



Phytochemical analysis and antibacterial activities of *Tylophora indica*

M Maheshwari^{1*}, P Vijayarengan²

^{1,2} Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

Abstract

Tylophora indica is a perennial twining herb belonging to the family Asclepiadaceae. The plant is commonly called Indian Ipecac and Kaluthai Palai in Tamil. It is used in Unani, Sidha and Ayurveda systems of medicine to treat various diseases. It is distributed from India to Sri Lanka and South East Asia. The plant has been naturalized in Mauritius and introduced in Africa. It is a valuable medicinal plant. Previous works on this plant revealed that it can be used to treat asthma, inflammation, bronchitis, diarrhoea, allergies etc. Three solvents were used to test the antimicrobial activities and to identify phytochemical compounds. Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids, glycosides, terpenoids, tannin, carbohydrates and amino acids. The antibacterial activity of different solvent extracts by the micro-dilution method revealed that *T.indica* leaves extract possess bioactive compounds, which inhibits the growth of microorganisms. Among the six pathogens tested the Gram positive bacteria *Bacillus subtilis* was most susceptible to the extracts and the rest of the pathogens showed almost similar kind of MIC values. The range of MIC to inhibit various microorganisms in order is *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

Keywords: *Tylophora indica*, phytochemical analysis, antibacterial activities, mic value

1. Introduction

Since ancient times, plants have been the source of crude material for medicines. A rich heritage of knowledge of medicinal plants is available in India amongst the common people. Generally, it is estimated that more than 3,500 plant species in India are used as a source of crude drugs. About 2,500 plants are ethno medicinally important. There are too many plants species which are considered as a weed, but they are also medicinally important. The phytochemical products obtained from plants are used in various traditional and modern methods of therapy. The advanced modern biotechnological and bioinformatic techniques using natural products as medicine have helped new drug discoveries.

The continued evolution of infectious diseases and the development of resistance by pathogens have led to the intensification of the search for new drugs against fungal, parasitic, bacterial, and viral infections [1]. Despite the recent advances in drug development through molecular modelling, combinatorial and synthetic chemistry, natural plant-derived compounds are still proving to be an invaluable source of medicines for humans [2]. Plant derived antimicrobials have a long history of providing the much needed novel therapeutics [3]. Besides, the emerging resistance of microorganisms requires the careful use of existing antimicrobial drugs. However, there is a need for the design of novel antimicrobial agents, particularly for the treatment of the infections of hospitalized patients and protection of immune-suppressed patients. A potential approach is the design of innovative drugs, with different mechanisms of action, in effort to avoid cross resistance [4]. Many antibacterial agents are available in the market, which are developed by the great scientists. But still the microbes are challenging the scientists by developing the resistance to the presently available drugs. Plants are known to produce a variety of compounds such as alkaloids to protect

themselves against a variety of pathogens and therefore considered as potential source for different classes of antimicrobial substances.

Tylophora indica (Burm f.) Merrill is a perennial twining medicinal plant belonging to the family Asclepiadaceae. It is a threatened medicinal climber native to the plain and hill forests of eastern and southern India up to an altitude of 900m. The plant has been traditionally exploited by tribes in certain regions of India for the treatment of various ailments [5]. It is used as a traditional folk medicine and as an ingredient in Ayurvedic and Unani medicines. It is used to treat asthma, inflammation, bronchitis, diarrhoea, allergies, rheumatism, dermatitis, syphilis, fever, eye diseases, urinary disorders [6], and burning sensation and also used in antitumor treatment [7]. The plant contains several phenanthroindolizidine alkaloids [8] and pharmacological investigations have confirmed the anti-asthmatic effects of its leaf extracts [9]. The demand of *Tylophora indica* is great 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. The plant contains certain bioactive compounds like alkaloids, flavonoids, tannins and saponins [10,11]. The phytochemical analysis from leaves and root explants was also reported by Mohan *et al* [12].

Therefore the present study was undertaken to investigate the phytochemical analysis and antibacterial activities of ethanol, acetone and ethyl acetate extracts of leaves of *Tylophora indica* (Burm f.) Merrill against six bacterial pathogens by micro-dilution bioassay.

