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Phytochemical Analysis, Antibacterial and Antioxidant Activity of *Tylophora indica*

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A B S T R A C T

The present paper reports the phytochemical analysis, antibacterial and antioxidant activity of a threatened plant *Tylophora indica* (Burm. F.) Merill is a threatened medicinal plant (climber) which belongs to the family Asclepiadaceae. is a slow growing perennial medicinal woody climber it is commonly used to treat Asthma and there is a growing demand for leaves of *T. indica* in the pharmaceutical trade due to its use as a remedy for diabetes and also as a tonic of the nerves and as a laxative other ailments. Phytochemical studies were taken up and the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids was confirmed by qualitative analysis and antibacterial activity of extracts of different *T. indica* explants were performed against gram positive and gram negative bacteria by the disk diffusion method. The activities of the compounds were compared with standard strain for antibacterial properties of the imine base and its solvent extract evaluated and presenting in indicate that the compounds are active in exhibiting antibacterial role also carried out. *T. indica* exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the roots and leaves extract is very much source of natural antioxidant agent.

Introduction

Plants have been used for the treatment of various diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs (Sofowora, 1982). Thus over 50% of these modern drugs are of natural products origin and as such play an important role in drug development in the pharmaceutical industry (Jeyachandran, 2007). *Tylophora indica* commonly known as antamool is a perennial twining medicinal herb belonging to family Asclepiadaceae. It is a threatened plant, is distributed in Assam, West Bengal and Peninsular India. It is used as a traditional folk medicine and as an ingredient in Ayurvedic and Unani medicine. It is used to treat asthma, inflammation, bronchitis, diarrhoea, allergies, rheumatism, dermatitis, syphilis, fever, eye diseases, urinary disorders (Gupta *et al.*, 1979), burning sensation and also used in antitumor treatment (Donaldson *et al.*, 1968). The herb contains certain bioactive compounds like Alkaloids, Flavonoids, Tannins and Saponins, (Rao, 1971; Benjamin, 1973). Phytochemical analysis from leaves and root explants was also reported by (Mohan *et al.*, 2014). There

is a great demand for *Tylophora indica* for production of traditional and modern medicine in pharmaceutical industries. Due to the over exploitation from its natural habitat, it has also been listed as a threatened plant.

Antibacterial l 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, cyotoxic or antitumour, antifeedant and insecticidal activities (Purohit *et al.,* 1995; Abubakar, 2009). 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 phytochemical analysis, antibacterial activity and to estimate the antioxidant potential and free radical scavenging properties of different parts such as root and leaves of *Tylophora indica* (Burm f.) Merrill methanolic extracts through DPPH in vitro assay.

Materials and Methods

Material: *Tylophora indica* plants were collected from Herbal garden, N. G. Ranga Agricultural University at Hyderabad. The collected plant materials were identified by Taxonomist Department of Botany. *Tylophora indica* is a slender climber with twining woody stem and opposite petiolate leaves, which are entire, shiny, smooth, varying in shape and size according to their age.

Flowers are small, in auxiliary and sessile racemes. The root is long, rigid and cylindrical. The plants were subjected to photochemical analysis (qualitative) and antibacterial activity of the plant extract was also taken up.

Preparation of extracts

Plant samples were washed with distilled water and air-dried at room temperature for 7-10 days, then oven-dried at 40 ^{0}C to remove the residual moisture. The dried plant parts were pulverized and stored in air-tight containers at 4 ^{0}C for future use. 50 g of powdered samples of bark, flowers and leaves were extracted with methanol by soxhlation method at 60 to 80 ^{0}C. The three filtrates were separately concentrated in water bath at 40 ^{0}C and evaporated under reduced pressure.

Qualitative analysis

Test for identification of alkaloids: The leaf extract was prepared (ground in 100 ml of water). It was dissolved in dilute HCl solution and clarified by filtration. The filtrate was tested with Drangendroff's and Mayer's reagent. The treated solution was observed for precipitation of white or creamy colour.

Test for identification of flavonoids: Ethyl acetate (5 ml) was added to the leaf extract and the mixture was shaken and allowed to settle. Production of green colour is taken as positive for flavonoids.

