Research Article**Phytochemical screening and Antimicrobial activity of wild and tissue cultured plant extracts of *Tylophora indica*****A. Vanitha, S. Vijayakumar, V. Ranjitha, K. Kalimuthu****Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, India*

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Abstract

Objective: To analyze phytochemical of wild and tissue cultured plant ethanol and methanol extracts of *Tylophora indica* through FTIR, GCMS and evaluation of antimicrobial activity against four human pathogens. **Materials and Methods:** Preliminary phytochemical screening was done by standard methods. Analysis of the wild and tissue culture plants were carried out through the potassium bromide (KBr) pellet (FTIR grade) method. The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector. Antimicrobial activity of ethanol and methanol extracts of wild and tissue cultured plant samples in *Tylophora indica* against bacterial pathogens and also two fungal pathogens by agar well diffusion method. **Results:** All the extracts of the selected plant was found to contain alkaloids, flavonoids, steroids and triterpenoids. Glycosides and Anthraquinones were present only in wild and tissue cultured plant ethanol extracts not in methanol extracts. The FT-IR spectrum showed the presence of alkyl halides (C-Br), aliphatic amines (C-N), alkyl halides, aromatic amine, alkanes, aromatics, amine and β - saturated esters. GCMS analysis of wild and tissue cultured plant of ethanol, methanol extracts were identified 56, 40, 111, 90 compounds with 6, 2, 9, 8 bioactive compounds respectively. The antimicrobial activity of ethanol extracts of wild and tissue cultured plant showed good antibacterial activity at the concentration of 60 μ l against *Bacillus Subtilis* (13 mm) and *Klebsiella pneumoniae* (10 mm) respectively. **Conclusions:** The results confirmed the fact that this plant possesses effective bioactive compounds useful for our health. Further isolation and biological screening will be providing the therapeutic potential of the compounds. . In addition, the results revealed that the tissue cultured plant extracts was effective in the same way as wild plant extracts.

Keywords: Antimicrobial, FTIR, GCMS, *In vitro* and *Tylophora indica*.

Introduction

Medicinal plants are globally valuable sources of new drugs (Chen et al., 2010; Chacko et al., 2010; Hamilton, 2000). Further, up to 80 % of people in developing countries are totally dependent on herbal drugs for their primary healthcare, and over 25% of prescribed medicines in developed countries are derived from wild plant species (Hamilton, 2000).

The secondary plant metabolites like alkaloids, flavonoids, terpenoids, glycosides, tannins etc. are biosynthesized by plants. These are active constituents at the plant and have marked pharmacological activity. These are very potent in action (Rao and Ravi Shankar, 2002). Secondary metabolites

are small biomolecules considered to be non-essential for the life of the producer organism (Agostini-Costa et al., 2012).

Many secondary metabolites have great importance for humans. They are widely used as active drug ingredients in medicine (Mousa and Raizada, 2013; Fischbach and Walsh, 2009; Khazir et al., 2013), as herbicides or phytotoxins in agriculture (Duke et al., 2000), as food additives (Rao and Ravi Shankar, 2002), fragrances, and even as precursors for the synthesis of plastics (Mooney, 2009). The various chemical substances present in the plants are responsible for its medicinal activity. The plant chemical constituents are the basic source for the establishment of several pharmaceutical industries. The proportion of the phytochemicals playing a significant role in the identification of crude drugs (Savithramma et al., 2011).

Gas chromatography is an ideal separator, whereas mass spectrometry is excellent for identification. The aim of an interfacing arrangement is to operate both a gas

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chromatograph and a mass spectrometer without degrading the performance of either instrument.

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection.

In many earlier investigations made in southern parts of India, it has been reported that Asclepiadaceae (presently treated as Subfamily Asclepiadoideae under the family Apocynaceae, as per the APG III system of classification), is one among the dominant families that includes plants with potential curative values for many health problems (Ganesan et al., 2006; Jeeva and Femila, 2012; Lingaraju et al., 2013; Reddy et al., 2009). Asclepiadaceae are 348 genera, with about 2900 species. They are mainly located in the tropics to subtropics, especially in Africa and South America.

