International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 206-212
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

SECONDARY METABOLITES FROM FUNGAL ENDOPHYTES OF *GYMNEMA SYLVESTRE* R.BR.

Ambika Vasantakumar and Vidyasagar G.M*

Medicinal Plants and Microbiology Research Laboratory, Department of P G Studies and Research in Botany, Gulbarga University, Kalaburagi-585106, Karnataka, India.

***Corresponding Author Email:** gmvidyasagar@gmail.com/ambikavasantakumar@gmail.com

ABSTRACT

Gymnema sylvestre (Asclepiadaceae) is a herb distributed throughout the world. The leaves showed antidiabetes, antimicrobial, anti hypercholesterolemic, 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 VAK-3. The presence of Phenols, alkaloids, flavonoids, tannins, terpenoids and saponins were detected in both plant and fungal extracts. The TLC profile of secondary metabolites of endophyte revealed similar bands as in host plant. Among the phytochemicals detected in VAK-3, flavonoids (4.8%) were found to be maximum followed by phenols (3.1%), alkaloids (2.05%), saponins (1.08%), tannins (0.8%), terpenoids (3.8%). 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.

KEY WORDS

Gymnema sylvestre, Endophytic Fungi, Phytochemicals, Secondary metabolites.

INTRODUCTION

Gymnema sylvestre (Asclepiadaceae) is a herb distributed throughout the world. The leaves showed antidiabetes, antimicrobial, anti-hypercholesterolemic, hepatoprotective (Rana Ac, Avadhoot Y. 1992) 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 (Kini R.M,et al.,1982. – Kini R.M, et al., 1982), snake bite (Komalavalli N, et al., 2000), diabetes and obesity (Liu, H. M, et al., 1992. - Kanetkar, et al., 2007). 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*

MATERIALS AND METHODS

Collection of the plant material

Gymnema sylvestre 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 was surface sterilized and the small segments were placed on PDA medium. The Petri dish were sealed using Parafilm and incubated at 23^{0}C. After incubation for 15 or more days, the fungal colonies grown were identified based on their morphology and literature available. Among the endophytes isolated, VAK-3 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±2°C.

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.

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 Alkaloids: Dragendorff's test, Hager's test,Wagner's test, (Saldanha C J, 1984).

Test for Phenols: Ellagic Acid Test,Ferric chloride test,(J.Memelink, et al., 200).

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

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

Test for Saponins: Foam test.

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

Test for Tannins: Ferric chloride test, Gelatin test, (Gibbs R.D.,1974).

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

Test for reducing sugars: Fehling's test,Benedict's test, (Treare GE, Evans WC,1985).

QUANTITATIVE ESTIMATION OF SECONDARY METABOLITES:

Flavonoids (Swain T, et al., 1959.), Phenols (Council of Europe, 2007. - Farmacopeia Brasileira, 2010.),Alkoloids Ikan,R.1981), Saponins (Sanchez, S. H.,et al., 1979),Tannins(Schanderi S H, 1970), Terpenoids(Ferguson, N.M., 1956), secondary metabolites were estimated by standard methods.

THIN LAYER CHROMATOGRAPHY (TLC):

The identification and separation of the components present in different extracts of *Gymnema sylvestre* and VAK-3 was carried out by Thin Layer Chromatography. The TLC of both the extract was performed using different solvent systems. The chromatograms were dried to remove the solvent, cooled and sprayed with the detecting reagents. The TLC profile of secondary metabolites of endophyte revealed similar bands as the host plant.

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 (Ferguson, N.M., 1956).

The presence of Phenols, alkaloids, flavonoids, tannins, terpenoids and saponins were detected in both plant and fungal extracts. The TLC profile of secondary metabolites of endophyte revealed similar bands as in host plant.

Among the phytochemicals detected in VAK-3, flavonoids (4.8%) were found to be maximum followed by phenols (3.1%), alkaloids (2.05%), saponins (1.08%), tannins (0.8%), terpenoids (3.8%) (Fig.4). 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.3). Several studies have described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds (Wiley and Sons, 1993 - Brown JE, 1998). 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 VAK-3 and the results are summarized in Table-1.

