

Full Length Research Paper

## Antifeedant and antimicrobial activity of *Tylophora indica*

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**Crude and pure extracts of *Tylophora indica* were investigated in view of antifeedant, antibacterial and antifungal properties. Leaf crude extract showed more antifeedant activity than stem and root against *Spodoptera litura*, a polyphagous pest on wide ranging crops. Among the pure compounds isolated, Tylophorine showed the highest antifeedant activity followed by Septicine, O-Methyl Tylophorinidine and simple aliphatic acid. Similarly the crude extracts of leaf has exhibited higher antibacterial activity than root and shoot against *Basillus subtilis*, *Staphylococcus aureus*, *Mycrococcus luteus* and *P. aergenosa*. In contrast, *Escherichia coli* was not inhibited even at higher concentrations of either crude extracts or extracted pure compounds of *T. Indica*. Pure compounds displayed strong antibacterial activity at lower concentrations in all tested bacterial strains except *E. coli*. While all the crude and pure compounds showed antifungal activity against *Aspergillus niger*, *Aspergillus fumigatus* and *Trichoderma viridae*, the pure compounds had strong antifungal activity compared to crude extracts.**

**Key words:** *Tylophora indica*, antifeedant activity, antimicrobial activity.

### INTRODUCTION

A considerable concern has been raised to adverse affects of pesticides affecting environment and resistance development. Hence, there is imperative need for development of safe alternative plant protections by botanical insecticides and antifeedants. The use of plants for medicinal and insecticidal purposes dates back to antiquity (Sofowora, 1984; Devanand and Usharani, 2008). Recent studies have focused on natural plant products as alternatives for disease control. Majority of rural dwellers in developing countries still depend on medicinal plants to prevent or eliminate diseases (Parekha and Chanda, 2008). Medicinal plants are cheaper, more accessible in the world. Thus, it is needed to encourage the use of medicinal plants as potential sources of new drugs. There has been an upsurge interest in herbal remedies, many of which with the herbal remedial being incorporated in to orthodox medical practice in several parts of the world (Satyanarayana et al., 1991; Ogbulie et al., 2007). Micro-

organisms and insect pests cause massive damage to the crops, horticultural plants, animals as well as humans. In tropical countries like India the damage potential of microorganisms and insects is immense due to congenial atmospheric factors such as temperature and humidity contributing to the growth of microorganisms and insect pests. Although chemical control measures are highly effective and have been in fact employed with tremendous success, there has been a renewed interest for botanicals possessing antimicrobial antifeedant and antibiotic properties (Makinde et al., 2007; Parekh and Chanda, 2008).

Whilst several natural insecticides were used in agriculture, only recently systematic investigations on antifeedant and other physiological, pharmacological and antibiotic activity of plant based compounds have been initiated. Several botanicals have been tested for their antifeedant activity (Dreyer and Jones, 1981; Devanand and Usharani, 2008). Compounds with antifeedant and medicinal properties have been identified from plants like *Reseda luteola*, *Trifolium incarnatum* (Robinson and Venkatachalam, 1929), *Morus alba* (Montgomery and Arn,

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1974), *Vitex negundo* and *Zingiber officinale* (Sahayaraj, 1998). Most of them displays antifeedant activity ranging strong to moderate, that this case opens a new eco-friendly source of plant based compounds for pest management.

Antimicrobial activities of several plant products have gained importance in recent times. Plant derived secondary metabolites like alkaloids, terpenoids and flavonoids have shown to interfere with many biological activities. They possess antibacterial, antifungal, cytotoxic or anti-tumour, antifeedant and insecticidal activities (Purohit et al., 1995; Muhammad, 2009). Folklore and Ayurvedic literature assert that some plants possess antiseptic and antimicrobial properties (Chatarjee et al., 1991). Satyanarayana et al. (1991) reported that crude extracts of *Flacourtia ramonhi* inhibited *S. aureus* and *E. coli*. Hiremath et al. (1993) also demonstrated antibacterial and antifungal activity from crude and different solvent extracts of *Acalypha indica*. The evaluation of solvent extracts of *Evolvulus alsinoids* against representative bacteria and fungi in *in vitro* conditions was reported by Purohit et al. (1995). Flavonoids are major groups of normal compounds due to their biological activity (Giesman, 1962). Antibacterial activity of some flavonoids was reported earlier by Sharma (1970). Alkaloids isolated from Rutaceae members such as Furoquinoline alkaloids, skimmianine isolated from *Fagara* species have shown antimicrobial activity (Tan et al., 1991). Although *T. indica* is a versatile medicinal plant, placing in restricted localities in Indian sub continents and parts of Africa, the information on the antifeedant, antimicrobial and antifungal activity of *Tylophora* species is insufficient. Hence the present study was carried out on antifeedant, antibacterial and antifungal activity of *T. indica*.