The species, *Tylophora indica* (Burm.f.) Merrill, (Asclepiadaceae) previously called as *Tylophora asthematica*, is an important indigenous medicinal plant. The plant is used as folk remedy in certain regions of India for the treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism, antiplasmodial and dermatitis (CSIR, 2003, Linz-Buoy et al., 2015). The powdered leaves, stem and roots contain several alkaloids including tylophorine (Gopalakrishnan et al., 1980) and tylophorinine which are pharmacologically active and anticancer tylophorinidine has also been isolated from the roots (Mulchandani et al., 1971). Several pharmacological companies (Acron Chemicals, Mumbai, India; Sabinsa Corporation, Piscataway, NJ, USA) are marketing *T. indica* extracts as anti asthmatic herbal drugs (Mohammad Faisal et al., 2007). The plant also has antibacterial and antioxidant activities (Thippeswamy et al., 2015; Ranemma et al., 2017). Apparently due to non-availability of sufficient quantity of planting materials, commercial plantations of this important aromatic and medicinal species have not been widely attempted and presently only the wild population is exploited for extraction purposes.

Hence comparative study has been made to scientifically document the medical properties through Preliminary phytochemical, FTIR, GCMS analysis and antimicrobial activities of wild and tissue cultured *Tylophora indica* plant.

Materials and methods

Preparation of plant extracts

100 g of *Tylophora indica* wild and 50 g of tissue cultured plants were subjected to soxhlet extraction followed successive

method using ethanol and methanol solvents (Elwekeel et al., 2013). Each plant extracts collected and evaporated in room temperature and finally stored in light sensitive bottle for further experiments.

Preliminary phytochemical studies

Phytochemical examinations were carried out for ethanol and methanol extracts as per the standard methods. The extracts were subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the plant material. Condensed extracts were used for preliminary screening of phytochemicals such as alkaloids (Ciulci, 1994), flavonoids (Sofowora, 1993), tannins (Ciulci, 1994), steroids (Ciulci, 1994), triterpenoids (Finar, 1986), saponins (Kokate, 1999), glycosides (Camporese et al., 2003), gum and mucilage (Whistler and BeMiller, 1993), fixed oil (Kokate, 1999) and anthraquinones (Sanker and Nahar, 2007).

Fourier-Transform Infrared Spectroscopy analysis (FTIR)

Analysis of the wild and tissue culture plants were carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using Jasco FT/IR-6300 Fourier transform infrared spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm^{-1} (JASCO, Tokyo, Japan).

Gas Chromatography Mass Spectrometry analysis (GC-MS)

Wild and tissue cultured plant ethanol and methanol extracts (1 μl) were used for GC-MS analysis. The GC-MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector.

Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 m x 0.25 μm DF of capillary column. The instrument was set to an initial temperature of 110 $^{\circ}\text{C}$, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 $^{\circ}\text{C}$, at the rate of an increase of 5 $^{\circ}\text{C}/\text{min}$, and maintained for 9 min. Injection port temperature was ensured as 200 $^{\circ}\text{C}$ and Helium flow rate as one ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Version –Year 2011 were used MS data library and comparing the spectrum obtained through GC-MS compounds present in the plants sample were identified.

Antimicrobial activity

Sample Preparation

10 mg of wild and tissue cultured plant ethanol and methanol extracts of *Tylophora indica* was weighed and dissolved in distilled 10 ml DMSO separately to a solution of 1 mg/ml concentration.

Test microorganisms

Streptococcus pyogenes, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* and human fungal pathogens like *Candida albicans* and *Trichoderma viride* were used as test organisms. The bacterial and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium at 37 °C and 28 °C respectively.

Preparation of Inoculum

The gram positive and gram negative bacteria *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* were pre-cultured in nutrient broth overnight in a rotary shaker at 37 °C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculums *Candida albicans*, *Trichoderma viride*, were prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores/ml.