Table 1: Phytochemical tests results obtained from *Gymnema sylvestre* and VAK-3

Phytochemical constituents	Petroleum ether		Chloroform		Methanol	
	G.sylveste	VAK-3	*G. sylvestre*	VAK-3	*G.sylveste*	VAK-3
Alkaloids						
Dragendorff's test	+	+	+	+	+	+
Hager's test	+	+	+	+	+	+
Wagner's test	+	+	+	+	+	+
Phenols						
Feric chloride test	+	+	+	+	+	+
Ellagic acid test	+	+	+	+	+	+
Flavonoids						
Shinoda test	+	+	+	+	+	+
Ferric chloride test	+	+	+	+	+	+
Zinc Hydrochloric acid reduction test	-	-	-	-	+	+
Alkaline reagent test	-	-	-	-	+	+
Lead acetate test	+	+	+	+	+	+
Terpinoids						
Liebermann - Burchard's test (LB test)	+	+	+	+	+	+
Salkowaski test	+	+	+	+	+	+
Saponins						
Foam test	+	+	+	+	+	+
Steroids						

Liebermann - Burchard's test (LB test)	-	-	+	-	-	-
Salkowaski test	-	-	+	-	+	-
Tannins						
Ferric chloride test	+	+	+	+	+	+
Gelatine test	+	+	+	+	+	+
Glycosides						
Baljet test	+	-	+	-	+	-
Keller-killiani test	+	-	+	-	+	-
Raymond'test	+	-	+	-	+	-
Bromine water test	+	-	+	-	+	-
Legal's test	+	-	+	-	+	-
Reducing sugar						
Fehling's test	-	-	-	-	-	-
Benedict's test	-	-	--	-	-	-

Alkaloids have different types of activities as painkillers, anti-microbial, stimulants, muscle relaxants, anaesthetics, antimicrobial, anti-diabetic, anti-cancerous, antioxidants. Flavonoids have an inherent ability to modify the bodys reaction to allergen, virus and carcinogens. They show anti-allergic, antimicrobial and anticancer activity. Tannins have general antimicrobial and antioxidant activities (Krings U, 2001), Current reports show that tannins may have potential value such as cytotoxic and antineoplastic agents (Rievere C, et al 2009), Saponins have antifungal properties (Aguinaldo A M, et al.,2005). 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 (Aboada O, 2001- Mandal P et al., 2005). Plant steroids have cardiotonic activity, possess insecticidal and antimicrobial properties. It is generally used in herbal medicines and cosmetic products (Manjunatha B K, 2006), phenolic compounds have anti-oxidative, antidiabetic, anticarcinogenic and anti-inflammatory (Callow, R.K. 1936 - Art,I.C,et al 2005). 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 (Scalbert et al.,2005), is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development (Deba F et al., 2008).

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 saponin.

Fig.2 TLC profile of both Plant and fungal extract.

