

Studies on Secondary Metaboloids From Potential Endophytic Fungi Isolated from *Gymnema sylvestre* R. Br

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Abstract: Medicinal plant *Gymnema sylvestre* (Asclepiadaceae) is a herb distributed throughout the world. The leaves showed antidiabetes, antimicrobial, antihypercholesterolemic, hepatoprotective and diuretic properties. Several active compounds such as, triterpenoid gymnemagenin, Gymnemic acid, Betaine, Beta-Carotene, Choline, Gymnemic-Acid, Niacin, Ascorbic-Acid have been reported from the leaves and root. In India leaves are mainly used as hepatoprotective and immunostimulatory agent, skin cosmetics and in the treatment of asthma, eye complaints, inflammations, snake bite, diabetes and obesity. *G. sylvestre* is a rare plant overexploited by traditional healers across the world. Therefore, endophytes can serve as the best alternate source for the host plant. Therefore, the present work was carried out to study the endophytic fungi of *G. sylvestre* and their role in the production of therapeutic active compounds. Present study was undertaken to evaluate the phytochemical constituents in the fungal endophyte VAG-4. The presence of Phenols, alkaloids, flavonoids, tannins, terpenoids and saponins were detected in both plant and fungal extracts. Among the phytochemicals detected in VAG-4, flavonoids (3.8%) were found to be maximum followed by phenols (2.1%), alkaloids (2.05%), saponins (1.08%), tannins (0.6%), terpenoids (2.8%). Similarly, the Plants reveals maximum of flavonoids (8.1%) followed by phenols (4.32%), alkaloids (3.75%), saponins (2.22%), tannins (1.2%) and terpenoids (5.8%). The fungal endophytes in isolation was found more efficient in producing secondary metabolites than its association with host plant.

Keywords: *Gymnema sylvestre*, Endophytic Fungi, Phytoconstituents, Secondary metabolites.

1. INTRODUCTION

In recent times, focus on plant research has increased all over the world and immense potential of medicinal plants used in various traditional systems has been highlighted [1]. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties [2]. *Gymnema sylvestre* (Asclepiadaceae) is a herb distributed throughout the world. The leaves showed antidiabetes, antimicrobial, antihypercholesterolemic, hepatoprotective [3]. and diuretic properties. Several active compounds such as, Betaine, Beta-Carotene, Choline, Gymnemic-Acid, Niacin, Ascorbic-Acid, triterpenoid gymnemagenin, Gymnemic acid, have been reported from the root and leaves. In India leaves are mainly used as hepatoprotective and immunostimulatory agent, skin cosmetics and in the treatment of asthma, eye complaints, inflammations[5-6], snake bite[4], diabetes and obesity[7-8]. As an alternative, the microbes which live such plants may offer tremendous potential sources of therapeutic compounds. Endophytes are microbes that inhabit plant tissues in their life cycle without causing any apparent harm to their host. Their presence implied a symbiotic interaction, in all the plants investigated until now, Endophytes were mentioned for the first time by Bray in 19th century. Endophytes have been found in nearly all plant families, It is estimated that there may be at least one million species of endophytic fungi alone. Recently endophytes are viewed an outstanding source of secondary metabolites and bioactive antimicrobial natural products. *G. sylvestre* (Fig.1) is a rare plant overexploited by traditional healers across the world. Therefore, endophytes can serve as the best alternate source material for the extraction of active compounds. Therefore, the present work was

carried out to study the endophytic fungi of *G. sylvestre* and their role in the production of therapeutic active compounds similar to host plant.



Fig.1 *Gymnema sylvestre*

2. MATERIALS AND METHODS

Collection of the plant material

Gymnema sylvestre stem and leaves were collected from the Botanical garden of Gulbarga University, Kalaburagi, and authenticated with the help of literature and herbarium specimens deposited in herbarium centre (HGUG-58), Department of Botany, Gulbarga university, Kalaburagi, Karnataka, India.

Preparation of plant crude extracts

Leaf material was brought to the laboratory, washed with tap water to remove the adherents, dried and powdered. The leaf powder was successively extracted using soxhlet apparatus in organic and inorganic solvents and used the crude extract for the detection of secondary metabolites.

Isolation of Endophytic Fungi

To isolate fungal endophytes, the plant material (stem) was surface sterilized and the small segments were placed on PDA medium. The Petri dish were sealed using Parafilm and incubated at 23⁰C. After incubation for 15 or more days, the fungal colonies grown were identified based on their morphology and literature available. Among the endophytes isolated, VAG-4 was selected for the present studies.

