International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 19-12-2021, Accepted: 04-01-2022, Published: 20-01-2022
Volume 7, Issue 1, 2022, Page No. 400-402

Phytochemical analysis and antibacterial activity *Tylophora indica* (Burm. F.) merill

Salikanti Saidaiah*, S Karnakar Reddy
Cytogenetics and Molecular Biology Laboratory, Department of Botany, Osmania University, Hyderabad, Telangana, India

Abstract
Tylophora indica is a threatened medicinal plant which belongs to the family Asclepiadaceae. *T. indica* is a medicinal woody climber it is commonly used to treat Asthma, 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. The present paper reports the phytochemical analysis, antibacterial and antioxidant activity of a threatened plant. 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.

Keywords: *Tylophora indica*, phytochemical analysis and antibacterial activity

Introduction
Tylophora indica (Burm. F.) Merill is a threatened perennial twining medicinal herb belonging to family Asclepiadaceae. It is commonly known as an antamool. The plant is distributed in Assam, West Bengal and peninsular India. 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) [6]. It is used as a traditional folk medicine and also used as an ingredient in Ayurvedic and Unani preparations in certain regions of India for treatment of various diseases like asthma, syphilis, allergies, rheumatism, dermatitis, fever, bronchitis, diarrhoea, inflammation, urinary disorders, eye diseases, burning sensation and also used in antitumor treatment (Gupta *et al.* 1979; Donaldson *et al*. 1968) [3, 4]. The herb contains certain bioactive compounds like Alkaloids, Flavonoids, Tannins and Saponins, (Rao *et al*. 1971, Benjamin and Mulchandani 1973, 1976) [1]. 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) [5].

Antibacterial 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) [7]. 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. 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. Hence the present study was carried out on phytochemical analysis, antibacterial activity.

Materials and Methods
Material
Tylophora indica plants were collected 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), antibacterial activity studies of the plant extract was also taken up.

Preparation of extracts
Tylophora indica collected plant samples leaves, root and stem were washed with distilled water and air-shad dried in open air saparately at room temperature for 10-15 days, then oven-dried at 40 ^{0} C to remove the residual moisture. Powder of the leaf is obtained by grinding them mechanically and dried plant parts were pulverized and stored in air-tight containers at 4 ^{0}C for future use. 50 to 80 g of powdered samples of leaf, root and stem were extracted with Methanol, Ethanol and Water extract by soxhlation method at 60 to 80 ^{0} C. After 50-70 hr the plant extracts were subjected to filtration, filtered with No 42 whatman filter paper separately. The three filtrates were separately concentrated in water bath at 40 50 ^{0} C and evaporated under reduced pressure. Concentrated extracts was preserved in sterilized air tight labeled bottles and preserved in refrigerator at 4 ^{o}C until required for further use. The extract was filtered under reduced pressure using rotary flash evaporator and subjected for further preliminary phytochemical tests. Different tests conducted for the identification of phytochemicals, antibacterial activity.

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 Glycosides

About 0.5 gm of methanol extract was taken in a test tube and 1 ml glacial acetic acid containing traces of ferric chloride was added to it. To this solution, 1 ml concentrated sulphuric acid was added and observed for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.

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. pnemone* 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}Cfor 24 h and the antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm).

Results and discussion

The present study comprises phytochemical studies in *T. indica* plant extract (leaf, stem and root) (methanol, ethanol 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 *T. indica* except Tannins in leaf and root, Steroids in team extract (Table-1and fig-1). Whereas, our study reports the absence of Tannins and Steroids (Meera *et al.*, 2009; and Kumar *et al.*, 2011) [8, 9]. indicated that were present in *T. indica* in the aqueous extract. Several medicinal properties have been attributed to Tannins and steroids by (Mohan *et al.*, 2014; Kumar *et al.*, 2011) but surprisingly, Tannins and Steroids 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) [8] who has attributed analgesic, anti-spasmodic and bactericidal effects. The present study also reports Saponins, similar to the report of (Meera *et al.*, 2009; Mohan *et al.*, 2014) [12, 14]. Alkaloids and Saponins are known to be effective for the treatment of syphilis and other venereal diseases, (Okwu 2004; Meera *et al.*, 2009) [10, 12] had earlier reported that Saponins have antibiotic properties and so help the body to fight infections and microbial invasion. These proteins were also reported in, hyperglycaemia, hyperchlolesterolaemia, anti-inflammatory, antioxidant, anti-fungal and weight loss and have antibacterial and anticancer properties. (Singh (2012) 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 Alkaloids and Flavonoids are reported in *T. indica* presently which is in similar studies agreement with (Meera *et al.*, 2009; Mohan *et al.*, 2014) [12, 14] who also reported the diuretic property of extracts of *T. indica* is very valuable information. 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. pnemone*) 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 Fig-2 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 (Vani *et al.*, 2016; Mohan *et al.*, 2014) [13, 14].

