EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 9
343-346
Year: 2018

ANALYSIS OF SECONDARY METABOLITES FROM ENDOPHYTIC FUNGI - PHYLLOSTICTA SP ISOLATED FROM AMARANTHUS RETROFLEXUS – A WEED

Sivasankaran Ramarajan[1] and Dr. Vardhana Janakiraman*

[1&*]Assistant Professor, Department of Biotechnology, SRM Arts and Science College, Chennai-603203.

***Corresponding Author: Dr. Vardhana Janakiraman**

Assistant Professor, Department of Biotechnology, SRM Arts and Science College, Kattankulathur, Chennai- 603203.

Article Received on 25/06/2018 **Article Revised on 15/07/2018** **Article Accepted on 05/08/2018**

ABSTRACT

To perform phytochemical analysis of Phyllosticta Sp, an endophytic fungi isolated from *Amaranthus Retroflexus* a weed .The tests for the presence of Flavanoids, Tannis, Phenol, Saponins, carbohydrates and steroids have been performed. The isolated endophytic fungus was identified based on the morphology and the structure of fruiting bodies. The isolated endophytic fungi showed the presence of active compounds like Tanins, Steroids and Saponins. The phytochemicals present in the endophytic fungi are effective alternative sources for antimicrobial drugs and antioxidant activity. Further Invitro and Invivo experiments are required to establish bioactivity and structural elucidation of these endophytic extracts.

KEYWORDS: Endophytic Fungi, Phytochemicals, Weed, Phyllosticta Sp, Antimicrobial activity.

INTRODUCTION

The term weed in broader sense is any plant growing where it is not wanted. Weeds have been a part of civilization and many ancient documents speak of humans battling weeds in the crops they grow. A weed is commonly defined as a plant that grows out of place and is competitive, persistent and pernicious.[1] Weeds are also found to be resistant to most of the microbial and pesticidal disease when compared to the crops which shows disease symptoms.[2] World Health Organization (WHO) has reported that more than 80 % of the world's population relies on the traditional medicine for their primary healthcare. This is because the plant kingdom represents an enormous reservoir of biologically active compounds called phytochemicals.

There is currently immense interest in natural antioxidants and their role in human health and nutrition. Similarly, Due to increased evolution of multiple antimicrobial resistant strains of organisms, there is need for continuous search for new antimicrobial agents.[3] Plants contains secondary metabolites which has anti-infective properties that be used as substitutes for antibiotics resistant to pathogenic bacteria and fungi. Since ancient times, plants have been utilized as an important source of medicines as they are a reservoir of chemical agents.[4] they have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles, it is reasonable to make use of locally available plants like weeds that could help replace the pharmaceutical preparations.

The phytochemical analysis of Medicinal and aromatic plants,[5] Iranian plants,[6,7] Plants of Qatar,[8] Nigeria,[9] Nepal[10] and Indian plants[11] were conducted. Endophytes are the microorganism which is living inside plant tissues and doing substantive harm or gain benefit other than residency.

Endophytes form a symbiotic relationship with their plant host. These microbes function as the biological defence for the plant against foreign phytopathogens. Antibiotics or hydrolytic enzymes can be released by endophytes prevents the colonization of microbial plant pathogens.[12] or prevent insects.[13] The present study was performed to study the phytochemical compounds present in the Endophytic Fungi isolated from the weed.

MATERIALS AND METHODS

The fresh and healthy leaves were collected from Mudichur (Tambaram), Chennai. Leaf samples are surface sterilized using 70% ethanol for 1 min, 0.1% mercuric chloride solution for 3 min, and sterile distilled water for 1 min and then allowed to surface dry on filter paper. The surface sterilized leaf samples are cut under sterile conditions into small pieces (2–3 cm).

Isolation of Endophytic Fungi

After proper drying 4 pieces of plant parts were inoculated in PDA plate supplemented with antibiotic (Chloromphenicol) and incubated at 28 ±10C for 5 to 7 days. Pure colonies were transferred on PDA slant. The fungal strains in the pure culture were preserved on

potato dextrose agar (PDA) slant at 4 to 5°C with proper labeling and were sub-cultured from time to time.[14]

Identification of Endophytes

Identification of fungal cultures involves both microscopic and macroscopic studies revealing the morphological features of the fungi. Pure culture of the fungi obtained was periodically examined for the morphology of the fungal culture, the mechanism of spore production and characteristics of the spore by following the standard mycological manuals. Also, the shape, method of spore production and the arrangements of spores (Conidial ontogeny) is examined. Macroscopically, colony features (Colour and Texture) were also noted.

Secondary Metabolite Production

Secondary metabolites are low molecular weight metabolites often have potent physiological activities produced by an organism that are not required for primary metabolic process. There are so many factors influencing the growth in turn to the production of secondary metabolites and hence preliminary studies are necessitated.

