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Phytochemical screening and GC-MS analysis of *Tylophora indica*

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Abstract
Tylophora indica belongs to the family Asclepiadaceae. The plants are widely used for important medicinal purpose worldwide for its ethno-medicinal property. It is a tropical medicinal plant used in treatment of Asthma, dermatitis and rheumatism. The present study describes the phytochemical investigation of *T.indica* using four different solvents such as hexane, acetone, ethyl acetate and methanol. The phytochemical studies revealed the presence of alkaloids, terpenoids, steroids, flavonoids, phenols, tannins, glycosides, carbohydrates, amino acids and saponins in the plant extracts. From the GC-MS analysis in *T.indica* using methanol extract totally 18 compounds were identified.

Keywords: medicinal plant *Tylophora indica*, phytochemical analysis and GC-MS analysis

1. Introduction
Herbal medicines were traditionally used by rural people in India especially in remote area. In India, since ancient times several medicinal plants were used to treat specific diseases. Thus over 50% of these modern drugs were of natural products origin, which plays an important role in drug development in the Pharmaceutical industry [1]. *Tylophora indica* is a valuable medicinal plant, belongs to the family Asclepiadaceae. Also known as "Antmul", it is a perennial, branching climber with long fleshy roots. It grows widely in plains and hilly areas of India up to an altitude of 1000 m in Bengal, Assam, Orissa and Southern India [2]. It is used for the treatment of asthma, bronchitis, whooping cough, inflammation, allergies, dermatitis, dysentery, diarrhoea and rheumatic gouty pains. It is used for its antitumor, anti-inflammatory, anti-anaphylactic properties and is also used to treat jaundice in certain parts of India [3] and Bhutan [4]. The plant extracts have been reported scientifically for their biological activities. Many phytochemical drugs could protect humans against certain diseases. Compounds present in *T. indica* shows a less side effects as compared to allopathic drugs. Phytoconstituents found in *T. indica* has various secondary metabolites like alkaloids, terpenoids, steroids, flavonoids, phenol, tannin, glycosides, carbohydrates, amino acids and saponins. The present study deals with phytochemicals and GC-MS analysis of *Tylophora indica*.

2. Materials and Methods
The leaves of *T.indica* were collected from Mannampandal at Mayiladuthurai in Nagapattinam district of Tamil Nadu, India during the month of April 2018. The collected plant materials were identified by Taxonomist, Department of Botany, Annamalai University. The leaves of the plant were subjected to Phytochemical analysis and the GC-MS analysis of the plant extract was also taken up.

2.1. Preparation of crude extract
The leaves of *T. indica* were collected and washed with distilled water and air-dried at room temperature for 10-15 days. After shade drying, the leaves were packed in brown cover and kept in an oven at 60^{o} C for an hour to make grinding easy. After an hour, the leaves were grinded using electrical blender. A hundred grams of the powder was extracted with different organic solvents like hexane, acetone, ethyl acetate and methanol for 8 hours, using the Soxhlet apparatus and the solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried powder was stored at 40^{o}C for further use.

2.2 Phytochemical analysis
The different extracts of *T. indica* were used for qualitative Phytochemical analysis for the presence of alkaloids, terpenoids, steroids, flavonoids, phenols, tannins, glycosides, carbohydrate, amino acids and saponins [5, 6].

2.3. GC-MS analysis
GC-MS analysis was carried out using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatography was fitted with DB 5 MS capillary column (30 m× 0.25 mm i.d., film thickness µ M). The injection temperature was set at 280^{O} C, and the oven temperature was initially at 45^{O} C then programmed to 300^{O} C at the rate of 10^{O} C/min and finally held at 200^{O} C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. One micro-liter of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of the composition of the compound was calculated by the GC peak areas. GC- Mass spectrometry (GC-MS) analysis of compound was performed using Varian 3800 gas chromatography equipped with Varian 1200 L single quadrupole Mass spectrometer. GC conditions were the same as reported for GC analysis and the same column 1000 amu. The compounds were identified based on the comparison of their retention indices (RI), retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data [7].

3. Result
The preliminary phytochemical studies revealed the presence of alkaloids, terpenoids, steroids, flavonoids,

phenols, glycosides, carbohydrates, amino acids and saponins in the plant extracts (Table 1). Phytochemical test using hexane indicates the presence of flavonoids, phenols and saponins. Acetone indicates the presence of alkaloids, steroids, phenol, glycosides, carbohydrates and amino acids. The ethyl acetate indicates the presence of alkaloids, steroids, flavonoids, glycosides, carbohydrates and amino acid. The methanol indicates the presence of more strong phytochemicals such as alkaloids, terpenoids, steroids, flavonoids, phenols, glycosides, carbohydrates, amino acids and saponins. The Phytochemical test doesn't show the presence of tannins and in methanol.

