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Studies on Phytochemical Screening and Antifungal Activity of Tinospora cordifolia

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Abstract: *Fruit rot is a very common and destructive disease that causes serious economic loss in production mainly of fruits and vegetables. Environmental friendly plant extracts have shown great potential as an alternative to synthetic fungicides. In the present study, premilinary phytochemical screening of Tinospora cordifolia revealed the presence of carbohydrates, proteins, aromatic amino acids, alkaloids, terpenoids, saponins, tannins, flavanoid s, phenolics, phlobatannins and sterols. The antifungal activity was tested against the two rot fungi i.e. Alternaria alternat a and Fusarium solani. Whole plant extract of T.cordifolia appeared significantly more effective to control the vegetative growth of Alternaria alternata than that of Fusarium solani.*

Keywords: *Phytochemical Analysis, Antifungal Activity, Tinospora cordifolia , Rot fungi*

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I. Introduction

Fruits play a vital role in human nutrition by supplying the necessary growth factors such as vitam ins and essential minerals in human daily diet and that can help to keep a good and normal health. Fruit rot is a very common and destructive disease that causes serious economic loss in production mainly of fruits and vegetables. It is estimated that abo ut 20-25% of the harvested fruits are decayed by pathogens during post - harvest handling even in developed countries [1 -2]. In developing countries, post - harvest losses are often more severe due to inadequate storage and transportation facilities Fungal fruits infection may occur during the growing season, harvesting, handling, transport and post -harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal deca yed [3]. The most important fungi causing post -harvest diseases include: *Penicillium* spp, *Aspergillus spp*, *Alternaria spp*., *Fusarium spp.*, *Botrytis cinerea*, *Monilinia lax* and *Rhizopus stolonifer* [4]. The excessive use of synthetic fungicides [5] complemented with high costs, residues in plants, and development of resistance, has left a negative effect on human health and the environment [6-7]. Environmental friendly plant extracts have shown great potential as an alternative to synthetic fungicides [8] and make excellent leads for development of new pesticides . But the actual use of these products for the control of post harvest pathogens of fruits is still limited. So, in the present study , an endeavour has been made to determine phytochemical profiles and antifungal activity of whole plant extract of *Tinospora cordifolia* against two rot fungi i.e. *Alternaria alternat a* and *Fusarium solani*.

II. Materials and Methods

Plant Material

The plant material was collected and shade-dried at room temperature for 10 –15 days. Dried plant samples were crushed and ground into fine powder with mortar and pe stle. 5gm of plant material is dissolved in 50ml of sterile water and left overnight . The crude plant extract was centrifuged at 5000 rpm for 15 minutes. The clear supernatant was filter sterilized and was stored at 4°C until use.

Phytochemical tests

Phytochemical analysis o f the water extract of *T. cordifolia* was carried out by using following standard methods [9].

Preparation of pure culture:

Diseased portion of the tomato fruit were scrapped with the aid of scalpel, the segments were plated on Czapek Dox agar media. Plates were incubated at 25ºC and observed d aily for emergence of colonies. The isolates were sub-cultured to get pure culture kept for further experimental purposes. The fungi isolates were identified under the microscope, using lacto phenol cotton blue as the staining agent based

on morphological characteristics.

Disc diffusion:

Czapek dox agar medium with different concentration (10percentage, 30percentage, 50percenta ge, 70percentage, 75percentage and 100percentage) of the plant extracts of the test plants were prepared. About 10 ml of the me dium and 10 ml of extract were poured into each Petri plate and allowed to solidify. Five mm disc of old culture of the test fungi were placed at the centre of the Petri plates and incubated at 25±2 ºC for 24 - 48 hours. After incubation the colony diameter was measured in millimeter. For each treatment two replicates were maintained. Czapek dox agar medium without the plant extract served as control. The fungi toxicity of the extracts in terms of percentage inhibition of mycelial growth was calculated by using the formula

Percentage growth inhibition = dc – dt/dc x 100

Where dc = Average increase in mycelial growth in control, dt = Average

Increase in mycelial growth in treatment.

III. Results

Phytochemical analysis

Phytochemicals are antimicrobial compounds and pesticides of antimicrobial agents which are found in aromatic and essential oil plants which have made great contribution for quick and effective management of plant disease and microbial contamination in several agricultural conditions. Premilinary phyt ochemical analysis revealed the presence of carbohydrates, proteins, aromatic amino acids, alkaloids, terpenoids, saponins, tannins, flavanoids , phenolics and phlobatannins (Table 1).

