



## Antimicrobial activity of the root extracts of *T. pumila* and *T. tinctoria* on clinical and phytopathogens

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### ABSTRACT

*Tephrosia* is a pantropical genus, known for its varied flavonoids and possesses insecticidal, piscicidal and many therapeutic properties. In the present study the methanolic extracts of the roots of *T. pumila* and *T. tinctoria* were examined for their antimicrobial activities. The methanolic root extract of *T. tinctoria* exhibited better antimicrobial activity than *T. pumila*. Both the plant extracts have showed good antibacterial properties at different dose levels (100mg/ml- 500mg/ml). Interestingly both the plant extracts have showed considerable activity against tested fungal pathogens except for *Candida albicans*.

**Keywords:** *T. pumila*, *T. tinctoria*, Antimicrobial activity, Flavonoids, Fabaceae

### INTRODUCTION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted secondary metabolites. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Microbial infections are important health problems throughout the world and the plants are termed to be the possible sources of antimicrobial agents. The increasing prevalence of multi drug resistant strains of Bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections which adds urgency to the search for novel infection fighting strategies.

The genus *Tephrosia* (Fabaceae) with about 400 species is distributed chiefly in Asia, Africa, Australia and America<sup>1, 2</sup>. About twenty-four species of *Tephrosia* were recorded in India<sup>3, 4</sup>. Most of the *Tephrosia*'s are herbs to undershrub and are grown as weeds. The genus is well known for its richness in prenylated flavonoids and is considered to possess insect repellent, larvicidal, piscicidal, antimicrobial and anticancer properties<sup>5, 6, 7, 8, 9</sup>. A formulation known as Tefroli (marketed by Orient Pharma Private Limited, Madras) is being used to treat various liver disorders. In the light of the above biological activities, the microbiological studies have been carried out on the methanolic extracts of the roots of *T. pumila* and *T. tinctoria* against a collection of plant pathogens.

### MATERIALS AND METHODS

The plant materials (roots, 2 kg each) of *T. pumila* and *T. tinctoria* were collected in and around Anantapur and Chittoor districts of Andhra Pradesh, India in March 2004 and authenticated by Prof. T. Pulliah, Taxonomist, Department of Botany, Sri Krishnadevaraya University, Anantapur, India. A voucher specimen (SG/TP/04/124) was deposited at the Herbarium, Department of Botany, Andhra University, Visakhapatnam, India. The coarsely powdered root materials were extracted separately with methanol and concentrated under reduced pressure, to get their corresponding residues respectively. The residues showed positive color reactions with Lieberman-Burchard test, ferric chloride test and Shinoda's test indicating the presence of sterols and flavonoids.

The methanolic extracts of *T. pumila* (yield: 12%) and *T. tinctoria* (yield: 13.4%) were tested for antibacterial activity. Phytochemical analysis of the root extracts of *T. pumila* yielded isoflavones, rotenone, tephroline, stigmaterol and lupeol<sup>10, 11, 12</sup>, while *T. tinctoria* yielded dehydrodeguelin, 2-Hydroxy tephrosin, lupinifolin,  $\beta$ -sitosterol and ursolic acid<sup>13</sup>. In view of the therapeutic uses of flavonoids, the work was further extended to test the crude extracts (methanolic) of the roots of *T. pumila* and *T. tinctoria* for antibacterial, antifungal activities.

The Test organism cultures used for the study were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh.

### Antibacterial activity:

Antimicrobial activity by the cup-plate agar diffusion method<sup>14</sup> was carried out for the methanolic root extracts of *T. pumila* and *T. tinctoria* in five dose levels of 100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml and 500mg/ml. For antibacterial activity, the test organisms are

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**Table 1. Antimicrobial activity of the *T. Pumila* root methanolic extract (mg/ml)**

Microorganisms	100	200	300	400	500	* Standard
<b>Bacteria</b>						
<i>Erwinia caratovora</i>	10	10	12	13	15	18
<i>Pseudomonas syringii</i>	13	14	15	18	19	20
<i>Xanthomonas campestris</i>	11	12	14	16	17	19
<i>Proteus vulgaris</i>	8	8	10	11	12	18
<i>Serratia marcesans</i>	7	8	9	10	10	19
<b>Fungi</b>						
<i>Rhizoctonia solanii</i>	12	13	14	16	19	20
<i>Pterosporella phaseolina</i>	14	15	16	19	21	30
<i>Bipolaris bicolor</i>	16	17	19	21	23	19
<i>Saccharomyces cerevesiae</i>	8	8	9	10	12	19
<i>Candida albicans</i>	-	-	-	9	9	20

Values are zone of inhibition in mm, including the diameter of the disc (5mm). Standard : Chloramphenicol (100 mg/ml); Nystatin (100 mg/ml).