Anti-bacterial Activity

The plant wild and tissue culture plant ethanol and methanol

extracts were tested by the well diffusion method (Anonymous, 1996). Different concentration of the extracts (30, 60, 90 µl) were prepared and used for this study. The test microorganisms were seeded into respective medium by spread plate method 10 µl (10 cells/ml) with the 24 h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Erythromycin (10 µg) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37 °C for 24 hrs. The diameters of the inhibition zones were measured in mm.

Antifungal Activity

The antifungal activity was tested by well diffusion method (Taylor et al., 1995). The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 100 µg concentrations of the extracts were placed on test organism-seeded plates. Erythromycin (10 µg well 1) used as positive control. The activity was determined after 72 hrs. of incubation at 28 °C. The inhibition zones diameter were measured in mm.

Results

Phytochemical analysis

The wild and tissue cultured plant ethanol and methanol extracts were subjected to various standard methods of chemical test for identification of the different constituent present with extracts. The results of presence or absence of compounds in there extracts were presented in the table 1. Alkaloids, flavonoids, steroids, triterpenoids were present in

Table 1. Preliminary phytochemical analysis of ethanol and methanol wild and tissue cultured plant extracts of *Tylophora indica*

Secondary metabolites	Tests	Wild plant ethanol extract	Tissue culture plant ethanol extract	Wild plant methanol extract	Tissue culture plant methanol extract
Alkaloids	Dragendroff's test	+	+	+	+
	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Hager's test	+	+	+	+
Flavonoids	10% HCl & 5% NaOH test	+	+	+	+
	Alkaline test	+	+	+	+
Tannins	5% FeCl ₃ test	-	-	-	-
Steroids	Libermann - Burchard's test	+	+	+	+
Triterpenoids	Libermann - Burchard's test	+	+	+	+
	Salkowski's test	+	+	+	+
Saponins	Foam test	-	-	-	-
Glycosides	Killer & Kilian test	+	+	-	-
Gum & Mucilages	Whistler & BeMiller test	-	-	-	-
Fixed oils	Spot test	-	-	-	-
Anthraquinones	NH ₄ OH test	+	+	-	-

+ indicate present; - indicate absent

the all extracts. Glycosides and Anthraquinones were present only in wild and tissue cultured plant ethanol extracts not in methanol extracts. Whereas tannins, Saponins, gum and mucilages and fixed oils were absent in both the extracts (Table 1).

Fourier-Transform Infrared Spectroscopy Analysis (FTIR)

Wild and tissue cultured plant samples of *T. indica* was subjected to FTIR spectrum analysis to identify the functional groups present in it Figure 1 and 2 represents the spectrum of wild and tissue cultured plant samples. Wild plant sample the prominent peaks were observed at around 443.63, 526.90, 1057.98, 1239.98, 1322.93, 1377.28, 1427.41, 1632.44 1728.86, 2311.02, 2926.80, 3401.07, 3766.97 and 3874.38 in the region of 500 to 3500 cm^{-1} . Whereas tissue cultured plant sample the intense peak in the spectrum are 439.18, 516.17, 1029.88, 1652.92, 2924.21 and 3733.69.

The peaks 526.90 and 516.17 having C-Br stretching band for alkyl halides, 1057.98 and 1029.88 having C-N stretch bond with aliphatic amines functional group found common in wild and tissue culture plant samples respectively. The other peaks and

compounds present in wild plant sample was 1239.98 (Alkyl halides), 1322.93 (Aromatic amine), 1377.28 (Alkanes), 1427.41 (Aromatics), 1632.44 (Amine), 1728.86 (α , β – unsaturated esters), 3401.07 (1, 2 amines, amides).

