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STUDIES ON PHYLLOPLANE MYCOFLORA AND MEDICINAL PLANTS

G. Angela, P. Usha Shri

[1,2]*Department of Botany, St. Ann's College for Women (Autonomous)*
Mehdipatnam, Hyderabad – 28, Telangana State, (India)

ABSTRACT

Phylloplane fungi are the mycota growing on the surfaces of leaves. The leaf surface is a complex terrestrial habitat that is characterized by a variety of microorganisms. The leaf surface is a suitable environment for microbial growth because of a thin film of nutrients deposited on the leaf. The microbial communities are influenced by external and/or internal factors such as nutrient availability, humidity, temperature, leaf age and type, and presence of inhibitors. In the present study fungi growing on four medicinally useful plants Hibiscus rosasinensis, Rauwolfia serpentina, Datura alba and Curcuma longa are considered. These plants were chosen for the study as they are medicinally useful and readily available. Phytochemical analysis was carried out to reveal the presence or absence of medicinally active constituents. The screening showed that the leaves were rich in glycosides, flavonoids, saponins and terpenoids. Some fungal species of Alternaria, Yeast, Aspergillus, Mucor, Cunninghammella, Cladosporiumm, Syncephalastrum, Trichoderma, Penicillium and Curvularia were isolated from surface sterilized leaves of the four plants.

Key words: *phylloplane, medicinal plants, active constituents*

I. INTRODUCTION

Microorganisms are known to be ubiquitous, their growth and survival depends on complex set of environmental and nutritional factors. The ubiquity of microorganisms is supported by Baas-Becking hypothesis: "everything is everywhere but the environment selects" [1] Phylloplane fungi are the mycota growing on the surface of leaves. There are two groups of phylloplane fungi: residents and casuals. Residents can multiply on the surface of healthy leaves without noticeably affecting the host whereas, casuals land on the leaf surface but cannot grow. According to [2, 3] the phylloplane, or leaf surface, represents an important terrestrial habitat that harbors a wide range of microorganisms. Filamentous fungi from the phylloplane may be either parasites, saprophytes, endophytes or epiphytes [4]. The leaf surface is a suitable environment for microbial growth because of a thin film of nutrients deposited on the leaf. The microbial communities are influenced by external and/or internal factors such as nutrient availability, humidity, temperature, leaf age and type, and presence of inhibitors (chemical compounds produced by the plant) [2, 5, 6, 7, and 8]. This complex relationship can either be beneficial or harmful.

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The medicinal plants have been used by humans from the pre-historical times. It has been well recognized that human health and well-being are directly dependent on biodiversity. Medicinal plants contribute substantially to health, cultural integrity and local economies, particularly among the poor, and particularly for women, children and the elderly. [9, 10]. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [11]. Medicinal plants produce metabolites which act as defense system to protect against diseases. Phytochemicals are divided into two groups, which are primary and secondary constituents, according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, phenolic compounds[12] and many more such as flavonoids, tannins and so on.

The following plants were selected for the study as they are medicinal plants having good properties and are also readily available in our college campus.

- *Datura alba*
- *Curcuma longa*
- *Hibiscus rosasinensis*
- *Rauwolfia serpentine*

II. MATERIALS AND METHOD

Fresh leaves of the above plants were collected, placed in sterile bags and brought to the laboratory.The mycoflora were grown on Potato Dextrose Agar medium. 200gm of peeled and sliced potatoes were taken in a conical flask and distilled water is added and boiled for 25-30 minutes. It is now filtered through muslin cloth and 20gm of dextrose and 15gm of agar are added, made upto to 1000ml with distilled water and shaken well to dissolve the contents. The pH of the medium is maintained at 5.6. The contents of the conical flask are autoclaved at 121^{o}C for 15 minutes. Leaf segments of all the four plants were taken into conical flasks and serial dilution technique was followed. Streptomycin was added to the medium in the petriplates.

