

Research Article**Isolation and identification of endophytic fungi from *Gymnema sylvestre* R. Br.**

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Abstract

Objective: Endophytes act as major reservoir of biologically active compounds for health care applications and an increasing number of novel bioactive compounds are being extracted from fungal endophytes. so the present study is isolation and identification of endophytic fungi from *Gymnema sylvestre*. **Material and methods:** Fresh leaves and stem were washed thoroughly in running water and with distilled water to remove dust and debris. The material was surface sterilized by mercury chloride. The leaf segments were placed in petri dishes (2–3 segments/petri dish) containing Potato Dextrose Agar (PDA) medium. The petri dishes were sealed using parafilm and incubated at 26±2 °C in the light chamber for 3 weeks. The fungi grown were transferred to PDA plate and identified based on their morphology and other features. **Results:** A total of 60 endophytic isolates were isolated from *Gymnema sylvestre* and among them 10 endophytes belonging to seven genera were identified. The other fungal isolates need further efforts to identify. **Conclusion:** A total of 10 endophytic fungi belongs to seven genera were isolated and identified from *Gymnema sylvestre* and they may be very much useful in the extraction of potent active compounds that the plant is known far. The study may be useful in protecting the highly useful medicinal plant by giving the alternate source for drug.

Keywords: *Gymnema sylvestre*, endophytes

Introduction

Gymnema sylvestre R. Br is a medicinal herb native to central and western India, tropical Africa and Australia. It is often called “gurmar” (destroyer of sugar), as chewing the leaves causes a loss of sweet taste (Yeh et al., 2003). *G. sylvestre* containing the triterpenoid gymnemagenin and Gymnemic acid, extracted from leaves and roots is mainly used in India and parts of Asia as a natural treatment for diabetes as it helps to lower the blood sugar (Duke et al., 1997). In addition, it possesses antimicrobial, antihyper cholesterolemic (Bishayee et al., 1994), sweet suppressing (Kurihara et al., 1992) and hepatoprotective (Rana et al., 1992) activities. It also acts as feeding deterrents to caterpillar, prodenia eridania (Granich et al., 1974), prevent dental caries caused by *Streptococcus mutans* (Hiji et al., 1990) and skin cosmetics (Komalavalli et al., 2000). Besides, it is also used in the treatment of asthma, eye

complaints, inflammations, snake bite (Kini et al., 1982). Gymnemagenin, the wonder drug for diabetes (Liu et al., 1992,) and obesity (Kanetkar et al 2007), also possess antiviral properties (Sinsheimer et al., 1971). They are being isolated from the leaves and roots of *Gymnema sylvestre*, which leads to the destruction of natural resources. Alternatively, gymnemagenin-producing endophytic fungi *Penicillium oxalicum* is isolated from *Gymnema sylvestre*, so the fungus can serve as a potential material for the production of gymnemagenin. As *G. sylvestre* has been used in the treatment of several ailments, it is assumed to have several active compounds such as, Betaine, Beta-Carotene, Choline, Gymnemic-Acid, Niacin, Ascorbic-Acid. Hence the present study was carried out to identify the fungal endophytes capable of producing the active compounds reported from *G. sylvestre*.

Materials and methods**Collection of the plant material**

G. sylvestre collected from Botanical garden of Gulbarga University, Kalaburagi, Karnataka and was authenticated with the help of flora of Gulbarga district (Seetharam et al., 2000). A specimen is deposited in the herbarium, Dept. of

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Figure 1. *Gymnema sylvestri*

Botany, Gulbarga University Kalaburagi (Voucher specimen No HGUG-58).

Isolation and identification of endophytic fungi

Fresh leaves and stem were washed thoroughly in running water and with distilled water to remove dust and debris. The material was surface sterilized by mercury chloride. Leaves and stem samples were cut into 2–3 cm segments under aseptic conditions, The leaf segments were placed in petri dishes (2–3 segments/petri dish) containing Potato Dextrose Agar (PDA) medium. The petri dishes were sealed using parafilm and incubated at $26 \pm 2^\circ\text{C}$ in the light chamber for 3 weeks. The plates were monitored every day to check the growth of endophytic fungal colonies on leaf segments. The fungi grown were transferred to PDA plate and identified based on their morphology and other features.