GC-MS analysis

The number of compounds from the GC fraction of the ethanol extract of *T. indica* wild plant was identified through GC-MS analysis. These compounds were identified through mass spectrometry attached with GC. The results of GCMS analysis of wild plant ethanol, methanol and tissue cultured plant ethanol and methanol extracts chromatogram showed 56, 40, 111 and 90 compounds respectively (Figure 3, 4, 5 and 6). Table 2 represents the compound nature, formula, CAS number of compounds present in the two or more than two extracts. In this + indicates present and – indicates the absent. Totally 38 compounds were present in either two extracts or more than two extracts. The compounds with bioactive nature are presented with formula and molecular weight in the table 3. Totally 56 compounds were identified through

Figure 1. FTIR analysis of wild plant of *Tylophora indica*

Figure 2. FTIR analysis of plant tissue cultured plant of *Tylophora indica*

Table 2. GC-MS analysis of wild and tissue cultured plant ethanol and methanol extracts of *Tylophora indica*

S. No	Compound name	Formula	CAS No	WPE	WPM	TCPE	TCPM
1	Ala.-beta.-ala, trimethylsilyl ester	C ₉ H ₂₀ O ₃ N ₂ Si	900333-69-0	+	+	-	-
2	1-Trimethylsilyloxy-2-Undecene (E)	C ₁₄ H ₃₀ OSi	900245-72-6	+	+	-	-
3	2-Butenethioic Acid, 3-(Ethylthio)-, S-(1-Methylethyl) Ester	C ₉ H ₁₆ OS ₂	56196-50-0	+	+	-	-
4	7-Oxooctanoic Acid, 2-Trimethylsilylethyl Ester	C ₁₃ H ₂₆ O ₃ Si	65690-30-4	+	+	+	-
5	E-2-Trimethylsilyloxy-3-Octene	C ₁₁ H ₂₄ OSi	86297-58-7	+	+	-	-
6	2-Oxovaleric Acid, Tert-Butyldimethylsilyl Ester	C ₁₁ H ₂₂ O ₃ Si	900353-15-8	+	+	-	-
7	Galacto-Heptulose	C ₇ H ₁₄ O ₇	900130-14-5	+	+	+	+
8	D-Glycero-D-Ido-Heptose	C ₇ H ₁₄ O ₇	900130-14-3	+	+	+	+
9	D-Glycero-D-Tallo-Heptose	C ₇ H ₁₄ O ₇	900130-14-7	+	+	+	+
10	D-Glycero-D-Galacto-Heptose	C ₇ H ₁₄ O ₇	77770-51-5	+	+	+	+
11	Sucrose	C ₁₂ H ₂₂ O ₁₁	57-50-1	+	+	+	+
12	1-Deoxy-D-Altritol	C ₆ H ₁₄ O ₅	68832-18-8	+	-	+	-
13	D-Erythro-Pentose, 2-Deoxy	C ₅ H ₁₀ O ₄	533-67-5	+	+	+	-
14	1,5-Anhydro-D-Mannitol	C ₆ H ₁₂ O ₅	900127-68-6	+	-	+	-
15	d-Mannoheptulose	C ₇ H ₁₄ O ₇	3615-44-9	+	-	+	-
16	N-Decanoic acid	C ₁₀ H ₂₀ O ₂	334-48-5	+	+	+	+
17	Undecanoic acid	C ₁₁ H ₂₂ O ₂	112-37-8	+	+	+	+
18	Tridecanoic acid	C ₁₃ H ₂₆ O ₂	638-53-9	+	-	+	+
19	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	1002-84-2	+	-	+	+
20	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	57-11-4	+	-	+	+
21	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	143-07-7	+	-	+	+
22	Pyrrole-2-Carboxylic Acid, 4-(1-Chlorodec-1-Enyl)-3,5-Dimethyl-, Ethyl	C ₁₉ H ₃₀ O ₂ NCl	900295-53-6	+	-	+	+
23	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	506-30-9	+	-	+	+
24	Nonadecanoic acid	C ₁₉ H ₃₈ O ₂	646-30-0	+	-	+	+
25	1-Monolinoleoylglycerol Trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	54284-45-6	+	-	+	-
26	Dl-Arabinose	C ₅ H ₁₀ O ₅	20235-19-2	-	+	-	+
27	2,3,4,5-Tetrahydroxypentanal	C ₅ H ₁₀ O ₅	53106-52-8	-	+	-	+
28	Lactose	C ₁₂ H ₂₂ O ₁₁	63-42-3	-	+	-	+
29	2-Hydroxymethyl-9-[.Beta.-D-Ribofuranosyl]Hypoxanthine	C ₁₁ H ₁₄ O ₆ N ₄	185377-94-0	-	-	+	+
30	Hydrazinecarboxylic acid, (2-ethoxy-1-methyl-2-oxoethylidene)-, ethy	C ₈ H ₁₄ O ₄ N ₂	91616-14-7	-	-	+	+
31	Pentane, 2-butoxy	C ₉ H ₂₀ O	62238-02-2	-	-	+	+
32	Pentanoic acid, 2-(Aminoxy)-	C ₅ H ₁₁ O ₃ N	5699-55-8	-	-	+	+
33	2-T-Butyl-4-Methyl-5-Oxo-[1,3]Dioxolane-4-Carboxylic acid	C ₉ H ₁₄ O ₅	900191-45-5	-	-	+	+
34	Cyclopropanetetradecanoic Acid, 2-Octyl-, Methyl Ester	C ₂₆ H ₅₀ O ₂	52355-42-7	-	-	+	+
35	Glucose	C ₆ H ₁₂ O ₆	50-99-7	-	+	-	+
36	l-Glucose	C ₆ H ₁₂ O ₆	921-60-8	+	+	-	+
37	Beta.-D-Glucopyranose, 4-O-.Beta.-D-Galactopyranosyl	C ₁₂ H ₂₂ O ₁₁	5965-66-2	-	+	+	+
38	N-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	57-10-3	+	+	+	+

