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# PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF FIVE MEDICINAL PLANTS AGAINST MYROTHECIUM SP

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## ABSTRACT

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science to have medicinal effects. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The main objective of the study is to isolate and identify the organism present in the infected bitter gourd leaves with NCBI BLAST homology and it was identified as organism belonging to *Myrothecium sp*., (Accession no. HM219863). Five medicinal plants, viz *Indigofera tinctoria, Piper nigum, Curcuma longa*, *Polygala elongata* and *Polygala glabra* in different solvent extracts are used in this study shows a good control to the growth of fungi *Myrothecium sp*., and all the five medicinal plants extracts were screened for their phytochemical content. Qualitative phytochemical tests were used to detect the presence of alkaloids, tannins, saponins, flavonoids, glycosides and phenols. Presence of these phytochemicals in the medicinal plants indicates the presence of disease preventive properties against *Myrothecium sp*.,

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## KEYWORDS

*Myrothecium sp*., Bitter gourd leaves, Medicinal plants, phytochemical properties.

## INTRODUCTION

*Myrothecium sp.,* generally cause round dark-brown leaf spot in cucurbits [1] which on later stage coalesces to form blighted areas on the leaves [2]. The discovery of medicinal plants in different parts of the world is important to agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits. Medicinal plants represents rich source from which antimicrobial agents may be obtained. The different parts used include root, stem, flower, leaves, fruit, twigs exudates and modified plant organs. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [3]. The antimicrobial activities of plant extracts may reside in a variety of different components. The beneficial medicinal effects of the plant materials typically result from the combinations of secondary product present in the plant [4]. In plants, these compounds are mostly secondary metabolities such as alkaloids, steroids, tannins, phenol compounds, flavonoids, steroids, reisns, and fatty acids which are capable of producing definite physiological action on disease causing organisms. The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in treatment of several diseases. A number of reports concerning the antifungal screening of plant extracts of medicinal plants have appeared in the literatures [5,6] .The present study is to isolate and identify the organism present in the infected bitter gourd leaves and to screen the phytochemical properties of five medicinal plant extracts; *Indigofera tinctoria, piper nigum, curcuma longa, Polygala elongata* and *Polygala glabra* which is responsible for the antimicrobial activity against the organism present in the infected bitter gourd leaves.

## MATERIALS AND METHODS

### *Isolation of culture*

The infected bitter gourd leaves which was collected from a farm, located near by VIT University, Vellore, Tamilnadu, India was crushed and carried out serial dilution and spread plated on fungal media, *Myrothecium* agar media [7] and incubated at room temperature for 1 to 2 weeks, and were subcultured to get pure fungal culture.

### *Morphological Characterization and Sequencing of culture*

The pure fungal culture isolated was carried out for morphological identification and Sequence analysis was performed with an automated sequence analyzer (ABI Prism 310 genetic analyzer; Applied Biosystems). Sequence similarities were assessed by a search for homology with GenBank sequences by using the BLAST search program. Sequence similarities of 98% or more over a range of at least 75% of the amplification product were considered significant [8].

### *Plant material and extract preparation*

Fresh medicinal plants were collected from the medicinal garden and were identified with the help of a botanist. Leaves of *Indigofera tinctoria, Polygala elongata, Polygala glabra* and Fresh Pepper corns and Turmeric were washed with distilled water, shade dried,

powdered and stored in air tight container separately. Aqueous and solvent extracts were prepared [9] using this plant material by transferring 1 g of the powdered plant material to 10 ml of aqueous and different solvents separately, which were allowed to soak for 24hrs at room temperature, after heating the extracts for an hour at 100°C. The mixture was then centrifuged at 2000 rpm for 10 min at 4°C. Supernatants were filtered through Whatman filter paper No.1 and the extracts were stored in sterile conditions separately for further use.

***Gel diffusion method***

Gel diffusion method for antifungal activity was carried out. Medicinal plants extract with different concentrations (50, 75, 100µl) in each well and with different solvents (aqueous, ethanol, methanol, and benzene) along with controls were plated in *Myrothecium* culture plates and the zone of inhibition was measured.

***Screening of Phytochemicals***

The Medicinal plant extracts were screened for the presence of phytochemical constituents which acts the antimicrobial agents.

Test for alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. 1 ml of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids [10].

Test for Flavonoids

To 5 ml of diluted ammonia solution, a portion of the aqueous filtrate of each plant extract was added. Followed, the addition of concentrated sulphuric acid, A yellow colouration observed in each extract indicated the presence of Flavonoids. The yellow colouration disappears on standby.

Test for Tannins

0.5 g of the dried powdered samples of each plant was boiled in 20 ml of water in a test tube and filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or a blue black colouration which indicates the presence of tannins [11].