Results and discussion

Isolation and identification of endophytic fungi

In the present study, a total of 60 isolates were isolated from the leaf and stem materials of *G. sylvestri*. Of which, ten fungal species belonging to seven genera were isolated and identified. *Cylindrocladium parvum*, *Cladosporium variabile*, *cochliobolus geniculatus* were recorded during September 2015, *Alternaria brassicicola*, *Colletotrichum capsici*, *colletotrichum dematium* were recovered during August and *Aspergillus fumigates*, *Aspergillus niger*, *Fusarium oxysporum* and *Alternaria alternata* were recorded during February month of 2015 (Figure 2). *Aspergillus niger* and *Alternaria alternata* were also reported from *Calotropis gigantean* (Srimathi et al., 2011) *Calotropis procera* (Rezwana et al., 2007), *Withania somnifera* (Rezwana et al., 2010), *Azadirachta indica* (Tenguria

et al., 2011) and *Melia azadirachta* (Kaushal et al., 2010). Ascomycota forms have been reported as endophytes (Blodgett et al., 2000; Suryanarayanan et al., 1998) and natural products from endophytes have a broad spectrum of biological activity (Zhang et al., 2006).

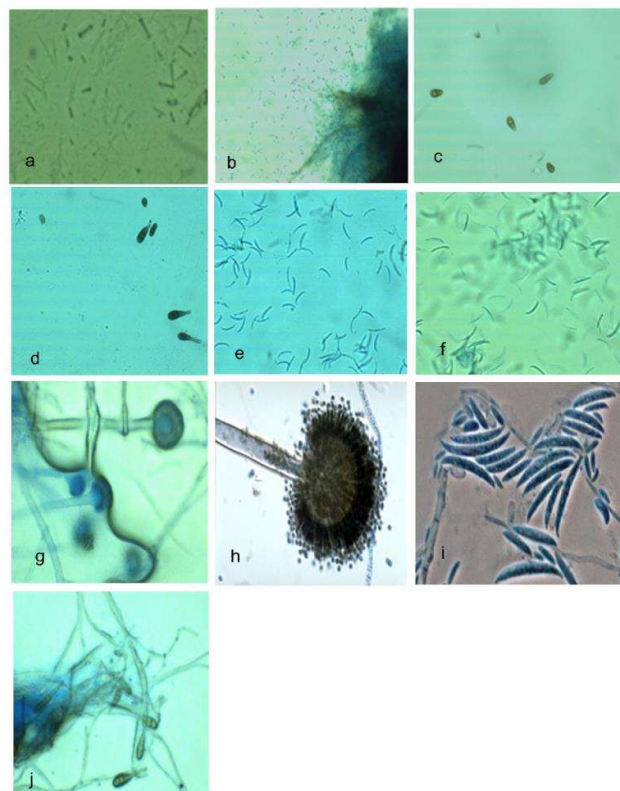


Figure 2. Endophytic fungi isolated from *Gymnema sylvestri*: (a) *Cylindrocladium parvum*, (b) *Cladosporium variabile*, (c) *Cochliobolus geniculatus*, (d) *Alternaria brassicicola*, (e) *Colletotrichum capsici*, (f) *Colletotrichum dematium*, (g) *Aspergillus fumigates*, (h) *Aspergillus niger*, (i) *Fusarium oxysporum*, (j) *Alternaria alternata*

Identification of endophytes

Endophytic fungi were identified according to their microscopic and macroscopic characteristics such as the morphology of fruiting structures and spore morphology under a bright-field microscope (10X and 40X) using Motic image microscope. Fungal features have been recorded with the help of a hand book of soil fungi by Nagamani (Nagamani et al., 2006) and illustrated genera of imperfect fungi by Barnett HL and Hunter BB (Barnet et al., 1972).

Conclusion

A total of 10 endophytic fungi belongs to seven genera were isolated and identified from *Gymnema sylvestri* and they may be very much useful in the extraction of potent active compounds that the plant is known far. The study may be useful in protecting the highly useful medicinal plant by giving the alternate source for drug.

Table 1. Fungal endophytes isolated from leaf and stem parts of *Gymnema sylvestre*

S. No	Name of the endophyte	Plant part used	Growth in diameter
1	<i>Cylindrocladium parvum</i>	Stem	2.5
2	<i>cladosporium variable</i>	Stem	2
3	<i>cochliobolus geniculatus</i>	Leaf	4.32
4	<i>Alternaria brassicicola</i>	Leaf	3.72
5	<i>Colletotrichum capsici</i>	Leaf	3.9
6	<i>colletotrichum dematium</i>	Stem	2.38
7	<i>Aspergillus fumigates</i>	Stem	4.16
8	<i>Aspergillus niger</i>	Stem	2.03
9	<i>Fusarium oxysporum</i>	Stem	2.06
10	<i>Alternaria alternata</i>	Stem	3.27

Conflicts of interest: Nil

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