Figure 3. GCMS analysis of wild plant ethanol extract of *Tylophora indica*

Figure 4. GCMS analysis of wild plant methanol extract of *Tylophora indica*

Figure 5. GCMS analysis of tissue cultured plant ethanol extract of *Tylophora indica*

Figure 6. GCMS analysis of tissue cultured plant methanol extract of *Tylophora indica*

GC-MS analysis of wild plant ethanol and methanol extracts. Among these 56 compounds 8 were high peak compounds are showed (Figure 3). In this N-Hexadecanoic acid having antioxidant, hypocholesterolemic nematocide, pesticide, anti-androgenic flavor, hemolytic, 5- α reeducates inhibitor activity.

The result of GCMS analysis of wild plant methanol extract showed 40 compounds and presented in figure 4. Among these 3, 7, 11, 15, 18-Pentaoxa-2, 19-Disilaicosane, 2, 2, 19, 19-tetramethyl, sucrose, inositol, 1-deoxy and N-Decanoic acid

were high peak compounds presented in figure 4. In this study Undecanoic acid having Manufacturing of a number of esters, some of which are used in perfumes, more in flavor composition.

Whereas in tissue cultured plant ethanol extract GC-MS analysis yield 111 compounds with 10 high peak compounds (Figure 5). The bioactive compounds N-Hexadecanoic acid, Undecanoic acid, Pentadecanoic acid and Tridecanoic acid was present in this extract with CAS No of 57-10-3, 112-37-8, 1002-84-2 and 638-53-9. Pentadecanoic acid having

Table 3. Bioactive uses of wild and tissue cultured plant ethanol and methanol extracts of *Tylophora indica*