2. Materials and Methods

2.1. Plant material

Tylophora indica (Burm f.) Merrill (Asclepiadaceae) was collected from Mannampandal, Mayiladuthurai during the

month of March 2018. The aerial parts of the plant samples were separated, and first washed under running water to remove the soil particles, then washed with distilled water, cut into small pieces, and shade dried. The dried parts were ground to a fine powder using a mixture grinder.

2.2. Preparation of extracts

One hundred grams of powdered plant material was successively extracted with acetone, ethyl acetate and ethanol by using Soxhlet apparatus for 8 hours^[13]. The extracts were filtered, pooled and the solvents were evaporated with the help of rotary evaporation (Heidolph, Germany) under reduced pressure at 40°C and the crude extracts were kept at 4°C in refrigerator for antimicrobial assay.

2.3. Qualitative analysis of Phytochemicals

The different extracts of *Tylophora indica* were used for qualitative phytochemical studies like alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, glycosides, tannins, carbohydrate and amino acids^[14, 15].

Test for alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's test

A fraction of each of the extracts was treated with Mayer's reagent (1.36 g mercuric chloride and 5g of potassium iodide in 100 mL of distilled water). Development of yellow coloured precipitate indicates the presence of alkaloids.

Test for Flavonoids

Alkaline Reagents Test

A fraction of each of the extracts was treated with 3 to 4 drops of sodium hydroxide solution. Development of dense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Test for steroids

Salkowski's test

A fraction of each of the extracts was treated with chloroform and filtered. The filtrates were treated with 3 to 4 drops of Conc.Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of steroids.

Test for terpenoids

Copper acetate test

A fraction of each of the extracts was dissolved in water and treated with 3-4 drops of copper acetate solution. Development of emerald green colour indicates the presence of terpenoids.

Test for carbohydrates

A fraction of each of the extracts were dissolved separately in 5 ml distilled water and filtered. The filtrates were used to test for the detection of carbohydrates.

Molisch's Test

Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Development of the violet ring at the junction indicates the presence of carbohydrates.

Test for amino acids

Xanthoprotein Test

The plant extracts were treated with a few drops of Conc.Nitric acid. Development of yellow colour shows the presence of proteins.

Test for glycosides

Extracts were hydrolysed with dilute HCL, and then subjected to test for glycosides.

Modified Borntrager's Test

A fraction of the extract was treated with ferric chloride solution and immersed in water bath for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The layer of benzene was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonial layer indicates the presence of glycosides.

Test for Saponins

Froth Test

A fraction of the extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Development of 1 cm thick layer of foam indicates the presence of saponins.

Test for tannins

Gelatins Test

To the extract, 1% gelatin solution containing sodium chloride was added. Development of white precipitate indicates the presence of tannins.

Test for Phenols

Ferric Chloride Test

A fraction of the extracts was treated with a few drops of ferric chloride solution. Development of green colour indicates the presence of phenols.

2.4. Microorganisms

The following Microorganisms were collected from Department of Clinical Microbiology, Raja Muthaiah Medical College Hospital, Annamalai University, Chidambaram: Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*.) bacterial stains.

2.5. Antibacterial activity

Minimum inhibitory concentrations (MIC) of compounds for antibacterial activity were determined using the microdilution bioassay as described by Eloff (1998)^[16]. Overnight cultures (incubated at 37°C in a water bath with an orbital shaker) of Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*.) bacterial strains were diluted with sterile Mueller-Hinton (MH) broth to give final inocula of approximately 10⁶ CFU/ml (colony forming units). The different extracts were resuspended in respective solvents to known concentrations (50mg/ml). One hundred microliters of each extract were serially diluted two-fold with sterile distilled water in a 96-well microtiter plate for each of the six bacterial strains. A similar two-fold serial dilution of Streptomycin (Sigma Aldrich) (0.1mg/ml) was used as a

positive control against each bacterium. One hundred microliters of each bacterial culture were added to each well. Water and different extracts used in the present study were included as negative and solvent controls respectively. The plates were covered with parafilm and incubated at 37°C for 24 h. Bacterial growth was indicated by adding 50µl of 0.2 mg/ml p-iodonitrotetrazolium chloride (INT) (Sigma–Aldrich) with further incubation at 37°C for 2 hours. When the colourless tetrazolium salt came across active organisms, it is biologically reduced to a red product. Therefore the MIC values were recorded as the concentrations in the last well in which no colour change was observed after adding the INT indicator. A reddish – pink colour was observed in the wells where the bacterial growth occurred. The assay was repeated two times with two replicates per assay.

