M1D medium was found to be the reservoir of *Phomopsis* sp. GJJM07 for the production of secondary metabolite (Chomcheon et al., 2005). *Phomopsis* species are the rich source of secondary metabolites possessing promising antimicrobial and antiviral activity (Du et al., 2008; Bunyapaiboonsri et al., 2010). The antibiotic potential against some bacteria and human tumor cells of secondary metabolites from *P. longicolla* has been previously reported (Wagenaar and Clardy, 2001).

**Fig. 2** Graphical representation of antimicrobial activity of *Phomopsis* sp. GJJM07 in different medium

To explore the effects of the *Phomopsis* sp.GJJM07 extract on *in vitro* antioxidant activity, the DPPH scavenging rate was studied. Antioxidants are compounds that inhibit or delay the oxidation process by preventing the initiation or propagation of oxidizing chain reactions. DPPH radical scavenging assay is a swift and sensitive method for the antioxidant activity. A number of methods are available for the determination of free radical scavenging activity but the assay of using the stable 2, 2- diphenyl-1-picryl-hydrazyl radical (DPPH) has received the utmost attention owing to the ease of use and its convenience (Moreno et al., 1998). Ascorbic acid was chosen as the standard antioxidant for this experiment. The DPPH radical contains an old electron, which is accountable for the absorbance at 517 nm and also for a visible deep purple color. DPPH is decolorized when it accepts an electron donated by an antioxidant compound, which can be quantitatively measured from the changes in absorbance. The crude extract of *Phomopsis* sp. GJ JM exhibited antioxidant activity with IC50 value, 31.25 µg/ml compared to the IC50 value of ascorbic acid, 11.11 µg/ml (Fig. 3). Scavenging of DPPH radical was found to rise with escalating concentration of the extracts (Hasan et al., 2009). Even though the DPPH scavenging aptitude of the extracts was found to be lower than that of the commercial antioxidant, ascorbic acid, it is still reached 91% inhibition at 100 µg/ml concentration. Thus this study suggests that the *Phomopsis* sp.GJJM07 has highlighted the potentiality of antimicrobial and also it is a persuasive resource of natural antioxidants.

**Fig. 3** Graphical representation of antioxidant activity of *Phomopsis* sp. GJJM 07

**Conclusion**

The present study concludes that the presence of bioactive compound in the extract which exhibited antimicrobial and antioxidant activity in *Phomopsis* sp.GJJM07. Furthermore, active crude extracts are being subjected to various pharmacological evaluations by several methods for the isolation and identification of active compounds may provide a better source for developing new therapeutic agents, which also indicates the potential...
applications of such compounds as natural antioxidants in different pharmaceutical products.

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