S. No	Compound name	Formula	Weight	Bioactive uses
1	Undecanoic acid	C ₁₁ H ₂₂ O ₂	186	Manufacturing of a number of esters, some of which are used in perfumes, more in flavor composition (Patel et al. 2017).
2	Tridecanoic acid	C ₁₃ H ₂₆ O ₂	214	Antibacterial, antifungal (Chandrasekaran et al. 2011) Anthelmintic, Anti-inflammatory and Antimicrobial activities and anti- cancerous activity (Kavitha and Mohideen 2017).
3	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242	Antioxidant (Elezabeth and Arumugam, 2014), Lubricants, Adhesive agents (Arora and Kumar, 2017).
4	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	Antifungal, Antitumor, Antibacterial (Arora and Kumar, 2017), Anti-inflammatory (Rajeswari, 2012; Vasudevan et al. 2012).
5	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200	Treatment of acne (Yamuna et al. 2017), Antimicrobial (Arora and Kumar, 2017), Antibacterial, antiviral, antifungal (Özçelik et al. 2005).
6	N-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Anti-inflammatory, nematocide, pesticide, lubricant, antiandrogeni, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant, hypocholesterolemic (Kumar and Rajakumar (2016). Antioxidant, hypocholesterolemic nematocide, pesticide, anti-androgenic flavor, hemolytic, 5- Alpha reductase inhibitor (Hema et al. 2011).
7	Caryophyllene	C ₁₅ H ₂₄	204	Anti-tumor, Analgesic, Antibacterial, Antiinflammatory, Sedative, Fungicide (Rency et al. 2015).
8	Decanoic acid, ethyl ester	C ₁₂ H ₂₄ O ₂	200	Flavour, Nematocide (Selvi and Basker 2012).
9	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	It is used in cosmetic and topical medicinal preparations where good absorption through the skin is desired (Jadhav et al. 2014).
10	Guanosine	C ₁₀ H ₁₃ O ₅ N ₅	283	Cytotoxic against T cells lines, Anti-viral against Vero cells infected with HSV-1 (Arora and Kumar, 2017).

Lubricants and Adhesive agents.

GC-MS analysis of tissue cultured plant methanol extract of *T. indica*. Chromatogram shows 90 compounds in different retention time. N-Hexadecanoic acid, the bioactive compound was present in this extract also. Among these 90 compounds 7 compounds were high peak compounds.

Antimicrobial activity

Antimicrobial activity of wild and tissue culture plant ethanol, wild and tissue culture plant methanol extracts of *T. indica* of

different concentrations (20, 40, 60 µl) was assayed against four species of bacteria (*Escherichia coli*, *Bacillus Subtilis*, *Salmonella paratyphi* and *Klebsiella pneumoniae*) and two species of fungi (*Aspergillus fumigatus* and *Verticillium lecanii*) by agar well diffusion method. Results of the microbial activity assay are presented in table 4 and 5; figure 7, 8, 9 and 10. In the present study the ethanol extracts of wild and tissue cultured plant showed good antibacterial activity at the concentration of 60 µl against *Bacillus Subtilis* (13 mm) and *Klebsiella pneumoniae* (10 mm) respectively.

Table 4. Antimicrobial activity of wild and tissue cultured plant ethanol extracts of *Tylophora indica*

Pathogenic bacteria	Zone of inhibition (mm)						Standard (Chloramphenicol)
	Wild plant ethanol			Tissue culture plant ethanol			
	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	
<i>Escherichia coli</i>	08	08	12	07	08	09	06
<i>Bacillus Subtilis</i>	06	11	13	06	08	09	06
<i>Salmonella paratyphi</i>	05	08	09	06	07	09	07
<i>Klebsiella pneumoniae</i>	06	08	09	08	09	10	07
<i>Aspergillus fumigatus</i>	04	05	06	05	06	07	05
<i>Verticillum lecanii</i>	05	05	06	04	05	06	05

Table 5. Antimicrobial activity of wild and tissue cultured plant methanol extracts of *Tylophora indica*

Pathogenic bacteria	Zone of inhibition (mm)						Standard (Chloramphenicol)
	Wild plant methanol			Tissue culture plant methanol			
	20 µl	40 µl	60 µl	20 µl	40 µl	60 µl	
<i>Escherichia coli</i>	05	07	09	06	08	09	06
<i>Bacillus Subtilis</i>	05	10	11	06	08	09	06
<i>Salmonella paratyphi</i>	05	09	11	05	08	11	07
<i>Klebsiella nemoniae</i>	05	09	12	06	08	09	05
<i>Aspergillus fumigatus</i>	05	06	08	06	07	08	06
<i>Verticillum lecanii</i>	04	05	07	06	08	09	05