Test for identification of phenols: The leaf extract was taken in a test tube (0.5 gm of roots ground in 100 ml of water) and warmed. To this 2 ml of ferric chloride was added and observed for formation of green or blue colour.

Test for identification of saponins: The root extract was taken in a test tube and shaken vigorously for about 30 sec and allowed to stand in vertical position and observed for 30 min. If honey comb froth above the surface of the liquid persists after 30 min, it indicates the presence of saponins.

Test for identification of steroids: The extract was mixed with 2 ml of acetic

anhydride. To this 1 or 2 drop of concentrated sulphuric acid was added slowly along the sides of the test tubes. An array of color change shows the presence of phytosterols.

Test for identification of tannins: The leaf extract was prepared and the solution was clarified by filtration. 10 % ferric chloride solution was added to the clear filtrate, and it was observed for a change in colour to blue.

Test for identification of terpenoids: 5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

Antibacterial activity

The disc diffusion method was used to evaluate the antibacterial activity of the synthesized compounds against four bacterial strains viz; *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus*. Each organism was cultured in nutrient broth at 37 ^{0}C for 24 h. Then 1 % broth culture containing approximately 106 colony forming units (CFU/mL) of test strain was added to nutrient agar medium at 45 ^{0}C and poured into sterile petri plates. The medium was allowed to solidify. 5 µL of the test compound (40 mg/mL in DMSO) was poured on 4 mm sterile paper discs and placed on nutrient agar plates. In each plate standard antibacterial drug (ampicillin) and metal complexes were added. The plates were incubated at 37^0 C for 24 h and the antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm).

Radical scavenging activity

The percentage of free radical scavenging activity is shown in figure 2. This assay is based on decrease in absorbance value of DPPH at 517 nm on addition of complex. The experiment involves diluting the working solution of the plant (root and leaves) methanol extracts and the ascorbic acid standard (700, 600, 500, 400, 300 and 200 µg/µL−1) in methanol. DPPH concentration was kept constant (2 mL, 0.004 %). To this varying concentration of plant extracts and standard were added. The mixture was shaken vigorously and kept in dark for 30 min at room temperature. Then the absorbance was measured at 517 nm in a spectrophotometer. The whole experiment was carried out using spectroscopic grade methanol solvent at 298 K. The radical scavenging activity has been measured by using the following Eq. 1;

$$\text{Suppression ratio (\%)} = [(A_0-A_i)/A_0)] \text{ X } 100\% \quad (1)$$

Where A_i=the absorbance in the presence of the plant sample extract, A_0=the absorbance in the absence of the plant sample extract.

Results and Discussion

The present study comprises phytochemical (qualitative) studies in *T. indica* plant extract (methanol and aqueous) was carried out for alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. All of the phytochemicals like alkaloids flavonoids, phenols, saponins, steroids and terpenoids were present in *Tylophora indica* except Tannins (Table 1 and Fig. 1). Whereas, our study reports the absence of Tannins (Mohan *et al.*, 2014; Kumar *et al.*, 2011), indicated that Tannins were present in *T. indica* in the aqueous extract. Several medicinal properties have been attributed to Tannins by (Okwu, 2004) but surprisingly, Tannins were not found in the present study. Alkaloids are however reported in the present study which agrees with the findings of (Meera *et al.*, 2009) who has attributed analgesic, anti-spasmodic and bactericidal effects. The present study also reports Saponins, similar to the report of (Mohan *et al.*, 2014). Alkaloids

and Saponins are known to be effective for the treatment of syphilis and other venereal diseases, had earlier reported that Saponins have antibiotic properties and so help the body to fight infections and microbial invasion. Also, it is used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins. These proteins were also reported in hyperchlolesterolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss and have anti-fungal properties (Singh *et al.,* 2010) reported the presence of tylophorine alkaloids in *T. asthamatica*. Investigation of *T. indica* for the presence of tylophorine is therefore needed in exclusive studies The presence of flavonoids are reported in *T. indica* presently which is in agreement with (Mohan *et al.,* 2014) who also reported the diuretic property of extracts of *T. indica* is very valuable information. The present study which agrees with the findings of Manjula *et al.,* (2013) and Rama *et al.,* (2014) reported about medicinal plants.