**Table 2 Antimicrobial activity of the *T. tinctoria* root methanolic extract (mg/ml)**

Microorganisms	100	200	300	400	500	* Standard
<b>Bacteria</b>						
<i>Erwinia caratovora</i>	12	12	15	17	19	23
<i>Pseudomonas syringii</i>	9	10	12	13	15	19
<i>Xanthomonas campestris</i>	11	11	12	13	14	20
<i>Proteus vulgaris</i>	8	9	9	10	12	16
<i>Serratia marcesans</i>	9	9	10	10	11	19
<b>Fungi</b>						
<i>Rhizoctonia solanii</i>	12	13	16	17	19	23
<i>Pterosporella phaseolina</i>	17	19	23	24	26	22
<i>Bipolaris bicolor</i>	12	14	15	17	18	28
<i>Saccharomyces cerevesiae</i>	8	9	10	12	12	19
<i>Candida albicans</i>	-	-	-	9	10	20

Values are zone of inhibition in mm, including the diameter of the disc (6mm). Standard : Chloramphenicol (100 µg/ml); Nystatin (100 µg/ml).

*Erwinia caratovora*, *Pseudomonas syringii*, *Xanthomonas campestris*, *Proteus vulgaris* and *Serratia marcesans* which are Phytopathogenic.

The nutrient agar medium (HIMEDIA) prepared was inoculated with 18 hours old cultures of the above mentioned test organisms and were transferred into sterile 15cm diameter petridishes. The medium in the plates were allowed to set at room temperature for about 10 minutes and allowed to solidify in a refrigerator for about 30 minutes. 5 cups of 5mm diameter were made in each plate at equal distance. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml and 500mg/ml respectively. 100µg/ml of each concentration were placed in the cups by means of sterile pipettes. In each plate one cup was used for control and standard. Antibiotic chloramphenicol (100µg/ml) was used as standard. The petridishes thus prepared were incubated for 24hrs at 37°C and were later examined by measuring the zones of inhibition and the results were tabulated in the tables 1 and 2.

#### Antifungal activity:

For testing antifungal activity the following fungal strains *Rhizoctonia solanii*, *Pterosporella phaseolina*, *Bipolaris bicolor*, *Saccharomyces cerevesiae* and *Candida albicans* were used. The nutrient PDA medium (HIMEDIA) was prepared and inoculated with

0.5 ml of aqueous suspension of the above mentioned test organisms, which were prepared from 48 hour cultures, are thus transferred into sterile petridishes. The medium in the plates were allowed to set at room temperature for about 10 minutes. 5 cups of 5mm diameter were made in each plate at equal distances. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml, 200mg/ml, 300mg/ml, 400mg/ml and 500mg/ml respectively. 100µg/ml of each of the above stock concentrations were placed in the cups by means of sterile pipettes. In each plate one cup was used for control and standard. Nystatin (100µg/ml) is used as reference standard. The petridishes thus prepared were incubated for 48 hours at 37° C and were later examined by measuring the zones of inhibition and the results were tabulated in the tables 1 and 2.

## RESULTS AND DISCUSSION

The methanolic root extracts of *T. pumila* and also *T. tinctoria* exhibited a broad spectrum of antibacterial activity against all the tested strains. The extracts of *T. pumila* showed good amount of activity against *Pseudomonas syringii* but showed mild response towards *Proteus vulgaris*. The root extracts of *T. tinctoria* displayed promising activity against *Erwinia caratovora*. Further both the extracts showed mild activity against *Serratia marcesans*. Both the methanolic extracts responded well against tested fungi, especially *T. pumila* extract showed promising activity against *Bipolaris bicolor* and *T. tinctoria* extract against *Pterosporella phaseolina*. Both the root extracts displayed mild activity towards *Saccharomyces Cerevesiae* and showed no activity against *Candida albicans*. The activity was found enhanced with increased doses.

The antimicrobial properties exhibited by the plant could be due to the presence of mixtures of active constituents (mostly prenylated flavonoids) which show a broad spectrum of biological and pharmacological activity. Most of the flavonoids are considered as constitutive antimicrobial substances recently termed as "Phytoanticipins" especially those belonging to prenylated flavonoids and isoflavones, but not excluding other classes of compounds. The antimicrobial effect of flavonoids is mainly attributed to the presence of phenolic hydroxyl groups that have affinity for proteins and therefore act as inhibitors of microbial enzymes as well as their biosynthetic pathways. In addition, substitution of the flavonoid ring system with prenyl groups is thought to increase their lipophilicity and consequently, enhances their antimicrobial activity through interaction with cellular membranes<sup>15</sup>.

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