The compounds present in the methanolic leaf extract of *T. indica* were identified by GC-MS analysis and a total of 18 compounds were identified (Table 2) such as 1-.Deta.-D-Ribofuranosyl-3-[5-Tetraazolyl]-1,2,4-Triazol, Methyl(Methyl 4-O-Methyl-.Alpha.-D-Mannopyranoside) Uronate, n-Hexadecanoic Acid, Phytol, 4-Hexadecen-6-Yne, (E)-,. Beta.-Sitosterol, 2,4-Cyclohexadien-1-One, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-, 5-Methyl-2-Phenylindolizine, 1,4-Bis (Trimethylsilyl) Benzene, Cyclotrisiloxane, Hexamethyl-, 1,4-Bis (Trimethylsilyl) Benzene, 1,4-Bis (Trimethylsilyl)Benzene, Alpha.-Amyrin, Trichothec-9-En-4-ol, 7,8:12,13-Diepoxy-, 2-Butenoate, [4.Beta.(Z),7.Beta.,8.Beta.]-, Benzene, 2-[(Tert-Butyldimethylsilyl)Oxy]-1-Isopropyl-4-Methyl-, Urs-12-En-24-Oic Acid, 3-Oxo-, Methyl ester, (+)-, [1,2,4] Triazolo [1,5-a] Pyrimidine-6-Carboxylic Acid, 4,7-Dihydro-7-Imino-, Ethyl Ester and Tert-Butyl (5-Isopropyl-2-Methylphenoxy)Dimethylsilane. Mass spectrum of the bioactive compounds with their retention time (RT) is shown in the (Figure 1).

4. Discussion

The present study comprises of the Phytochemical studies in *T. indica.* It was observed that the extracts contain alkaloids, terpenoids, steroids, flavonoids, phenols, glycosides, carbohydrates, amino acids and saponins. All the Phytochemicals except tannins were present in the leaves of *T. indica.* Mohan *et al*., 2014 [8] and Kumar *et al*., 2011 [9] indicated that tannins were present in *T.indica* in the aqueous extracts. The secondary metabolites, flavonoids have antibacterial activity [10] and phenols showed antioxidant activity [11], Tannins were known to have antiviral, antitumor, wound healing and anti-parasitic activities [12]. Saponins were the precursors for the synthesis of steroidal drugs, sex hormones and contraceptives [13]. The ethanol extract of the wild plant showed 56 bioactive compounds with 8 high peak compounds. In this, the bioactive compounds n-Hexadecanoic acid with antioxidant, pesticide, anti-androgenic flavor, hemolytic, 5- alpha reductase inhibitor activity [14]. The GC-MS analysis of *Tylophora indica* leaves revealed the presence of 18 compounds. The identified compounds possess many biological properties such as antitumor, immunostimulant, perfumery, pesticide and antimicrobial, anticancer, anti-inflammatory and diuretic agent [15]. 9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z, Z, Z) -, n-Hexadecanoic Acid, 1,2-Benzenedicarboxylic Acid and Di-Isooctyl Ester were present in *Caesalpinia sappan* ethanol extract [16].

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

Sl no.	Phytochemical compounds	Hexane	Acetone	Ethyl acetate	Methanol
1	Alkaloids	-	+	+	+
2	Terpenoids	-	-	-	+
3	Steroids	-	+	+	+
4	Flavonoids	+	-	+	+
5	Phenol	+	+	-	+
6	Tannin	-	-	-	-
7	Glycosides	-	+	+	+
8	Carbohydrates	-	+	+	+
9	Amino acids	-	+	+	+
10	Saponins	+	-	-	+

(+) =Positive (Present), (-) = Negative (Absent)