The antibacterial screening of the *T. indica* leaf extracts were performed against gram positive (*S. aureus*) and gram negative bacteria (*E. coli*, *P. aeruginosa* and *K. pneumoniae*) by the disk diffusion method. The activities of the compounds were compared with standard ampicillin for antibacterial activity. The antibacterial properties of the imine base and its solvent extract evaluated and presenting in figure 1 and table 2, indicted that the compounds are active in exhibiting antibacterial role like leaf 0.4, 0.2, 0.5 in gram negative bacteria and leaf 0.6 in gram positive bacteria. Study confirms the antibacterial activity of leaf extract of *T. indica* the extract found effective bacterial strain, the activity of leaf extract antibacterial activity higher than in gram negative bacteria, where as more when compare to in gram positive bacteria. However reported in the present study which agrees with the findings of were reported in medicinal plants like (Reddy *et al.,* 2009; Vani *et al.,* 2016; Mohan *et al.,* 2016).

The model of scavenging the stable DPPH radical is a widely used technique to screen antioxidant properties by spectrophotometer in a very short time period. When the reaction between antioxidant molecule and DPPH radical occurs, it results in decrease in absorbance at 517 nm. This is because the radical is scavenged by antioxidants through donation of hydrogen to form the reduced form (DPPH-H), and this property is also visually noticeable as the color changes from purple to yellow. The more rapidly the absorbance decreases, the more potent is the antioxidant compound. In the present study the antioxidant activity of root and leaves extract was evaluated by scavenging stable DPPH radical (Fig. 2).

Table.1 Qualitative analysis of *T. indica*

S.No	Test for Phytochemicals	Test results	
		Leaf	Root
1	Alkaloids	***+ve***	***+ve***
2	Flavonoids	***+ve***	***+ve***
3	Phenols	***+ve***	***+ve***
4	Steroids	***+ve***	***+ve***
5	Tannins	***-ve***	***-ve***
6	Terpenoids	***+ve***	***+ve***
7	Saponins	***+ve***	***+ve***

Table.2 Minimum inhibition zone (mm) complexes (µg/ml) leaf extract of *Tylophora indica*.

Bacterial inhibition zone (mm) Gram (+)			Bacterial inhibition zone (mm) Gram (-)
E. coli	***P. aeruginosa***	***K. pneumoniae***	***S. aureus***
0.4	0.2	0.5	0.6

Fig.1 Antimicrobial activity of leaf extract of *Tylophora indica* (A) *E. coli*, (B) *P. aeruginosa* (C) *K. pneumoniae* (Gram Negative) and (D) *S. aureus* (Gram Positive) ampicillin as positive control