3. Results

The phytochemical screening of crude extract of leaves of *Tylophora indica* reveals the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, glycosides, saponins, steroids, phenols, carbohydrate and amino acids. Among the secondary metabolites alkaloids, glycosides, carbohydrates and amino acids were present in all the tested solvent system and tannin was not present in any of the solvents (Table 1). Whereas, (Mohan *et al.*, (2014)^[12] and Kumar *et al.*, (2011)^[17], indicated the presence of tannins in the aqueous extract which possesses several medicinal properties have been attributed to tannins^[18], but surprisingly, tannins were not found in the present study. The antibacterial activity of acetone, ethyl acetate and ethanol extracts of *Tylophora indica* were tested against six human pathogens *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes* (Gram positive) *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Gram negative) bacterial strains and the results were presented in table 2. In general all the extracts possess antibacterial activity. Minimum Inhibitory Concentration (MIC) was estimated for all the compounds over the test pathogens. The range of MIC to inhibit various microorganisms is *Bacillus subtilis* (3.125 - 0.78µg/ml), *Staphylococcus aureus* (12.5- 3.125 µg/ml), *Streptococcus pyogenes* (3.125- 1.56 µg/ml), *Escherichia coli* (3.125- 1.56 µg/ml), *Pseudomonas aeruginosa* (12.5-3.125 µg/ml), and *Salmonella typhi* (3.125 µg/ml). Among the six pathogens tested the Gram positive bacteria *Bacillus subtilis* was most susceptible to the ethanol extracts of *Tylophora indica* and the rest of the pathogens showed almost similar kind of MIC values. Among the different solvents tested, ethanol was most active followed by ethyl acetate and acetone extract showed moderate inhibitory effect to the test pathogens.

Table 1: A Preliminary Phytochemical analysis of different extracts of leaves of *Tylophora indica*

S.no	Phytochemical compounds	Acetone	Ethyl acetate	Ethanol
1	Alkaloids	+	++	+++
2	Flavonoids	-	+	+++
3	Glycosides	+	+	+
4	Saponins	-	-	+
5	Steroids	+	+	++
6	Terpenoids	-	-	++
7	Phenols	+	-	+
8	Tannins	-	-	-
9	Carbohydrates	+	+	++
10	Amino acids	+	+	+

(+++)= more strong; (++)= strong; (+)= positive (present); (-)= negative (absent)

Table 2: Minimum inhibitory concentrations (MIC - µg/ml) of different extracts of leaves of *Tylophora indica* against the selected bacterial strains

Sl. No.	Bacterial strains / solvent	Acetone	Ethyl acetate	Ethanol
1	<i>Bacillus subtilis</i>	3.125	3.125	0.78
2	<i>Staphylococcus aureus</i>	12.5	6.25	3.125
3	<i>Streptococcus pyogenes</i>	3.125	1.56	3.125
4	<i>Escherichia coli</i>	3.125	1.56	3.125
5	<i>Pseudomonas aeruginosa</i>	12.5	3.125	3.125
6	<i>Salmonella typhi</i>	3.125	3.125	3.125