## MATERIALS AND METHODS

### Preparation of methanolic extracts

Fresh leaves, stem and roots of *T. indica* were collected from forests of Telangana region, Andhra Pradesh, India and these were shade dried and powdered. Nearly 10 gr of each crude powders were extracted with methanol. The methanolic extract was distilled in a rotation-vapour to obtain a concentrated sample. A yield of 6 - 9% was obtained and this was used as sample for further tests to be carried out.

### Purification of secondary metabolites from methanolic extracts

About 2 kg of shade dried and powdered plant material was extracted with 20 L of methanol in a soxhlet-apparatus, filtered and concentrated. The semisolid dark colored methanolic extract was macerated thrice with 2N HCl (100 ml) and allowed to stand for about 15 min. The aqueous HCl layer was collected, filtered and extracted with ethyl acetate in a separating funnel. The aqueous acidic solution was treated with NH<sub>4</sub>OH until the solution was alkaline and extracted thrice with ethyl acetate in separating funnel. The upper ethyl acetate fraction was collected, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and decanted the ethyl acetate layer into a clean dry china dish, concentrated under reduced pressure. The ethyl acetate

soluble part of the methanolic extract of the leaves of *T. indica* was fractionated by column chromatography on neutral alumina (200 mesh, ACME). A total of 278 fractions were collected by eluting with increasing order of solvents like petroleum ether, (Fraction 1 - 33), petroleum ether and Benzene 1:1 (Fraction 34 - 49), benzene (fraction 50 - 69), Benzene and ethyl acetate 9:1 (fraction 70 - 110), Benzene and ethyl acetate 8:2 (fraction 111 - 141), Benzene and ethyl acetate 7:3 (142 - 202), ethyl acetate (fraction 203 - 252) and ethyl acetate and methanol (fraction 253 - 278). All the fractions with uniform RF values upon TLC were pooled together. The IR, UV, NMR and Mass spectra revealed the presence of simple aliphatic acid in fractions 50 - 69, Septicine in 70 - 110, Tylophorine in 111 - 141, O-Methyl Tylophorinidine in 142 - 202 (Data not shown here).

### Culture medium

Nutrient broth /agar medium for bacterial cultures and potato dextrose agar medium for fungal cultures was prepared and sterilized as instructed by the manufacturers (Himedia, India).

### Microorganisms

The bacterial and fungal cultures were obtained from IMTECH, Chandigarh, India.

### Insect antifeedant activity

The antifeedant activity was evaluated by non-choice test method of Debey et al. (1991) with a modification. The pre-starved larvae for six hours have been employed instead of unstarved larvae used in the leaf disc method. The test insect *Spodoptera litura*, a polyphagous pest on wide ranging crops was maintained in the laboratory conditions, at 27 ± 2°C and 70 ± 5% Relative humidity. The IV instar larvae were fed on their natural food, castor leaves. In non-choice test method, leaf discs of 10 cm diameter were cut from fresh-castor leaves and treated with acetone solution of test compounds at 250, 500, 750 and 1000 ppm. These leaf discs were air dried for 3 to 5 s and placed in separate Petri dishes and one preserved IV instar larvae were released simultaneously into the jars containing test leaf discs maintained independently. The consumption of leaf was evaluated at the end of 24 h. The leaf area consumed by the insect in both control and treated was measured by planimeter. The mean percentage of damage and protection was calculated and expressed as percentage of protection (antifeedant activity) using the formula of Singh and Pant (1980).