Test for Saponins

50 mg of the dried powdered samples of each plant was diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 min. A 2 cm of foam indicates the presence of saponins.

Test for Cardiac Glycosides

About 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% $FeCl_3$. This was under laid with conc. $H_2SO_4$. A brown ring obtained at the interface indicated the presence of a deoxy sugar, characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form just above ring and gradually spreads throughout this layer [12].

Test for phenols

50 mg of each powdered medicinal plant samples were dissolved in 5 ml of distilled water. To this few drops of neutral ferric chloride solution was added. A dark green colour indicated the presence of phenols.

## RESULTS

In the present study the organism isolated from the infected bitter gourd leaves was identified as *Myrothecium sp.,* according to the microscope (Fig-1) and culture morphology (Fig-2).

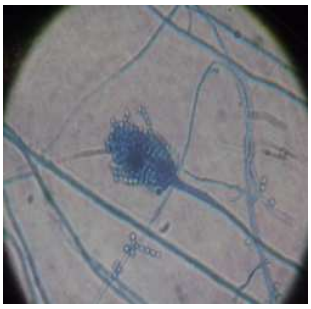


**Fig-1**
***Microscope Morphology of Myrothecium sp***

**Fig-2**
***Culture Morphology of Myrothecium sp***

The isolated culture was further confirmed as the organism

belonging to the *Myrothecium sp* by hybridization which showed sequence similarity (Table 1) with GenBank sequences below 98 % (Accession no. HM219863).

**Table 1**
**Sequence of *Myrothecium sp***

| |
|---|
| 1 gttcgtacta gcgagcccac ctcccacccg tgtttactgt accttagttg cttcggcggg |
| 61 cccgccattc gtggccgccg ggggctctca gccccgggcc cgcgcccgcc ggagacacca |
| 121 cgaactctgt ctgatctagt gaagtctgag ttgattgtat cgcaatcagt taaaactttc |
| 181 aacaatggat ctcttggttc cggcatcgat gaagaacgca gcgaaatgcg ataactagtg |
| 241 tgaattgcag aattccgtga atcatcgagt ctttgaacgc acattgcgcc ccctggtatt |
| 301 ccgggggggca tgcctgtccg agcgtcattg ctgcccatca agcacggctt gtgtgttggg |
| 361 tcgtcgtccc ctctccgggg gggacgggcc ccaaaggcag cggcggcacc gcgtccgatc |
| 421 ctcgagcgta tggggctttg tcacccgctc tgtaggcccg gccggcgctt gccgaacgca |
| 481 aatcaatctt ttccaggttg acctcggatc aggtaaggga aa |

Antimicrobial properties of medicinal plants were studied against *Myrothecium sp*., *Curcuma longa* in methanol and ethanol extracts found to be very active against the fungi *Myrothecium*. Medicinal plants in aqueous and benzene extract do not show any inhibition against the fungal culture. All the medicinal plants in methanol and ethanol extract shows good anti fungal activity against *Myrothecium sp*., (Table 2 & Graph 1). Combinations of Plant extracts were also carried out. In this, the combinations containing *Curcuma longa* shows good inhibition against the fungi in 75µl concentration. (Table 3).

**Table 2**
***Effect of plant extracts on control of Myrothecium***

| Sl. No. | Medicinal Plant | Solvents | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Water | | | Benzene | | | Ethanol | | | Methanol | | |
| | | 50 µl | 75µl | 100µl | 50µl | 75µl | 100µl | 50µl | 75µl | 100µl | 50µl | 75µl | 100µl |
| 1. | *Indigofera tinctoria* | - | - | - | - | - | - | 1mm | 2mm | 3mm | - | 12mm | 12mm |
| 2. | *Piper nigum* | - | - | - | - | - | - | - | 1mm | 2mm | 11mm | 12mm | 17mm |
| 3. | *Curcuma longa* | - | - | - | - | - | - | 4mm | 7.5 mm | 6mm | 12mm | 13.5 mm | 15mm |
| 4. | *Polygala elongata* | - | - | - | - | - | - | - | 3mm | 4.5 mm | 11.5 mm | 12.5 mm | 14mm |
| 5. | *Polygala glabra* | - | - | - | - | - | - | - | - | 3.5 mm | 10mm | 11mm | 15mm |

