

SCREENING AND EVALUATION OF ANTI-MICROBIAL ACTIVITY IN *TYLOPHORA INDICA*

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Abstract: In present study, aqueous and alcoholic extract of both parental and *in vitro* medicinal plant *Tylophora indica* was selected for evaluate antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus species*. Agar well diffusion was used to evaluate antimicrobial activity. Results indicated antibacterial activity of the aqueous and alcoholic extracts of *in vitro* raised calli of *Tylophora indica* against the tested gram-positive bacteria are shown in the table 1. Significant activity ($P < 0.05$) was observed against *Staphylococcus aureus* and *Staphylococcus epidermidis* in the alcoholic leaf callus extract. Against these gram positive bacteria no significant activity was exhibited by the aqueous leaf callus extract. No activity was observed against the tested gram-positive bacteria in alcoholic as well as aqueous extracts of root and nodal calli.

Keyword: Antimicrobial activity, *Tylophora indica*

INTRODUCTION

Plants are the first medicines for mankind since the ancient times and they are the veritable source of drugs (Schippmann *et al.*, 2002). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Janovská *et al.*, 2003).

Tylophora indica belongs to the plant family Asclepiadaceae. This *Tylophora indica* is also known as Indian ipecac (English); Svasaghni, Lataksheeri, Anntmool, Anthrapachaka (Sanskrit); Antamul, Jangli pikvan (Hindi); Antamul (Bengali); Adumuttada, Nepala (Kannada); Vallippala (Malayalam); Kharaki-rasna, Anthamul, Pitmari (Marathi); Mendi, Mulinied (Oriya); Koorinja, Peyppalainadu (Tamil); Verripala, Kukka-pala (Telugu) (Bhavan 1992; Chopra *et al.*, 1956 a,b,c). *Tylophora indica* is an extensively used Indian traditional medicine to cure a variety of human ailments. It acts as a folk remedy for the treatment of bronchial asthma (Bielory and Lupoli, 1999), allergies, rheumatism and dermatitis (Gupta and Bal, 1956; Shivpuri *et al.*, 1969; Dhananjayan *et al.*, 1974; Mathew and Shivpuri, 1974; Haranath and Shyammalkumari, 1975; Thiruvengadam *et al.*, 1978; Gupta *et al.*, 1979; Nayampalli and Sheth, 1979; Gore *et al.*, 1980). It also seems good remedy in traditional medicine used for the treatment of psoriasis, seborrheic dermatitis, anaphylaxis and leucopenia. Few studies have been conducted for ascertaining the antimicrobial properties of *Tylophora indica*. The alcoholic and aqueous extracts of *Tylophora indica* were tested against various bacterial pathogens and significant activity was found against *Klebsiella pneumonia* (Parekh and Chanda, 2007). The alcoholic extract of *Tylophora*

indica showed maximum inhibitory effects were shown against *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Escherichia coli* whereas mild to moderate activity was found against *Klebsiella pneumoniae* and *Staphylococcus aureus* (Uma Reddy, 2010). Aim of present study is screening and evaluation of anti-microbial activity in *Tylophora indica*.

MATERIAL AND METHOD

Collection of plant material: Fresh leaf, stem and nodal segments were collected from 6 years old plant of *Tylophora indica* grown in botanical garden, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

***In vitro* shoot regeneration (for *in vitro* plant extract):** This process was down according to Deshwal and Siddiqui (2011).

Plant Extraction: The antibacterial activity was tested in both aqueous and alcoholic extracts of these plants. By following the method of Singh and Singh (2000) the extraction was done with few modifications mentioned by Shahid *et al.* (2007).

(a) Aqueous and Alcoholic Extract: Two extracts such as Aqueous extract, Alcoholic extract was selected for the present study. These extracts were prepared according to Deshwal and Siddiqui (2011a). These aqueous and alcoholic extracts were used immediately for experimentation.

Testing of Bacterial Strains: The bacterial strains tested are *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus species*.

Antimicrobial Susceptibility Test: Mueller-Hinton Agar (M 173; Hi media, India) was employed for ascertaining antimicrobial susceptibility test. For fastidious organisms like *Streptococci*, the blood agar used is composed of 5% defibrinated sheep blood.

Agar well diffusion method was used for determining antimicrobial activity (Shahid *et al.*, 2007).