### Antimicrobial activity

Antimicrobial activity was tested against three gram positive and two gram negative bacterial cultures obtained from IMTECH, Chandigarh, India. They include *B. subtilis*, *S. aureus*, *M. luteus*, *E. coli*, *P. aeruginosa*. Antifungal activity was assayed against *A. niger*, *A. fumigat* and *T. viridae* (from IMTECH). The standard method of paper disc diffusion on agar plates was employed using a concentration range of 50 to 1000 µg /ml of test compound (Mathew, 2006). The presence or absence of growth inhibition zone around each disc was recorded by comparing with the standard antibiotic disc (streptomycin). Formation of a clear zone around the disc indicated the inhibition of microbial growth. The compounds tested were viz., (1) leaf crude extract (2) Stem crude extract (3) Root crude extract (4) Simple aliphatic acid (5) Septicine (6) Tylophorine (7) O-methyl tylophorinidine.

**Table 1.** Antifeedant activity <sup>φ</sup> of crude and pure extracts of *T. indica* of different concentrations.

Compound	250 ppm	500 ppm	750 ppm	1000 ppm
Leaf crude extract	45.36 ± 2.45 ***	71.85 ± 1.21 ***	93.34 ± 1.83 **	95.85 ± 0.91 **
Stem crude extract	40.46 ± 2.20 ***	62.90 ± 1.67 ***	90.12 ± 1.32 **	90.82 ± 1.21 **
Root crude extract	38.38 ± 1.14 ***	56.00 ± 2.92 ***	80.64 ± 1.31 ***	81.75 ± 1.43 **
Simple aliphatic extract	55.21 ± 1.38 ***	72.68 ± 1.55 ***	92.28 ± 1.25 **	93.34 ± 1.28 **
Septicine	70.69 ± 2.15 ***	90.35 ± 1.39 **	98.51 ± 1.03	98.88 ± 0.99
Tylophirine	95.63 ± 1.09	100 ± 0.59	100 ± 0.04	100 ± 0.12
O- Methyl Tylophorinidine	85.68 ± 1.34 ***	99.43 ± 0.53	100 ± 0.57	100 ± 0.62

\*\*\* p < 0.005 Values are significant at 5% level when compared to control value, \*\* P < 0.001 Values are significant at 1% level when compared to control value, <sup>φ</sup> Antifeedant activity measured in terms of area of leaf remained unconsumed by insect.

### Statistical analysis

The standard deviation and analysis of variance (ANOVA) was calculated where ever necessary from the data obtained in the study.

## RESULTS AND DISCUSSION

The antifeedant activity of crude extracts and pure compounds of *T. indica* was tested against *Spodoptera litura* and listed in the Table 1. With ascending concentration of crude or pure compounds, antifeedant activity also increased. Among the crude extracts tested in the present study, leaf showed maximum antifeedant activity by 71.85% followed by stem (62.90%) and root extracts (56.04%) at 500 ppm at 24 h interval. These three crude extracts, however, showed antifeedant activity of 95.85, 90.82 and 81.75 per cents respectively at 1000 ppm. Of the pure compounds used in the study Tylophorine and O-methyl tylophorinidine have shown cent per cent antifeedant activity followed by Septicine (90.35%) and simple aliphatic acid (72.68%) at 500 ppm at 24 h interval (Table 1). Similar reports on the insect antifeedant activity of Tylophorine was observed by Verma et al. (1986), Thirpathi et al. (1990) against *Spilosoma oblique walker*. Arivudainambi and Nachiappan (1993) observed that extracts of *Ipomoea* have shown antifeedant property against the semilooper, *Achaea janata* Linn. The antifeedant properties of indigenous medicinal plants against the larvae of *Heliothis armigera* (Hubner) was reported by Dubey et al. (1991).