Table: 1 Phytochemical analysis of water extract of *Tinospora cordifolia*

S.No	Compound	Status
1	Carbohydrates	Present
2	Proteins	Present
3	Aromatic amino acid	Present
4	Alkaloids	Present
5	Terpenoids	Present
6	Saponins	Present
7	Tanins	Present
8	Flavanoids	Present
9	Phenolics	Present
10	Phlobatannins	Present
11	Sterols	Present

Antifungal activity of whole plant extract

The data revealed the significant reduction of vegetative growth of two rot fungi i.e *Alternaria alternata* and *Fusarium solani with* increase in concentration of whole pla nt water extract of *Tinospora* cordifolia. The radial mycelial diameter of *Alternaria alternata* observed at 10% of concentration of water extract 25.63±0.65mm which was reduced to 9.43±0.77mm at 100percentage concentration of water extract. The maximum percent growth inhibition *observed* was 66.11%. Whereas the radial mycelial diameter of 20.70± .081mm of *Fusarium solani* was observed at 10% of concentration of water extracts which was reduced to 9.33±0.43mm at 100 percentage of water extract concentration. The maximum percentage growth inhibition of mycelia exhibited was (61.38%). In our study, it was observed that the higher the concentration of aqueous extract, the higher the potency on the inhibition of mycelia growth of the fungal strains tested (T able 2 and 3). In the present investigation the whole plant extract of *T.cordifolia* tested appeared significantly more effective to control the vegetative growth of *Alternaria alternate* than that of *Fusarium solani.*

Table 2 Effect of different concentration of w ater extract of Tinospora Cordifolia on mycelial growth of Fusarium solani

Concentration of	Diameter of fungus	Percent growth

plant extract (percentage)	**mycelia (mm)**	**inhibition (%)**
0	24.16± 0.84	0.00
10	20.70± .081	14.32
30	17.10 ±.081	29.22
50	15.30±0.79	36.67
70	14.63±0.24	39.44
75	12.66±1.21	47.59
100	9.33±0.43	61.38

Table 3 Effect *of different concentration of water extract of Tinosp ora Cordifolia on my celial growth of Alternaria alternata*

Concentration of plant extract (percentage)	**Diameter of fungus mycelia (mm)**	**Percent growth inhibition (%)**
0	27.03±0.12	0.00
10	25.63±0.65	5.17
30	22.33± 1.35	17.38
50	19.86±0.12	26.52
70	16.36±0.21	39.47
75	14.91±0.059	44.83
100	9.43±0.77	66.11

IV. Discussion

Knowledge of the phytochemical constituent s of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for t he synthesis of complex chemi cal substances [10]. Alkaloids have proved to have various pharmacological properties such as hypotensive activity [11], anticonvulsant activity [12], and antiprotozoal , antimicrobial and antimalarial activity. Polyphenols are the major plant compounds with antioxidant activity. Presence of phenolics and flavonoids suggests the antioxidant activity of *T.cordifolia* due to their ability to reduce free radical formation and to scavange free radicals [13]. In addition the presence o f phenolics compounds indicates that the

plants are antimicrob ial agents [14].The anti-inflammatory effects of flavonoids isolated from Caesalpinia pulcherima have been reported [15]. It might be correlated with the obtained result that *T. cordifolia* has shown the presence of the flavanoids and the terpenoids in the whole plant extract.

Phytochemical screening of aqueous extract revealed the presence of carbohydrates, proteins and aromatic amino acids that might attributed to the nutritional value of this plant. Shrub also contains saponins. This compound has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant pr operties [16]. Hence this plant could be suitable for this purposes. Tannins exhibit the property of astringency which fastens the healings of wounds and inflamed mucous membranes [17]. Presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent [18]. It should be noted that steroidal compoun ds are of importance and interest in pharmacy due to their relationship with such compounds as *sex* hormones [19]. The presence of phlobatannins suggests the diuretic property of the plant [20]. The foregoing would suggest the possible utilization of *T.cordifolia* as diuretic agent. Hence present study highly recommended the whole plant extracts of *T. cordifolia* for the herbal preparations to the traditional medicinal practioners and for the pharmaceutical industries for the mass scale extract ions of the therapeutic agents.

The data on antifungal activity of T.cordifolia revealed the significant reduction of vegetative growth of two rot fungi i.e *Alternaria alternata* and *Fusarium solani with* increase in concentration of plant diffusates. Our results are suppor ted by studies reported by [21-23].Capsicum frutescence and Zingier officinal plant extract against Penicillium digitatum, Aspergillus niger and Fusarium sp. key post-harvest pathogens in citrus [24]. The use of biocontrol agents in plant disease control with plant extracts like lemon, citronella, clove, mint, thyme and oregano oils has been employed[25] as alternative control measures to replace the conventional synthetic pesticides. The report of different authors attributed the antifungal properties of medicinal plant to its phytochemical components. Among the great variety of secondary compounds found in plants, phenolics and terpenoids represent the main antimicrobial agents currently known. So the antifungal properties of *Tinospora cordifolia* may also be due to their active phytochemical components which caused inhibitory effects on the mycelia growth of the fungi. However,
the inhibitory potency may depend on the mode of extraction and the concentration of the extract used. The differential sensitivi ty of mycelial growth of the fungus to plant extract may be due to their chemical configuration and active ingredient, slower or faster absorption and detoxification after absorption on account of metabolic activity of the fungus [26].

V. Conclusions

Fungitoxicity of the plant extracts have been found to be promising against plant pathogens like *Fusarium solani* and *Alternaria alternata* and can be increased further by using the plant extract at higher concentrations. The finding of the present investigation su ggests isolation and characterization of the antifungal agent and its further use for crop protection strategies. Thus ascertains the value of this plant in quick and effective management of post harvest pathogens of various fruit diseases.

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