0.2ml of the sample was collected and uniformly spread on the medium taken in petriplates. The petriplates were kept undisturbed at room temperature for 5days. The fungal colonies were observed and slides were prepared from the colonies to study the fungi. The mycelial strands were picked from the cultures, a drop of lactophenol is placed on it, the hyphae were separated using sterile needles and a coverglass is placed on it. The slide is gently heated to drive out air bubbles and it is sealed. The slides were observed under Wesvox stereo binocular microscope.

The identification of the fungi is done using standard laboratory manuals, the manual of soil fungi [13] and Dematiaceous Hyphomycetes and More Dematiaceous Hyphomycetes [14].

2.1 Phytochemical Analysis

Phytochemical tests were carried out on the aqueous and ethanol extract and on the powdered specimens using standard procedures to identify the constituents as described by [15, 16, 17].

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2.2 Processing of plant samples

The leaves of all the four plants under study were thoroughly washed with distilled water. They were dried in an oven at a temperature of 30-35^{0}C for 3days. The dried leaves of each plant were powdered separately and stored in air tight containers protected from moisture and sunlight to be used for analysis.

2.3 Qualitative analysis

2.3.1 Test for steroids

An ethanol extract of 0.5g of each of the leaf extracts was taken and 2ml of acetic anhydride and 2ml of sulphuric was added. The colour changed from violet to blue or green indicating the presence of steroids.

2.3.2 Test for Terpenoids

To each of the leaf extracts (5ml), 2ml of chloroform was added and then carefully 3ml of conc.H_2SO_4 was added. A reddish brown colouration at the interface was formed indicating the presence of terpenoids.

Test for Cardiac Glycosides (Keller Killani test)

To each of the leaf extracts (5ml), add 2ml of glacial acetic acid containing one drop of ferric chloride solution. Now by adding 1ml of conc.H_2SO_4 along the sides of the test tube a brown ring is formed at the interface indicating the presence of cardiac glycosides.

2.3.3 Test for flavonoids

A small quantity of an aqueous filtrate of each plant is taken and 5ml of dilute ammonia solution is added. This is followed by the addition of conc.H_2SO_4. Appearance of yellow indicated the presence of flavonoids.

2.3.4 Test for saponins

From each of the powdered samples of the leaf 2g are taken and boiled in 20ml distilled water in a water bath and filtered.5ml of distilled water is added to 10ml of the filtrate in a test tube and shaken thoroughly until froth is obtained. The froth is then mixed with 3 drops of olive oil. An emulsion is formed indicating the presence of saponins.

III. RESULTS

3.1 Biodiversity

A total number of 10 fungal species were isolated from surface sterilized leaf segments of four important medicinal plants such as *Hibiscus rosasinensis, Rauwolfia serpentina, Datura alba and Curcuma longa* by dilution plating technique. On the phylloplane of *Hibiscus rosasinensis* were found *Alternaria alternata, species of Cunninghamella, Mucor and Yeast. Rauwolfia serpentina* was inhabited by *Syncephalastrum racemosum* and *Aspergillus flavus*. *Datura alba* leaves had species of *Trichoderma* and *Penicillium* growing on them. Phylloplane of *Curcuma longa* was inhabited by a species of *Cladosporium and Curvularia lunata.*

Table 1: Fungi isolated from the Phylloplane of medicinal plants

S.No	Medicinal plants	Fungal species
1	*Hibiscus rosasinensis*	*Alternaria alternata* *Yeast* *Cunninghamella sp.* *Mucor*
2	*Rauwolfia serpentina*	*Aspergillus flavus* *Syncephalastrum racemosum*
3	*Datura alba*	*Trichoderma sp.* *Penicillium sp.*
4	*Curcuma longa*	*Cladosporium sp.* *Curvularia lunata*

3.2 Phytochemical Analysis

The present study carried out in the plant samples revealed the presence (or) absence of medicinally active constituents. Saponins were present in *Hibiscus rosasinensis, Rauwolfia serpentina* and *Curcuma longa.* They were absent in *Datura alba.* Terpenoids and steroids were present in all the four medicinal plants. Favonoids, and cardiac glycosides,were found in *Hibiscus rosasinensis, Rauwolfia serpentina* and *Curcuma longa.* .