Preparation of Fermentation Medium

1L Erlenmeyer flask containing 300ml of PDB was autoclaved at 121°C for 15 mins at 15lb pressure. The flask was inoculated with *Phyllosticta* species and was incubated for 21 days at 26°C condition. The flasks were observed everyday for the growth of the fungus. At the end of the period, after 21 days of culture, inoculated flasks were collected and the culture fluid was passed through four layer cheese cloth to remove solids and extracted with solvent.[15] 300ml of culture filtrate was processed for organic solvent extraction using Dichloromethane.

Phytochemical Analysis from Endophytic Fungal Extract

Test for Flavonoids: In the test tube, containing 1 ml of fungal crude extract a few drops of 20% NaOH solution was added. A change to yellow colour was observed which on addition of acid changed to colourless solution depicted the presence of flavonoids

Test for Phenols: The fungal extract was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

Test for Tannins: The fungal extract was treated with alcoholic $FeCl_3$ reagent. A bluish black colour, which disappears on addition of a little dilute H_2SO_4, was followed by the formation of yellowish brown precipitate indicated the presence of tannins.

Test for Steroids: Libermann-Burchard reaction was performed to assess the presence of steroids. The fungal extract was added in 1 ml of chloroform solution. The mixture was treated with acetic anhydride and few drops of concentrated H_2SO_4 were added. A blue green ring indicated the presence of steroids.

Test for Saponins: The presence of saponins was determined by frothing test. The fungal extract was vigorously shaken with distilled water and was allowed to stand for 10 min. Formation of a fairly stable emulsion indicated the presence of saponins

Test for Carbohydrates: The presence of carbohydrates was determined by heating the sample. The fungal extract was taken and added with conc.H2SO4 in alcohol heated. A black colour indicates the presence of carbohydrates.[16,17]

RESULT AND DISCUSSION

Identification of Endophytic Fungi

The isolated endophytic fungi was identified as *Phyllosticta* Sp (Fig 1), based on the, morphological and the structure of fruting bodies. *Phyllosticta* is a very common and important pathogen of numerous plants including woody and herbaceous ornamentals. *Phyllosticta* only infects plant foliage causing leaf spots. It also can be commonly found as a saprophyte on decaying plant tissue. *Phyllosticta* causes small tannish-gray leaf spots with dark, brown to purple borders.

Pycnidia (fungal fruiting structures) are produced within the leaf spot, often in a ring pattern. Pycnidia are light to dark brown in color and partially embedded within plant tissue. Pycnidia contain short, simple conidiophores that produce the fungal spores. Spores are extruded through an opening (ostiole) at the top of the pycnidium. Conidia are very small, colorless (hyaline) and single celled. Often spores form a "tendril" as they are compressed and extruded through the pycnidia's ostiole. It is difficult to distinguish Phyllosticta from Phoma, another common leaf-spotting fungus.[18]

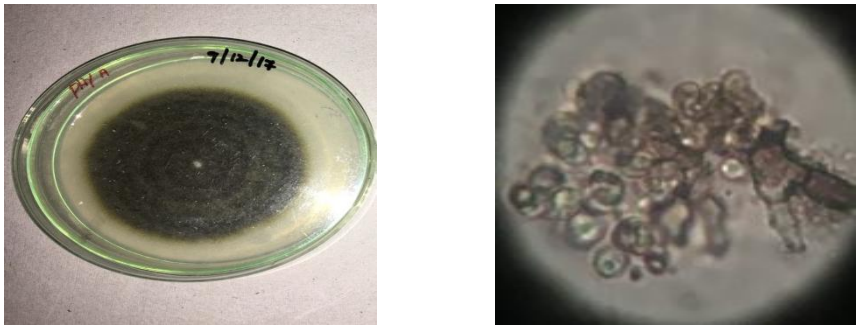

Fig. 1: Culture and Conidia of *Phyllosticta* sp.

Phytochemical Analysis

The isolation of endophytic fungi was made on PDA medium (Potato dextrose agar) and the identification was based on macroscopic and microscopic observations of the different strains isolated and using identification keys. The qualitative phytochemical analysis of Dichloromethane extracts of the endophytes was carried out using standard procedures. From the phytochemical analysis it was found that the Endophytic fungi shows the presence of active compounds like Tanins, Steroids and Saponins. Other compounds such as Flavanoids, Phenols and Carbohydrates were Absence (Fig 2 & Table 1).

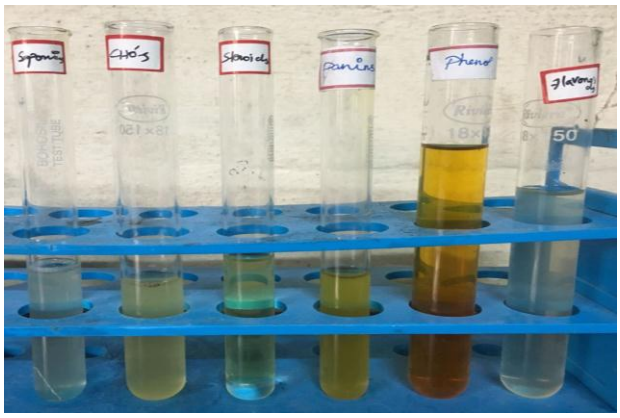

Fig. 2: Phytochemical analysis of the fungal extract.