The bacterial cultures in Petri plates were incubated along with test compounds, which were checked for growth inhibition zone of organisms after 24 h. The antibacterial activity of crude extracts and pure compounds of *T. indica* was studied and listed in Table 2. Among the pure compounds used in the present study, Tylophorine and O-Methyl Tylophorinidine showed higher antibacterial activity (10 - 20 mm diameter) than other compounds. Of the five bacterial cultures used in the study, except *E. coli* all strains resulted in inhibition by *T. indica* (Table 2). Pure compounds exhibited maximum antibacterial activity compared to the crude compounds. Antibacterial activities

of *D. stemonium* and *T. indica* were evaluated on bacterial strains *S. aureus*, *P. vulgaris*, *P. aeruginosa* and *E. coli*. (Uma, 2009). These extracts exhibited significant zone of inhibition and good antimicrobial activity. Similar results were obtained with the crude ethanol extracts of *Euphorbia hirta* showing antibacterial activities against a variety of species (Ogbulie et al., 2007). Garg and Jain (1998) reported that, essential oils extracted from *Curcuma caesia* have showed significant antibacterial activity.

The antifungal activity of *T. indica* was studied against three fungal species, *A. niger*, *A. fumigatus* and *T. viride* and listed in Table 3. Both crude extracts and isolated pure compounds showed antifungal activity. However, except stem crude extract against *A. niger* and root crude extract against *A. fumigatus*, antifungal activity was shown by all the extracts. Compared to crude extracts, isolated pure compounds exhibited maximum antifeedant activity. Of the pure compounds tested in the study, Tylophorine and O-Methyl Tylophorinidine showed maximum antifeedant activity (10 - 15 mm diameter of zone of inhibition) against *A. niger* and *T. viride* followed by Septicine and simple aliphatic acid (5 - 10 mm zone of inhibition). *A. fumigatus* was less inhibited even by pure compounds. Similar report of antifungal activity was earlier observed from the crude extracts of *A. indica* (Hiremath et al., 1993). Antibacterial and antifungal activity of the aqueous and methanol extracts of *C. alata* leaves was also evaluated (Makinde et al., 2007). According to studies of Uma Reddy (2009), the crude extract of *T. indica* effectively inhibited the growth of fungal strains *A. niger* and *Fusarium* species.

## Conclusion

The present work clearly indicates that crude and pure extracts of *T. indica* showed potent antifeedant and antimicrobial activity. Leaf crude extracts showed antifeedant, antibacterial and antifungal activity at higher concentrations; where as pure compounds are highly effective at lower concentration against tested organisms, except *E. coli* which inhabits human intestine. It suggests that

**Table 2.** Anti bacterial activity of crude and pure extracts of *T. indica*.

Compound (120 µg/ml)	<i>B. subtilis</i>	<i>S. aureus</i>	<i>M. luteus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Leaf crude extract	+	+	+	-	+
Stem crude extract	-	+	-	-	+
Root crude extract	+	+	+	-	-
Simple aliphatic acid	++	++	+	-	+
Septicine	++	++	++	-	++
Tylophorine	+++	+++	++	-	+++
O-Methyl Tlophorinidine	+++	+++	++	-	++
Streptomycin	+++	+++	++	+	+++

- = No inhibition, + = ≤ 5 mm diameter of zone of inhibition, ++ = 5 - 10 mm diameter of zone of inhibition, +++ = 10 - 20 mm diameter of zone of inhibition.

**Table 3.** Anti fungal activity of crude and pure extracts of *T. indica*.

Compound (120 µg/ml)	<i>A. niger</i>	<i>A. fumigatus</i>	<i>T. viridi</i>
Leaf crude extract	+	+	+
Stem crude extract	-	+	+
Root crude extract	+	-	+
Simple aliphatic acid	++	+	++
Septicine	++	+	++
Tylophorine	+++	+	+++
O-Methyl Tlophorinidine Streptomycine (Control)	+++	+	+++

- = No inhibition, + = ≤ 5 mm diameter of zone of inhibition, ++ = 5-10 mm diameter of zone of inhibition, +++ = 10-15 mm diameter of zone of inhibition.

these extracts can be employed for therapeutic purposes as oral medicine without having any adverse affects on normal bacterial strains present in human gut. Hence the extracts of *T. indica* would also serve as novel antibacterial in addition to anti fungal and antifeedant agent.

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