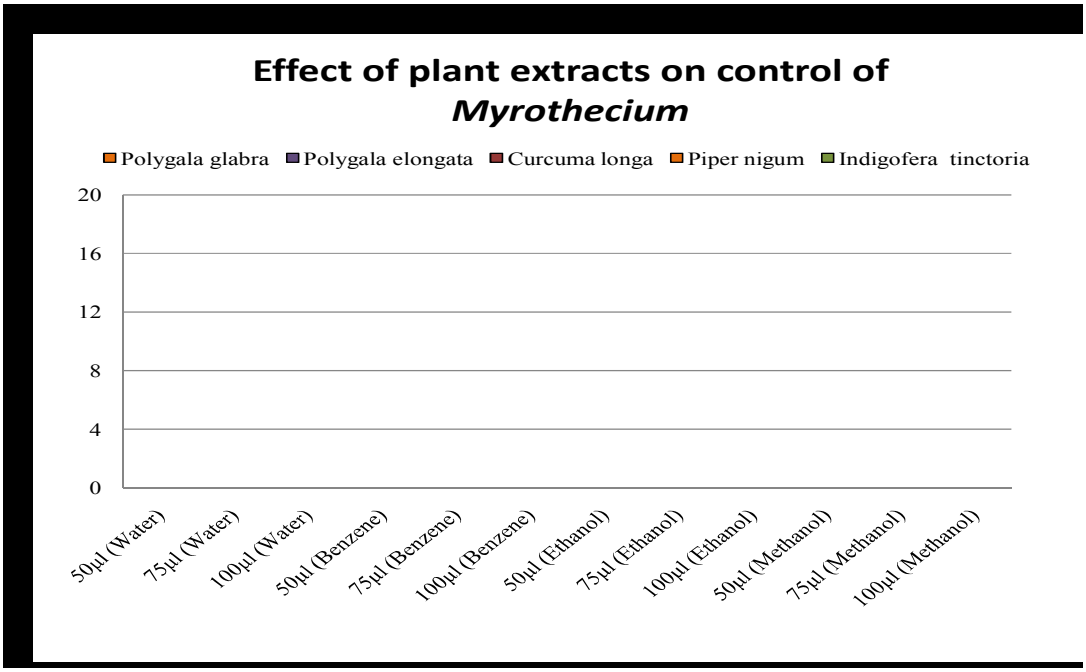
- No zone was observed

**Table 3**
***Effect of combination of plant extracts on control of Myrothecium***

| S.No | Extracts | AB | AC | AD | BC | BD | CD | ABC | BCD | ACD | ABD | ABCD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Methanol | 7mm | 11.5 mm | 7mm | 9mm | 1.5 mm | 4.5 mm | 6mm | - | 11mm | 5.5 mm | 14mm |
| 2. | Ethanol | - | 3mm | 9mm | 10mm | - | 2mm | 4mm | - | 9mm | - | - |

***A****-Indigofera tinctoria;* ***B****-Piper nigum; C- Curcuma longa; D-Polygala elongata.*

**Graph 1**
**Effect of plant extracts on control of *Myrothecium***




Phytochemicals constituents were screened for each plant extract. Except for saponins in all the plant extract, alkaloid in *Curcuma longa*, Flavonoids in *piper nigum* and Tannin in *Curcuma longa*, all other Medicinal Plant extracts shows positive for phytochemical constituents (Table 4).

**Table 4**
***Screening of phytochemical constituents in medicinal plant extracts***

| Medicinal plants / Phytochemicals | Alkaloids | Flavonoids | Tannins | Saponins | Cardiac glycosides | Phenols |
|---|---|---|---|---|---|---|
| *Indigofera tinctoria* | ✓ | ✓ | ✓ | × | ✓ | ✓ |
| *Piper nigum* | ✓ | × | ✓ | × | ✓ | ✓ |
| *Curcuma longa* | × | ✓ | × | × | ✓ | ✓ |
| *Polygala elongata* | ✓ | ✓ | ✓ | × | ✓ | ✓ |
| *Polygala glabra* | ✓ | ✓ | ✓ | × | ✓ | ✓ |

*✓- Positive, × - Negative*

## DISCUSSION

Thus results show wide difference in the growth inhibition of medicinal plants extract against *Myrothecium sp.* The inhibition effect of medicinal plants may be due to active ingredients in the plants. These classes (such as alkaloids, saponins, tannins, flavonoids and glycosides) of compounds are known to have curative activity against several pathogens and therefore could suggest the use traditionally for the treatment of various illness [13,14] .The activities of tested plant depends on their kind, mode of extraction and used species tested. This finding can form the basis for further studies to prepare an optimize preparation of the herbal extract to further evaluate them against a wider range of fungal cultures. The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. The result of present study clearly indicates that the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity.

## CONCLUSION

This study demonstrated that folk medicine can be effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. From the present study it has been concluded that antifungal activity of these medicinal plants provide hope that they can form the basis for an alternative biocontrol agent in the management of bitter gourd infection.

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