Table 1: Phytochemical analysis of the fungal extract.

Phytochemicals	Test	Observation	Inference
Flavanoids	Aluminium chloride test	Reddish pink colour	-
Phenol	Ferric chloride test	Dark green	-
Tannins	Ferric chloride test	Blackish blue colour	+
Steroids	Liebermann-Burchard test	Blue green test	+
Carbohydrates	Conc. sulphuric acid test	Black colour	-
Saponins	Frothing test	Foam formation	+

CONCLUSION

The present study reveals that the phytochemicals present in the endophytic fungi *Phyllosticta* Sp, are effective alternative sources for antimicrobial drugs and antioxidant activity. Further Invitro and Invivo experiments are required to establish bioactivity and structural elucidation of these endophytic extracts. The study also suggests that these phytochemicals can be purified to find out the exact activity of each chemical components.

REFERENCES

1. James L, Evans J, Ralphs M, Child R, editors. Noxious Range Weeds. West view Press. Boulder, CO., 1991.
2. Udayaprakash NK, Bhuvaneswari S, Aravind R, Kaviyarasan V, Sekarbabu, H. A comparative study on antibacterial activity of common weeds. *Intl J of Phar & Bio Sci.*, 2011; 2(1): 677-83.
3. Sazada, S., A. Verma, A. A. Rather, F. Jabeen and M.K. Meghvansi. Preliminary phytochemical analysis of some important medicinal and aromatic plants. *Adv. in Biol. Res.*, 2009; 3: 188-195.
4. Adegoke AA, Adebayo-tayo BC. Antibacterial activity and phytochemicalanalysis of leaf extracts of *Lasienthera africanum*. *African Journal of Biotechnology*, 2009; 8(1): 77-80.
5. Mishra N, Behal KK. Antimicrobial activity of some spices against selected microbes. *Intl J of Pharm Pharm Science.*, 2010; 2(Suppl 3): 187-196.
6. Salehi, S. M.H., Y.Aynehchi, G. H. Amin and Z.Mahmoodi. Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins. IV. *Daru*., 1992; 2: 281-291.
7. Mojab, F., M. Kamalinejad, N. Ghaderi and H.R. Vahidipour. Phytochemical Screening of Some Species of Iranian Plants. *Iran. J.Pharm. Res.*, 2002; 77-82.
8. Rizk, A.M. Constituents of plants growing in Qatar. I. A chemical survey of sixty plants. *Fitoterapia*, 1982; 52: 35-44.
9. Ayoola, G.A., Sofidiya, T., Odukoya, O and Coker, H. A. B. Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. *J. Pharm. Sci. & Pharm. Pract*, 2006; 8: 133-136.

10. Panthi, M.P. and Chaudhary, R.P. Antibacterial activity of some selected folklore medicinal plants from west Nepal. *Scientific World*, 2006; 4(4): 16- 21.
11. Kantamreddi, V. S. S. N., Y. Nagendra Lakshmi and V. V. V. Satyanarayana Kasapu. Preliminary phytochemical analysis of some important Indian medicinal plant species. *Intl Journ. Pharm. and Bio Sci*., 2010; 1(4): 351-358.
12. Hallmann J, Berg G and Schulz B Isolation procedures for endophytic microorganisms. In: Schulz B, Boyle C, Sieber TN (eds) *Soil biology*, Microbial Root Endophytes Springer-Verlag, Berlin Heidelberg, 2006; 9: 299–319.
13. Azevedo JL, Walter MJ, Pereira JD and Araujo WL "Endophytic microorganism: a review on insect control and recent advances on tropical plants" *Electron J Biotechnol*, 2000; 3: 40–65.
14. Rajagopal K and Suryanarayanan TS Isolation of endophytic fungi from leaves of neem (*Azadirachta indica A. Juss*.) *Current Science*, 2000; 78: 1375-1378.
15. Strobel G, Yang X, Sears J, Kramer R, Sidhu RS and Hess WM Taxol from *Pestalotiopsis microspora* of *Taxus wallachiana*. *Microbiology*, 1996; 142: 435-440.
16. Akanksha Bharadwaj Phytochemical screening of endophytic fungi isolated from spikes *pinus roxburghii*. *Clinical microbiology*, 2015; 6: 3-1.
17. Nameirakpam nirjanta Phytochemical analysis and enzyme analysis of endophytic fungi from *Centella asiatica*. *Journal of tropical biomedicine*, 2012; S1280-S1284.
18. Plant Pathology: Extension & Outreach: Plant Disease Library.