Extraction of crude extracts from Endophytic Fungi

The fungus was inoculated in to 500 ml conical flasks containing 300 ml Potato dextrose broth and kept for incubation at 21±2⁰C. The culture filtrate was discarded and the mycelial mat was used for extraction of secondary metabolites by using organic and inorganic solvents. The filtrate thus obtained was used as crude material for Phytochemical analysis.

3. PHYTOCHEMICAL SCREENING

The preliminary phytochemical studies were performed for testing the different chemical groups present in petroleum ether, chloroform and methanol extracts of both plant and fungal extracts .

Test for Phenols : Ellagic Acid Test, Ferric chloride test, [10].

Test for Triterpenoids: Liebermann - Burchard's test (LB test), Salkowaski test.

Test for Flavonoids: Shinoda's test, Ferric chloride test, Zinc-Hydrochloric acid reduction test, Alkaline reagent test, Lead acetate solution test,

Test for Alkaloids: Dragendorff's test, Hager's test, Wagner's test, [9].

Test for Saponins: Foam test.

Test for Steroids: Liebermann-Burchard's test, Salkowaski reaction.

Test for Tannins: Ferric chloride test, Gelatin test, [11].

Test for glycosides: Keller-Killiani test, Legal's test.

Test for reducing sugars: Fehling's test, Benedict's test, [12].

QUANTITATIVE ESTIMATION OF SECONDARY METABOLITES:

Flavonoids [14], Phenols [16-17]. Alkaloids [13], Saponins [18], Tannins [15], Terpenoids [19], secondary metabolites were estimated by standard methods.

4. RESULT AND DISCUSSION

Phytochemical analysis conducted on the plant and fungal extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [19]. The presence of flavonoids, alkaloids, Phenols, terpenoids, tannins, and saponins were detected in both plant and fungal extracts.

Among the phytochemicals detected in VAG-4, flavonoids (3.8%) were found to be maximum followed by phenols (2.1%), alkaloids (2.05%), saponins (1.08%), tannins (0.6%), terpenoids (2.8%) (Fig.2). Similarly, the Plants reveals maximum of flavonoids (8.1%) followed by phenols (4.32%), alkaloids (3.75%), saponins (2.22%), tannins (1.2%) and terpenoids (5.8%). The fungal endophytes in isolation was found more efficient in producing secondary metabolites than its association with host plant (Fig.2) . Several studies has described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds [20-21]. A preliminary phytochemical investigation was carried out according to the conventional method to identify the types of phytoorganic constituents that are present in *Gymnema sylvestre* and VAG-4 and the results are summarized in Table-1.

Table 1: Phytoconstituents tests results obtained from *Gymnema sylvestre* and VAG-4

| Phytochemical constituents | Petroleum ether | | Chloroform | | Methanol | |
|----------------------------|--------------------|-------|---------------------|-------|--------------------|-------|
| | <i>G. sylveste</i> | VAG-4 | <i>G. sylvestre</i> | VAG-4 | <i>G. sylveste</i> | VAG-4 |
| Phenols | | | | | | |
| Feric chloride test | + | + | + | + | + | + |
| Ellagic acid test | + | + | + | + | + | + |
| Alkaloids | | | | | | |
| Dragendorff's test | + | + | + | + | + | + |
| Hager's test | + | + | + | + | + | + |
| Wagner's test | + | + | + | + | + | + |

| | | | | | | |
|--|---|---|----|---|---|---|
| Terpinoids | | | | | | |
| Liebermann - Burchard's test (LB test) | + | + | + | + | + | + |
| Salkowaski test | + | + | + | + | + | + |
| Flavonoids | | | | | | |
| Shinoda test | + | + | + | + | + | + |
| Ferric chloride test | + | + | + | + | + | + |
| Zinc Hydrochloric acid reduction test | - | - | - | - | + | + |
| Alkaline reagent test | - | - | - | - | + | + |
| Lead acetate test | + | + | + | + | + | + |
| Tannins | | | | | | |
| Ferric chloride test | + | + | + | + | + | + |
| Gelatine test | + | + | + | + | + | + |
| Saponins | | | | | | |
| Foam test | + | + | + | + | + | + |
| Steroids | | | | | | |
| Liebermann - Burchard's test (LB test) | - | - | + | - | - | - |
| Salkowaski test | - | - | + | - | + | - |
| Glycosides | | | | | | |
| Baljet test | + | - | + | - | + | - |
| Keller-killiani test | + | - | + | - | + | - |
| Raymond's test | + | - | + | - | + | - |
| Bromine water test | + | - | + | - | + | - |
| Legal's test | + | - | + | - | + | - |
| Reducing sugar | | | | | | |
| Fehling's test | - | - | - | - | - | - |
| Benedict's test | - | - | -- | - | - | - |