4. Discussion

Various studies have been undertaken previously by different researchers to analyze the antibacterial potential of *T. indica*. Reddy *et al.* (2009)^[19] reported pure compounds exhibited maximum antibacterial activity compared to the crude extracts of *T. indica*. Among the pure compounds tested Tylophorine and O-Methy Tylophorinidine showed higher antibacterial activity (10 - 20 mm diameter). Of the five bacterial cultures used in their study, except *E. coli* all strains resulted in inhibition by *T. indica*. In the present study *E.coli* also inhibited by all the extracts with MIC ranged from 3.125- 1.56 µg/ml. Antibacterial activities of *D. stemonium* and *T. indica* were evaluated on bacterial strains *S. aureus*, *P. vulgaris*, *P. aeruginosa* and *E. coli*.^[20] These extracts exhibited significant zone of inhibition and good antimicrobial activity. Noor Jahan *et al.*^[21] evaluated the MIC of the alcoholic leaf extract of *in vitro* raised *T. Indica*, determined by broth microdilution method. MIC against gram positive bacteria ranged from 3.05 to 12.0 µg/ml and MIC against gram negative bacteria ranged from 1.53 to 24.0µg/ml. In the present study also similar range of MIC values was obtained against the tested bacterial pathogens. Sangeetha *et al.*^[22] showed significant activity of leaf extract of *T. indica* against *S. aureus* only and no activity against *E. coli* and *P. aeruginosa*. These findings are in contrast with our study. This could be due to different concentrations of extracts used in their study as well as variation in active

metabolites present in plant extracts derived from different places.

The presence of alkaloids, carbohydrates, steroids, saponins, tannins and triterpenes in the extracts are exhibiting various pharmacological activities^[23, 24] and possess antimicrobial activities^[25, 26, 27]. The different antimicrobial property of solvent extracts of medicinal plants has been well reported by Parekh *et al.*^[28] and Nair *et al.*^[29]. In our study, Gram positive bacteria were more susceptible to the solvent extracts than Gram negative bacteria. This might be due to the presence of outer membrane that appears as effective lamina in Gram negative bacteria^[30].

5. Conclusion

Tylophora indica is an important medicinal plant with a variety of ethnic medicinal uses. In the present study the medicinal value was investigated by preliminary phytochemical analysis and antibacterial potential. The qualitative phytochemical analysis of *T. Indica* shows the presence of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, tannins, glycosides, carbohydrate and amino acids. The antibacterial activities of crude extracts showed significant antibacterial activity against the tested pathogens of which *Bacillus subtilis* was most susceptible to the extracts. Therefore the plant extractive studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds of this plant for industrial drug formulation.