Figure 7. Antimicrobial activity of wild plant ethanol extract of *Tylophora indica*

The second best inhibition was observed at 60 µl concentration against *Escherichia coli* (12 mm) and *E. coli* (9 mm), *Bacillus Subtilis* (9 mm) and *Salmonella paratyphi* (9 mm) respectively against control (Figure 7 and 8). Both the extracts showed good antifungal activity at 60 µl concentration against *Aspergillus fumigatus* (6 mm) and *Verticillium lecanii* (6 mm) (Figure 7 and 8).

Whereas wild and tissue cultured plant methanol extracts also showed effective antibacterial activity against *Klebsiella pneumoniae* (12 mm) and *Salmonella paratyphi* (11 mm) at 60 µl concentrations respectively. The second best inhibition zone was observed at 60 µl concentration against *Bacillus Subtilis* (11 mm), *Salmonella paratyphi* (11 mm) and *Bacillus Subtilis* (9 mm), *Klebsiella pneumoniae* (9 mm) respectively against control (Figure 9 and 10). These extracts inhibit the fungal growth moderately (Table 5; Figure 9 and 10) at 60 µl concentration with 8 mm, 7 mm and 8, 9 mm against *Aspergillus fumigatus* and *Verticillium lecanii* respectively.

Discussion

Many medicinally important secondary metabolites like alkaloids, flavonoids, steroids and triterpenoids were present in wild and tissue culture plant ethanol and methanol extracts.

Figure 8. Antimicrobial activity of tissue cultured plant ethanol extract of *Tylophora indica*

Glycosides and Anthraquinones were present in wild and tissue culture plant ethanol extracts. This is many be due to the type and nature of the explants. These phytochemicals are known to have therapeutic importance since they have biological activities. Among the secondary metabolites, flavonoids have antibacterial activity (Cushnie and Lamb, 2005), phenols showed antioxidant activity (Chakraborty et al., 2012), Tannins are shown to have antiviral, antitumor, wound healing and antiparasitic activities (Danial et al., 2011; Amokaha et al., 2002), Saponins are the precursors for the synthesis of steroidal drugs, sex hormones and contraceptives (Danial et al., 2011). The phytochemical study helps the future investigators regarding the selection of the particular explant for further study of isolating the active principles (Mishra et al., 2010).

In plants, an FTIR technique was used for evaluation of the type of organic and inorganic complex. The analysis was carried out on drying and low acting temperature material of different parts of plants (Theivandran et al., 2015). The FTIR analysis of wild and tissue culture plants of *Tylophora indica* infra-red spectrum shows a frequency range from 526-516 representing the C-Br stretching vibrating, presence of alpha

Figure 9. Antimicrobial activity of wild plant methanol extract of *Tylophora indica*

halides and the frequency ranges from 1057-1029 peaks are representing C-N stretch bond with aliphatic amines. Here the peaks are less (6 peaks) in tissue culture plant sample when compared to wild plant (14 peaks). This may be due to the age of the tissue culture plant material. Similar type of analysis has been documented in *Myxopyrum Smilacifolium* (Praveen et al., 2015), *Nicotiana tabacum* (Chinnadurai Vajjiram et al., 2017). The other peaks of wild plant represent alkyl halides, aromatic amine, alkane's aromatics, amines and unsaturated esters.