The stock cultures (both standard and clinical) were first thawed and 2-3 identical colonies were immediately suspended into nutrient broth. It was kept in an incubator at 37°C. The turbidity of the bacterial suspension was adjusted to that of 0.5 McFarland Barium Sulphate tube. Then with the help of sterile swabs, two sets of Mueller-Hinton Agar plates (one for aqueous and the other for alcoholic extracts) were then lawn cultured with respective bacterial suspensions.

In each of the plates seven wells of 5 mm diameter were made with the help of sterile borer. 20 µl of the plant extracts (aqueous as well as alcoholic extracts) were poured into the wells with the help of micropipette. The blank controls for the aqueous and alcoholic extracts used were sterile DDW and ethanol respectively. These plates were kept in the upright position for 5-10 minutes so that the solution diffuses completely into the medium. After this the plates were incubated aerobically at 37°C overnight. After overnight incubation period, the streptococci cultures were suspended into Brain-Heart infusion (BHI) broth and lawn cultured on two sets of 5% sheep blood agar (one for aqueous and the other for alcoholic extracts). With the help of sterile borer, seven wells of 5 mm diameter were done in each plate. 20 µl of plant extracts (both aqueous and alcoholic) were poured in these wells with the help of micropipette. For about 5-10 minutes, these plates were kept in upright position so that the poured solution (extracts) diffuses into the medium. These

plates were incubated in atmosphere enriched with 10% CO₂ at temperature of 37°C. The diameter of the zone of inhibition was recorded as the result of activity. Each of these experiments was performed in triplicate.

RESULT AND DISCUSSION

The antibacterial activity of the aqueous and alcoholic extracts of *in vitro* raised calli of *Tylophora indica* against the tested gram-positive bacteria are shown in the table 1. Significant activity ($P < 0.05$) was observed against *Staphylococcus aureus* and *Staphylococcus epidermidis* in the alcoholic leaf callus extract. Against these gram positive bacteria no significant activity was exhibited by the aqueous leaf callus extract. No activity was observed against the tested gram-positive bacteria in alcoholic as well as aqueous extracts of root and nodal calli. Similarly, Joshi *et al.* (2011) reported that aqueous ethanolic extract of *Eugenia caryophyllata* (Clove) was found to be the most effective against bacterial pathogen. Deshwal and Siddiqui (2011a, b) screened the antimicrobial activity in *Tylophora indica*, *Cassia sophera*, *Coleus forskohlii* and *Stevia rebaudiana*. Similarly, Hindumathy *et al.* (2011) also reported that alcohol and water extracts of *Cymbopogon citratus* effectively inhibited the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus*. Our finding clearly indicates that medical plants are good choice to control diseases.

Table 1. Antibacterial activity of aqueous and ethanolic extracts of *in vitro* raised calli of *Tylophora indica* against the tested pathogenic Gram-positive bacteria

Organisms	Zone of Inhibition(mm)±SE					
	Ethanol (control for alcoholic extracts)	DDW (control for aqueous extracts)	Leaf callus		Root callus	Nodal callus
			Aqueous#	Alcoholic	Alcoholic	Alcoholic
<i>Streptococcus pyogenes</i>	7.67±0.33 ^{bc}	5±0.0 ^a	0±0.0 ^a	0.0±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b
<i>Streptococcus agalactiae</i>	7.67±0.33 ^{bc}	5±0.0 ^a	0±0.0 ^a	0.0±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b
<i>Staphylococcus aureus</i> ATCC 25923	8.33±0.33 ^{ab}	5±0.0 ^a	0±0.0 ^a	11.67±0.33 ^a (P=0.020)	0.00±0.00 ^b	7.67±0.33 ^a
<i>Staphylococcus epidermidis</i>	8.33±0.33 ^{ab}	5±0.0 ^a	0±0.0 ^a	11.33±0.33 ^a (P=0.027)	7.67±0.33 ^a	0.00±0.00 ^b
<i>Enterococcus faecalis</i>	7.33±0.33 ^c	5±0.0 ^a	0±0.0 ^a	0.0±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b
<i>Bacillus species</i>	9.33±0.33 ^a	5±0.0 ^a	0±0.0 ^a	0.0±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b

*DDW=Double Distilled Water; # same value in aqueous root callus, nodal callus

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