Flavonoids have an inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, antimicrobial and anticancer activity. Alkaloids have different types of activities as painkillers, antimicrobial, stimulants, muscle relaxants, anaesthetics, antimicrobial, anti-diabetic, anti-cancerous, antioxidants. Saponins have antifungal properties [24]. Tannins have general antimicrobial and antioxidant activities [22]. Current reports show that tannins may have potential value such as cytotoxic and antineoplastic agents [23]. These contents show different types of activities against different pathogens. Therefore, it can be used in the treatment of diseases. Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and Weight loss etc. According to medical field, it is bioactive antibacterial agents of plants [25-26]. Plant steroids have cardiogenic activity, possess insecticidal and antimicrobial properties. It is generally used in herbal medicines and cosmetic products [27], phenolic

compounds have anti-oxidative, antidiabetic, anticarcinogenic and anti-inflammatory [28-29]. Terpenoids are generally the secondary metabolites occurring in the plants, Terpenoids are the largest group of natural products which have proved increased interest in researcher for their commercial use. thus, these preliminary qualitative tests according to [30], is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development [31].

Preliminary phytochemical analysis of organic solvents extracts of both plant and fungal extracts were carried out for the evaluation of presence or absence of the phytochemicals, Both extracts showed the presence of phenols, alkaloids, flavonoids, tannins, terpenoids and saponins.

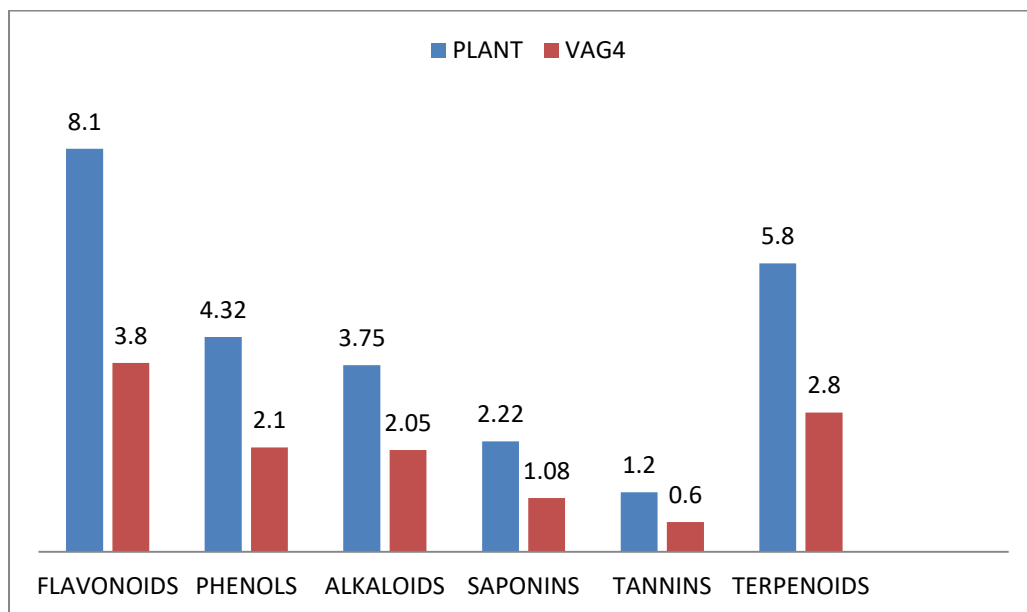


Fig: 2 Quantitative estimation of secondary metabolites of Plant and VAG-4

5. CONCLUSION

The present study clearly revealed the capable of producing phytoconstituents similar to the host plant, the selected fungus can serve as an alternate source for *G. sylvestre* in the production of some therapeutic compounds. plant phenolics and Flavonoids and tannins active molecules are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of in future it may be an agent for treating oxidative stress related disease along with microbial infections.

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