Endophytes

Phenols

Host plant

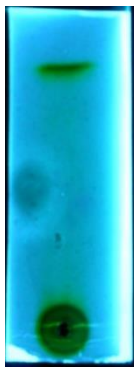
Phenols

Alkaloids

Alkaloids

Flavonoids

Flavonoids



Tannins

Tannins

Terpenoids

Terpenoids

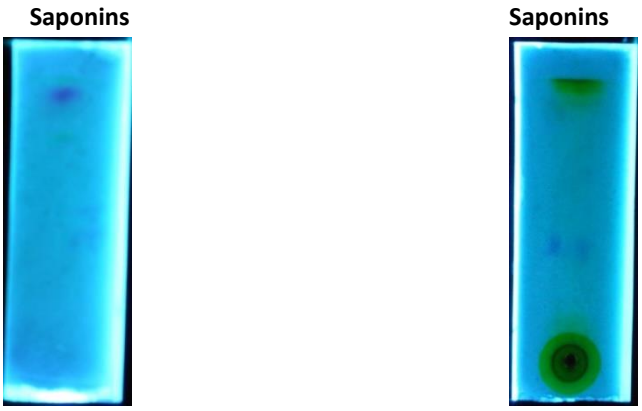


Fig:3 Quantitative estimation of secondary metabolites of Plant

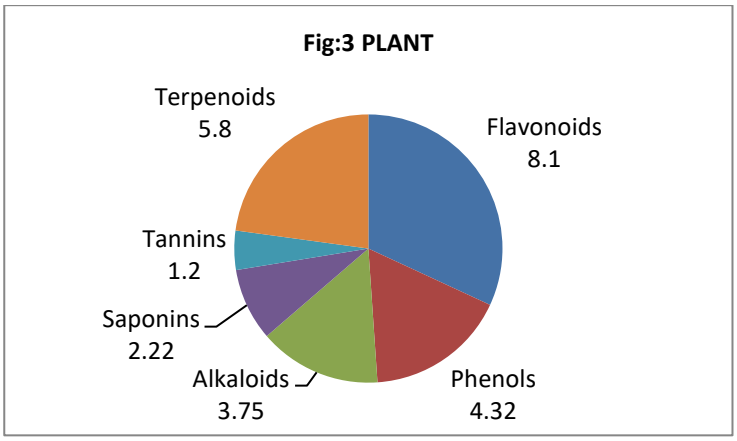
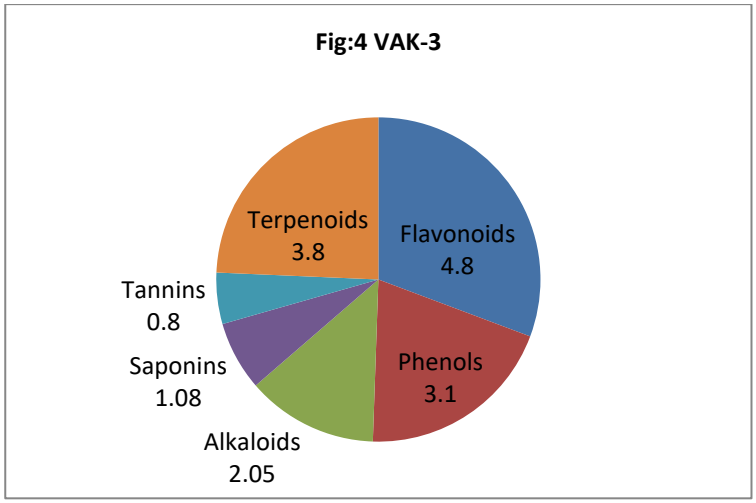


Fig:4 Quantitative estimation of secondary metabolites of VAK-3



CONCLUSION:

The purpose of the study is capable of producing the active compounds similar to the host plant, the selected fungus can serve as an alternate source for *G. sylvestre* in the production of some therapeutic compounds. Flavonoids and tannins compounds and plant phenolics 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.

REFERENCES

Rana Ac, Avadhoot Y. 1992. Experimental evalution of hepatoprotective activity of *Gymnema sylvestre* and *Curcum Zedoaria* . Fitoterapia. 63:60.

Komalavalli N, Rao M.V. 2000. In vitro micropropagation of Gymnema sylvestre-A multipurpose medicinal plant. Plant Cell Tiss org.61:97-105.

Kini R.M,Gowda T.V. 1982. Studies on sanke venom enzymes: Part I purification of ATPase, a toxic component of *Naja naja* venom and its inhibition by potassium gymnemate. Indian J.Biochem.Biophys. 22(2):152-154.

Kini R.M,Gowda T.V. 1982. Studies on sanke venom enzymes: Part II Partial characterization of ATPase from Russell's viper (vipera russelli) venom and their interaction with potassium gymnemate.Indian J.Biochem.Biphys. 19(5):342-46.