6. Reference

- Gibbons S. Anti-staphylococcal plant natural products. *Natural Product Reports*. 2004; 2(1):263-277.
- Salim AA, Chin YW, Kinghorn AD. Drug discovery from plants. In: Ramawat, K.G and Mérillon, J.M. (Eds.), *Bioactive Molecules and Medicinal Plants*. Springer-Verlag, Berlin, Heidelberg, 2008.
- Avila M, Ojcius DM. The book on infectious diseases. *Microbes Infection*. 2008; 10:942-947.
- Pfultz RF, Wilkinson BJ. The Escalating challenging of vancomycin Resistance in *Staphylococcus aureus*. *Current Drug Target- Infectious Disorder*. 2004; 4(4):273-294.
- Anonymous. *Wealth of India, Raw materials*. CSIR, New Delhi, 1976, 398-399.
- Gupta S, George P, Gupta V. *Tylophora indica* in bronchial asthma-a double blind study. *Ind. J. Med. Res.* 1979; 69:981-989.
- Donaldson GR, Atkinson MR, Murray AW. Inhibition of protein synthesis in Ehrlich ascites-tumor cells by the phenanthrene alkaloids tylophorine, tylocrebrine, cryptolepine. *Biochem. Biophys. Res. Commun.* 1968; 31:104-109.
- Gellert ETR, Govindachari MV, Lakshminathan IS, Ragade R, Rudzats N, Viswanathan J. The alkaloids of *Tylophora crebriflora*: structure and synthesis of tylanthocrebrine, a new phenanthroindolizidine alkaloids. *J. Chemical Society*, 1992, 1008-1014.
- Shivpuri DN, Singhal SC, Prakash D. Treatment of asthma with an alcohol extract of *Tylophora indica*. A cross over double study. *Ann. Allergy*. 1972; 30:407-412.
- Rao KV, Wilson RA, Cumming B. Alkaloids of *Tylophora*. 3 new alkaloids of *Tylophora indica* (Burm. f) Merrill and *Tylophora dalzielii* Hook. *J. Pharm. Science*. 1971; 60(11):1725-1726.
- Benjamin BD, Mulchandani NB. Studies in Biosynthesis of Secondary Constituents in Tissue Culture of *Tylophora indica*. *Planta Medica*. 1973; 23:394-397.
- Mohan, Rama Devi B, Manjula P, Prathibha Devi B. Phytochemical investigation and micropropagation of *Tylophora indica* (Burm. f) Merrill from nodal explants. *J. Indian Bot. Soc.* 2014; 93(1&2):42-49.
- Vogel. *Textbook of practical organic Chemistry*. The English language book society Longman, London, 1978, 1368.
- Harborne JB. *Methods of plant analysis*. In: *Phytochemical methods*. Chapman and hall London, 1973, 74-79.
- Trease GE, Evans WC. *Pharmacognosy 2nd Edn*. Braille Tiridel and Macmillan Publishers, 1989, 242-245.
- Eloff JN. A sensitive and quick microplate methods to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 1998; 64:711-713.
- Kumar S, Kaushik N, Edreda-Ebel R, Prokcsch P. Isolation, Characterization and Bioactivity of endophyte Fungi of *Tylophora indica*. *World J. Microbiology Biotechnology*. 2011; 27(3):571-577.
- Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* Linn. *Plant parts*. *J. Sustain. Agric. Environ.* 2004; 6:140-147.
- Reddy BK, Balaji M, Reddy PU, Sailaja G, Vaidyanath K, Narasimha G. Antifeedent and antimicrobial activity of *Tylophora indica*. *Afric. J. Biochem. Res.* 2009; 3:393-397.
- Uma B, Prabhakar K, Rajendran S, Sarayu Y. Lakshmi "Antimicrobial Activity and Phytochemical Analysis of *Coriander sativum* Against Infectious Diarrhea," *Ethnobotanical Leaflets*. 2009; 5:Article 4.
- Noor Jahan, Razia Khatoon, Siraj Ahmad, Anwar Shahzad. Evaluation of antibacterial potential of medicinal plant *Spilanthes acmella* Murr. and its *in vitro* raised callus against resistant organisms especially those harbouring *blagenes*. *J. App. Pharm. Sci.* 2013; 3(10):119-124.
- Sangeetha G, Rajeshwari S, Venkatesh R. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Prog. Nat. Sci. Mater. Int.* 2012; 22:693-700.
- Patel NJ, Gujarati VB, Gouda TS, Rao NV, Nandakumar K, Shantakumar SM. Antidiarrhoeal activity of alcoholic and aqueous extracts of root of *Tylophora indica* (Weight and Arn.) in Rodents. *Pharmacol.* 2006; 1:9-29.
- Gujrati V, Patel N, Rao VN, Nandakumar K, Gouda TS, Shalam MD. Hepatoprotective activities of alcoholic and aqueous extract of leaves of *Tylophora indica* in rats. *Indian J. Pharmacol.* 2007; 39:43-47.
- Nadukwe G, Achimuga MO, Amako NF. Phytochemical and antimicrobial screening of crude extracts from the stem bark of *Invingia gabonesis*. *J. Pest Dis. Vector Mang.* 2005; 6:391-397.

26. Cowan MM. Plant product as antimicrobial agents. *Clin. Microb. Rev.* 1999; 12(4):564-582.
27. Lewis K, Ausubel FM. Prospects for plant derived antibacterials. *Nat. Biotechnol.* 2006; 24(12):1504-1507.
28. Parekh J, Nair R, Chanda S. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J. Pharmacol.* 2005; 37:408-409.
29. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. *Turk. J. Biol.* 2005; 29:41-47.
30. Nikaïdo H. Microdermatology; cell surface in the interaction of microbes with the external world. *J. Bacteriol.* 1999; 181:4-8.
31. Fowa AB, Djouedam FG, Njateng GS, Tsafack DN, Sokoudjou JB, Kodjio N, Gatsing D. In vitro antisalmonellal activities of leaf extracts of *Adenia lobata* Jacq. (Passifloraceae) and mechanism of action of the most active extract on *Salmonella Typhi* ATCC6539. *In vitro.* 2019 Jul;4(3).