Table 1: Qualitative analysis of *T. indica.*

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

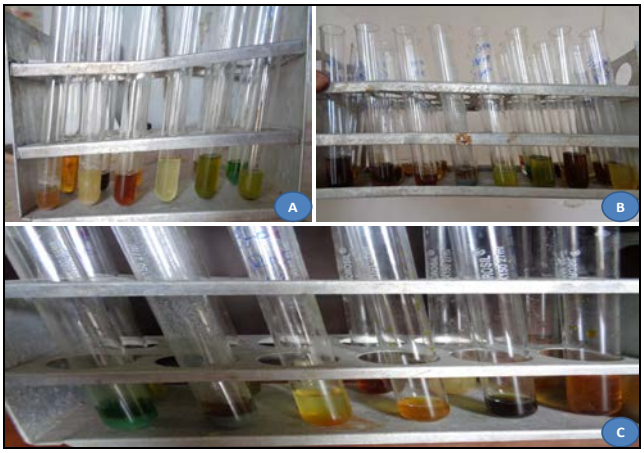

Fig 1: Phytochemical analysis of *Tylophora indica* methanol extract of leaves, stem and root.

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. pnemone*	*S. aureus*
0.4	0.2	0.5	0.6

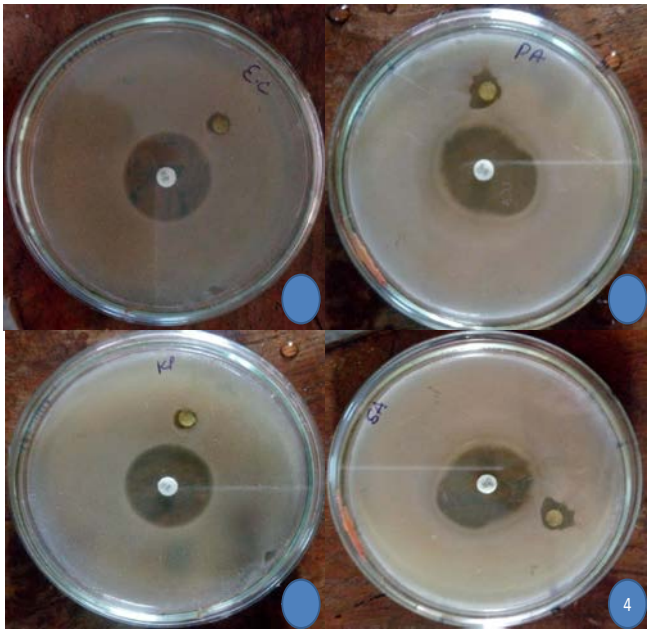

Fig 2: Antimicrobial activity of leaf, stem and root extract of *Tylophora indica* (A) *E. coli*, (B) *P. aeruginosa* (C) *K. pnemone* (Gram Nagative) and (D) *S. aureus* (Gram Positive) ampicillin as positive control.

Conclusion

It is concluded that *Tylophora indica* is an important medicinal plant with a variety of ethnic medicinal uses. The qualitative and quantitative 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 in gram negative bacteria and leaf 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. 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.

References

1. Benjamin BD, Mulchandani NB. Studies in biosynthesis of secondary constituents in tissue culture of *Tylophora Indica.* Plant A Med,1973:23:394-397.
2. Rao PS, Narayanaswamy S. Morphogenetic investigations in callus cultures of *Tylophora indica*. Physiol Plant,1972:27:271-276
3. Gupta S, George P, Gupta V *et al*. *Tylophora indica* in bronchial asthma—a double blind study.*Indian J Med Res*,1979:69:981-989.
4. Donaldson GR, Atkinson MR, Murray AW. Inhibition of protein synthesis in Ehrlich ascites-tumor cells by the Phenanthrene alkaloids tylophorine, tylocrebrine cryptopleurine. *Biochem Biophys Res. Commun*,1968:31:104-109.
5. Jeyachandran R, Mahesh A. Antimicrobial evaluation of Kigelia africana (Lam). Res. J. Microbiol,2007:2(8):645-649.
6. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd Edn. Ibadan John Willey and Sons ltd, 1982, 8-14.
7. Purohit MG, Santhaveerappa BK, Shrisailappa B, Swamy HKS. Anti microbial activity of various extracts of Evolvulus alsinoids, Ind. J. Microbiol,1995:35(1): 77-78.
8. Meera R, Devi P, Muthumani P, Kameshwari B, Eswarapriya B. Evaluation of Diuretic activity from *Tylophora indica* leaves extracts. J Pharm Sci and Res,2009:1(3):112-116.
9. Kumar S, Kaushik N, Edrada-Ebel R, Ebel R, Proksch P. Isolation, characterization and bioactivity of endophytic fungi of *Tylophora indica.* World Journal of Microbiology and Biotechnology,2011:27(3):571-577.
10. Okwu DE Okwu ME. Chemicalcomposition of Spondias mombin Linn.Plant parts. J Sustain Agric Environ,2004:6:140-147
11. Singh A, Duggal S, Sabkar Katekhaye. *Tylophora asthmatica* Wightand Arn. – *Revie.* Nature of Pharmaceutical Technology,2010:1(1):1-4.
12. Mohan, Rama devi, Manjula, Kiran kumar, Naresh, Prathibha devi. Phytochemical investigations and Micropropagation of *tylophora indica* (burm. F.) Merill from nodal explants J. Indian bot. Soc,2014:93(3-4):25-32.
13. Vani, Kistamma, Srinivas, Narshimha Reddy, Mohan. Antibacterial activity, antioxidant activity and micropropagation of *Gymnema sylvestre* R.Br. a valuable medicinal plant, Journal of Pharmacognosy and Phytochemistry,2016:5(2):207-210.