Liu, H. M, Kiuchi, F., & Tsuda, Y. 1992. Isolation and structure elucidation of gymnemic acids, anti-sweet principles of Gymnema sylvestre. Chemical and Pharmaceutical Bulletin. 40, 1366–1375.

Kanetkar, P. V, Singhal R. S, & Kamat, M. Y. 2007. *Gymnema sylvestre*: a memoir. Recent advances in Indian herbal drug research.Journal of Clinical Biochemistry and Nutrition.41,77–81.

Saldanha C J, 1984. Flora of Karnataka, Oxford and IBH Publishing Co, New Delhi, vol.1. J.Memelink, R. Verpoort,J.W. Kigine, 2001.Organisation of jasmonate responsive gene expression in alkaloid metabolism. Gibbs R.D., 1974. Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen's University Press, Montreal and London.

Treare GE, Evans WC. 1985. Pharmacognosy 17th edn., Bahiv Tinal, London. P 149.

Ikan, R., 1981. Natural products: A laboratory guide, Academic Press, London.

Swain T, Hillis WEJ. 1959. Phenolic constituents of Prunus domestica I.Quantitative analysis of phenolic constituents. J Sci Food Agric 10:63

Schanderi S H. 1970. Methods in food anaiysis Academic press, New York, p.709 Council of Europe. 2007. Determination of tannins in herbal drugs. In European Pharmacopoeia, 6thed.European. Directorate for the Quality of Medicines: Strasbourg, France, p. A286.

Farmacopeia Brasileira, 2010. 5th ed.; Agência Nacional de Vigilância Sanitária: Brasília, Brazil.

Sanchez, S. H., Vidal, J. C. & Paladini, A. C..1979. Arch. Biol. Med. Exp. 12, 284.

Ferguson, N.M., 1956. At text book of Pharmacognosy, Mac Millan Company, 191.

Sofowora A,Medicinal plant and traditional medicine in Africa.New York: John Wiley and Sons;1993 191-289.

Brown JE,Rice-Evans CA.Luteolin rich artichoke extract protects low density lipoprotein from oxidation in in vitro. Free Radic Res 1998;29:246-55.

Krings U, Berger RG. Antioxidant activity of roasted foods.Food Chem 2001;72: 223-9

Rievere C, J H van Nguyen, L Pieters, B Dejaegher, Y V Heyden, C V Minh, J Quetin-Leclercq. Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*. Phytochemistry 2009;70: 86-94.

Aguinaldo A M, El-Espeso, B Q Guovara, M G Nanoto2005. Phytochemistry. In: Guevara B.Q (ed) A. Guide book to plant screening phytochemical and biological. University of Santo Tomas, Manila, Philippines.

Aboada O O, and B M Efuwape Antibacterial properties of some Nigerian species. Biol Res Comm 2001; 13,183-88.

Mandal P, S P Sinha Babu and N C Mandal Antimicrobial activity of saponins from *Acacia auriculiformis*. Fititerapia 2005;76:(5) 462-65.

Manjunatha B K Antibacterial activity of *Pterocarpus santalinus.* Ind JPha Sci 2006;68:(1) 115-6.

Callow, R. K, 1936.Proc, Royal, Soc London Series.157:194.

Art,I.C,and P.C. Hollman. Polyhenols are disease risk in epidemiological studies. Amer J Clin Nut 2005;81:317-325.

Scalbert, A C Manach, C Morand, C Remesy and L Jimenez. Dietary olyhenols and the prevention of diseases.Rev Food Sci Nutr 2005;45:287-306.

Deba F, T D Xuan, M Yasuda and S Tawatu, Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. Var. Radiata, Food Control, 2008;19: 346-2.

Vaghasiya, Y R Dave and S. Chandan,Phytochemical analysis of some medicinal plants from Western region of India Res J Medic Plant 2011;5:567.

Received:02.05.18, Accepted: 05.06.18, Published:01.07.2018

***Corresponding Author:**
Vidyasagar G.M*
Email: gmvidyasagar@gmail.com/ambikavasantakumar@gmail.com