Table 2: GC-MS analysis of methanolic leaf extract of *Tylophora indica*

Peak No	R. Time	Name of Chemical Compounds	Molecular formula	Molecular weight (g/mol)	Area %
1	6.087	1-Beta-D-Ribofuranosyl-3-[5-Tetraazolyl]-1,2,4-Triazole	$C_8H_{11}N_7O_4$	269.22	2.75
2	8.573	Methyl (Methyl 4-O-Methyl-Alpha-D-Mannopyranoside) Uronate	$C_9H_{16}O_7$	236.22	11.78
3	11.741	n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.42	2.71
4	13.169	Phytol	$C_{20}H_{20}O$	296.5	1.40
5	13.433	4-Hexadecen-6-Yne, (E)-	$C_{16}H_{28}$	220.39	2.84
6	15.873	Beta-Sitosterol	$C_{29}H_{50}O$	414.7	9.44
7	16.818	2,4-Cyclohexadien-1-One, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-	$C_{14}H_{22}O_2$	222.32	8.34
8	17.083	5-Methyl-2-Phenylindolizine	$C_{15}H_{13}N$	207.27	2.03
9	17.461	1,4-Bis (Trimethylsilyl)Benzene	$C_{12}H_{22}Si_2$	222.47	6.34
10	17.679	Cyclotrisiloxane, Hexamethyl-	$C_6H_{18}O_3Si_3$	222.46	1.78
11	17.896	1,4-Bis (Trimethylsilyl)Benzene	$C_{12}H_{22}Si_2$	222.47	4.73
12	18.038	1,4-Bis (Trimethylsilyl)Benzene	$C_{12}H_{22}Si_2$	222.47	2.28
13	18.265	Alpha-Amyrin	$C_{30}H_{50}O$	426.7	11.14
14	19.371	Trichothec-9-En-4-ol, 7,8:12,13-Diepoxy-, 2-Butenoate, [4. Beta. (Z), 7. Beta., 8. Beta.]-	$C_{19}H_{24}O_5$	332.4	2.20
15	20.610	Benzene, 2-[(Tert-Butyldimethylsilyl) Oxy]-1-Isopropyl-4-Methyl-	$C_{16}H_{28}OSi$	264.48	3.90
16	22.217	Urs-12-En-24-Oic Acid, 3-Oxo-, Methyl ester, (+)-	$C_{31}H_{48}O_3$	468.7	11.99
17	22.435.	[1,2,4] Triazolo [1,5-a] Pyrimidine-6-Carboxylic Acid, 4,7-Dihydro-7-Imino-, Ethyl Ester	$C_8H_9N_5O_2$	207.19	11.55
18	24.250	Tert-Butyl(5-Isopropyl-2-Methylphenoxy) Dimethylsilane	$C_{16}H_{28}OSi$	264.48	2.83

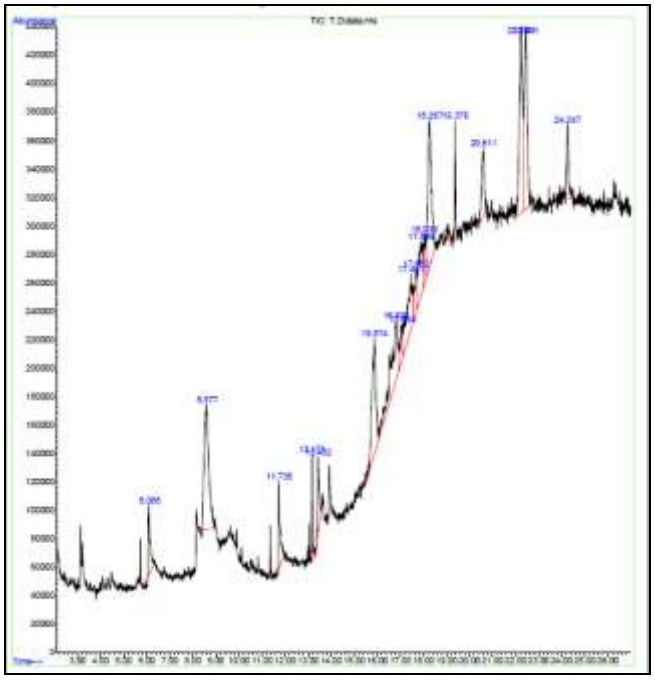

Fig 1: GC-MS Analysis of Methanolic leaf Extract of *Tylophora indica*

5. Conclusion

Tylophora indica is widely used as a medicinal plant. The medicinal values are due to the presence of bioactive compounds present in the plants. Mostly it is used to treat respiratory disorders, mainly asthma; hence it has a synonym *Tylophora asmatica.* It is also used to treat various ailments like sinusitis, digestive discomforts, etc. The preliminary phytochemical studies revealed the presence of alkaloids, terpenoids, steroids, flavonoids, phenols, glycosides, carbohydrates, amino acids and saponins in the plant extracts. The GC-MS analysis of the methanolic leaf extract of *T. indica* showed the presence of 18 compounds. The bioactive compounds are to be isolated to know more about their pharmaceutical values.

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