Through the FTIR spectrum we can confirm the presence of functional group in the plant part extracts, identify the medicinal materials from the adulterate and can evaluate the quantity of medicinal materials (Kumar and Prasad, 2011). The present results of FTIR spectroscopic revealed the functional constituent present in the wild and tissue culture plant samples of *T. indica*. It also showed the similarity and variation between these two samples based on the functional group with the help of absorption spectrum. Many researchers used the FTIR spectrum as a tool for classifying and discriminating closely related plants (Helm et al., 1991; Ellis et al., 2002). In the present study we applied the FTIR studies to identify and differentiate the wild and tissue cultured plants of *Tylophora indica*. The tissue culture plant showing some

Figure 10. Antimicrobial activity of tissue cultured plant methanol extract of *Tylophora indica*

of the similar functional groups with wild plant could be taken into advantage that without exploiting the wild plant, tissue cultured plants could be used for further studies.

The GC-MS analysis is useful tool to understand the nature of active principles present in various plant extracts. The chromatogram obtained from wild and tissue culture plant ethanol and methanol extracts of *T. indica* with relative concentration of various compounds separated along with retention time. The height of the peak appeared on the chromatogram based on the relative concentration.

The wild plant ethanol extract showed 56 bioactive compounds with 8 high peak compounds. In this, the bioactive compound N-Hexadecanoic acid with antioxidant, pesticide, anti-androgenic flavor, hemolytic, 5-alpha reeducates inhibitor activity (Hema et al., 2011). Whereas in wild plant methanol extract 40 compounds were identified. Among these four high peak compounds were present. The bioactive uses of these compounds were not studied or not known. The bioactive compound N-Hexadecanoic acid was present with 1.387 peak area.

Tissue culture plant ethanol and methanol extracts GC-MS

analysis yield 111 and 90 compounds with 10 and 7 high peak compounds. Like wild plant ethanol and methanol extracts these tissue culture plant ethanol and methanol extracts also, the bioactive compound N-Hexadecanoic acid and Undecanoic acid was present. The peak area of N-Hexadecanoic acid of wild ethanol and tissue culture plant ethanol and methanol extracts were 5.519, 1.387 and 12.072 respectively. The peak area was high in tissue culture plant methanol extract. In general the known bioactive compound N-Hexadecanoic acid was present in all the extracts. Even though many compounds were present in all the extracts but the number of known bioactive compound were ten. The activity of the phytochemicals identified in this study was also observed in the GC-MS studies of *Baccharoides anthelmintica* (Kalimuthu et al., 2016). Both wild and tissue culture plant extracts having many similar compounds. It shows that tissue culture plant extracts also almost equal effect like wild plant extracts. Presence of various bioactive compounds in all the extracts proved the purpose of *T. indica* for various ailments. However individual compound isolation, characterization proceeds to find a novel drug. Also the tissue culture plant can be used for isolation of compounds instead of natural plants.

The presences of phytochemical in the plants are responsible for the bioactivity. Plants rich in flavonoids are having antiviral (Mehrangiaz et al., 2011), antimicrobial (Maria Lysete and Maria Raquel, 2009) properties. The effectiveness of the extracts various according to the peaks, nature of the plant, solvent used and concentration and production of secondary metabolites (Shah et al., 2015). The present result was in accordance with results of (Krishna Reddy et al., 2009).

Antimicrobial activity of wild and tissue culture ethanol and methanol extracts of *T. indica* used against four species of bacteria and two species of fungi by agar well diffusion method. All the extracts showed good antimicrobial activity against bacteria and fungi. When compared to wild plant extracts, tissue culture plant extracts showed moderate inhibition against all the bacterial and fungal strains. This is in agreement with the results of *Ceropegia juncea* *in vivo* and *in vitro* plant extracts and *T. indica* (Saraswathy et al., 2017; Ranemma et al., 2017). The secondary metabolites flavonoids present in plants have been found *in vitro* to be effective antimicrobial substances against a wide range of microorganisms. Many lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya et al., 1996).

Conclusion

Based on the results obtained in this study, it is clear that the ethanol, methanol extracts of wild and tissue cultured plants of *T. indica* indicated the presence of secondary metabolites, and also confirmed the antimicrobial properties. In addition, the results revealed that the tissue cultured plant extracts was effective in the same way as wild plant extracts.

Conflicts of interest: Not declared.

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