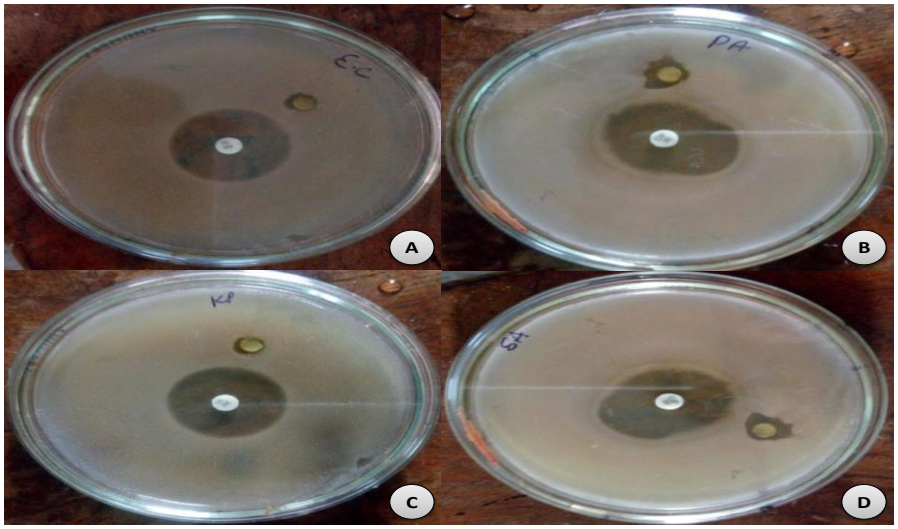
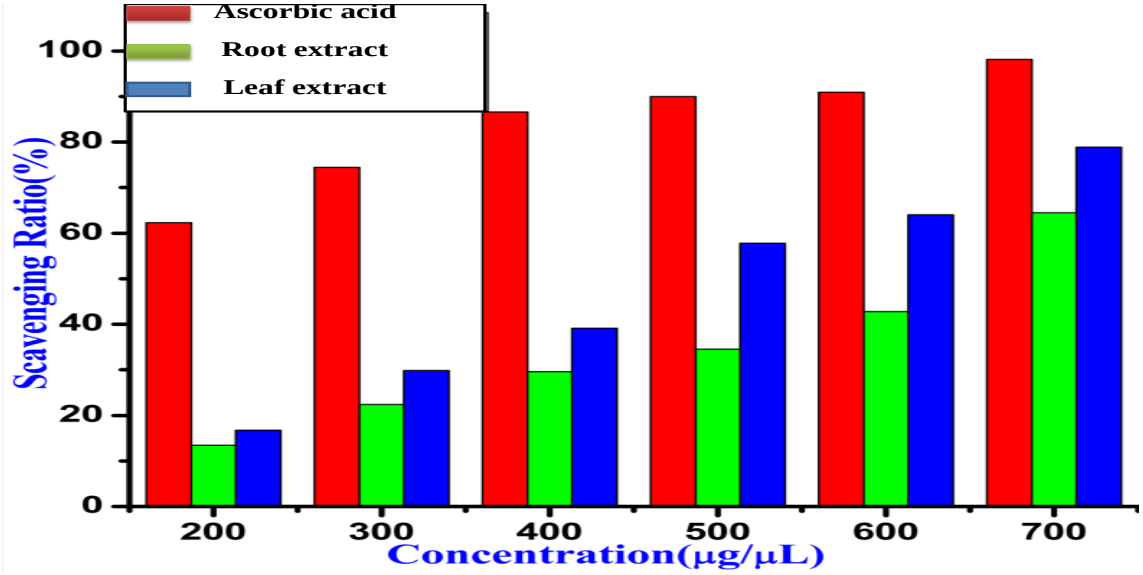


Fig.2 Radical-scavenging activity of the root and leaf extract of *T. indica* on DPPH radicals (%)



The DPPH radical scavenging activities were found to be 63.03 % for ascorbic acid, 12.85 % for root and 17. 24 % leaf extract, at concentration of the 200 µg/µL-1. Ascorbic acid exhibited higher DPPH scavenging activity than the compound at all concentrations. At the concentration of 700 µg/µL^{-1} scavenging activities were found to be 89.85 %, 80.15 % and 64.12 % for Ascorbic acid, root and leaf extract of respectively. The compounds scavenging activity which is the measure of antioxidant property at the concentration of above compounds at 200 µg/µL^{-1} follows the order: Ascorbic acid > root > leaf extract of while at higher concentration the same order is followed by root and leaf extraction exchanged their position the present study results which agree and similar with the findings of (Sharma *et al.,* 2014; Aruna, 2013; Vani *et al.,* 2016).

In conclusion, *Tylophora indica* is an important medicinal plant with a variety of ethnic medicinal uses. The present study describes the valuable medicinal plant which is used in treating various disorders. The qualitative analysis of *T. indica* shows the presence of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids. Antibacterial properties of the imine base and its solvent extract evaluated and presenting in indicted that the compounds are active in exhibiting antibacterial role like leaf 0.4, 0.2, 0.5 in gram negative bacteria and leaf 0.6 in gram positive bacteria. Study confirms the antibacterial activity of leaf extract of *T. indica* the extract found effective bacterial strain, the activity of leaf extract antibacterial activity higher than in gram negative bacteria, where as more when compare to in gram positive bacteria is a plant with a variety of ethnic medicinal uses. *T. indica* exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the roots and leaves extract is very much of *T. indica* can be used as an accessible source of natural antioxidant agent. This is valuable information for preparation of drugs in pharmaceutical industry and stress the need for more intensive research since they play a great role in healthcare.

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