Table- 2: Preliminary phytochemical screening of *Hibiscus rosasinensis , Rauwolfia serpentina, Datura alba, Curcuma longa*

Phytochemical constituents	***Hibiscus rosasinensis***	***Rauwolfia serpentina***	***Datura alba***	***Curcuma longa***
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	-	+
Flavonoids	+	+	-	+
Saponins	+	+	-	+

(+) = Positive (-) = Negative

IV. DISCUSSION

Biodiversity is the variation of life forms within a given ecosystem. Biodiversity is often used as a measure of the health of biological systems. Biodiversity of fungi is essential for anyone collecting or monitoring any fungi. Fascinating and beautiful fungi are vital components of nearly all ecosystems and impact human health and our economy in a myriad of ways. Standardized methods for documenting diversity and distribution have been lacking. One third of fungal diversity of the globe exists in India. Out of 1.5 million of fungi, only 50% are

characterized till now. Unfortunately, only around 5 – 10% of fungi can be cultured artificially. Fungi play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bio remediation and many other ways fungal biodiversity has become an integral part of the human welfare [18].

In the present study totally 10 species of fungi were isolated from the phylloplane of medicinal plants by dilution plating technique. Although the present study deals with the diversity of fungal communities associated with some medicinal herbs, the actual diversity may depend on the methods used for gathering and handling leaf samples, size of the leaf fragments and culture.

The number of fungal colonies and the percentage cover of phylloplane fungi can be estimated by direct observation using light microscopy [19]. The leaf washing method cannot provide a quantitative description of the species richness of the phylloplane fungi as leaf washings only indicate the amount of porpagules, but not the fungal biomass. Thus the leaf washing method is suitable for qualitative studies only [20].

Phytochemical Screening

In the present investigation the phytochemical screening of the medicinal plant studies showed that the leaves were rich in flavonoids, terpenoids, cardiac glycosides and saponins. They were known to show medicinal activity as well as exhibiting physiological activity.

Flavonoids, terpenoids, saponins, cardiac glycosides and steroids were reported from *Hibiscus rosasinensis* . Young leaves and flowers of *Hibiscus* are used in case of headache. Decoction of leaves, root and fruits are helpful in treatments of arthritis, boils and coughs, and the fruit is used externally in cases of sprains, wounds and ulcers. Alkaloids in the plants reduce blood pressure, depress activity of the central nervous system and act as hypnotics .

The phytochemical analysis of *Rauwolfia serpentina* flavonoids, terpenoids, saponins, cardiac glycosides and steroids were reported. The high saponin content of *Rauwolfia serpentina* substantiates the use of this extracts to stop bleeding and in treating wounds.It has calming effect over mind and brain. It induces sleep. It also relieves excited state of mind, hence useful in schizophrenia (insanity).It decreases blood pressure. Juice of leaves can be applied to the eyes as a remedy for corneal opacity.

*Datura alba h*as been used extensively in medicine . Steroids and terpenoids were reported in phytochemical screening. It is a strange ploy played by nature that a plant known almost exclusively for its toxicological effects finds its use in the medical field. It has been used as an anaesthetic for setting bones, treating bruises and wounds, skin ulcers, hemorrhoids, asthma, rheumatism, whooping cough, muscle spasm, sciatica, painful menstruation, etc. [21]. Most of these uses have been proved effective by modern medicine.

In *Curcuma longa* steroids, terpenoids, cardiac glycosides and flavonoids were reported. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and anticancer activities and thus has a potential against various malignant diseases, diabetes, allergies, arthritis, Alzheimer's disease, and other chronic illness.

V. CONCLUSION

From the present study, it may be concluded that phylloplane flora is highly sensitive to environmental factors. Phylloplane flora with a specific area quickly responds to change in environmental conditions from locality to locality. Generally, most phylloplane microorganisms show little specificity to their plant host; hence changes during their life cycle and in the environment may affect their number. The incidence of fungi like *Cladosporium, Curvularia, Aspergillus Mucor*, were the most common. There is need for investigation of the complete phylloplane mycoflora of a particular host, its development and the factors affecting its size and composition as the actual diversity depends on many factors.

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