PHYTOCHEMICAL ANALYSIS AND TLC PROFILE OF *TYLOPHORA SUBRAMANII* A.N. HENRY (ASCLEPIADACEAE) - AN ENDEMIC MEDICINAL PLANT SPECIES OF SOUTHERN INDIA

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Abstract: ***Tylophora subramanii*****, a climbing herb belongs to Family Asclepiadaceae. This is endemic Species habitat along the southern Western Ghats in India. It is used as traditional and folklore medicine for treating various disease like fever, cold, cough, diarrhoea, ulcer, external tumour, cut wounds and headache. The present study, primarily aims to carry out a preliminary phytochemical screening so as to detect the major class of compounds present in *T.subramanii* leaf and stem to perform thin layer chromatography (TLC) profiling of all sequential extracts. Phytochemical analysis of the different solvent extracted samples suggested the presence or absence of various metabolites including, alkaloid, saponin, flavonoid, carbohydrate in varying concentration. TLC profiling of the *T.subramanii* was constituted different coloured phytochemical compound with different RF values. The present study provides evidence that solvent extracts if *Tylophora subramanii* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.**

Keywords: ***Tylophora subramanii*****, Phytochemicals, TLC, Rf value, Alkaloid.**

1. INTRODUCTION:

Genus Tylophora R.Br. is a pantropical genus distributed in tropical and subtropical Asia, Africa, India to Australia about 60 species (Tseng and Chao, 2011). In India it is reported to have 21 species (Jagtap & Singh, 1999; Karthikeyan et al., 2014), of which 14 species occur in Tamil Nadu (Srinivasan, 1987). *Tylophora subramanii* is a native plant of southern India commonly found in evergreen forest areas of Theni, Tirunelveli and Kanyakumari districts of Tamil Nādu up to 1200 m elevation (Ravichandran et al., 2016). It is a slender branched climber with smooth pubescent bark. Leaves, watery latex and root part of the plant has been used for treating various local health care systems. The genus Tylophora have been used for treating various diseases like asthma, leukorrhea, dysentery, fever and headache. Root of this genus is acrid and is said to be emetic (Karuppusamy, 2007). The plant is used to cure nervous disorders among Kani tribe community of Agastiyamalai hills in Tamil Nadu. The plant is having watery latex in all over the body to have a number of secondary metabolites and high hydrocarbon content. The fruits and leaves of the plant have possessed the antioxidant capacity due to the presence of secondary metabolites. *Tylophora subramanii* plant stem and leaf was selected for this study is based on its traditional medicinal use (Jayakumar et al., 2015). The purpose of the present study is to investigates the phytochemical analysis and TLC profile of different extracts of *T.subramanii* stem and leaf. The obtained result provides a support for the use of this plant in traditional medicine and its further investigation and isolation of phytoconstituents.

2. MATERIAL AND METHODS

2.1. COLLECTION OF PLANT MATERIAL

Plant was selected for this study is based on its traditional medicinal use. Fresh stem and leaf were collected from Megamalai Wildlife Sanctuary of Theni district, Tamil Nadu (Fig.1). in India, in the month of September 2021. The plant voucher specimen's identification was done with the help of Dr. Ravichandran. Senior preservation Assistant, Botanical Survey of India, Southern Regional Centre, Coimbatore. The Identified plant was arranged alphabetically with their botanical name with author citation, family and habit referring to Flowering plants of Kerala (Nayar et al., 2006) Flora of Presidency of Madras (Gamble et al., 1957) and Flora of Presidency of Tamil Nadu (Matthew et al., 1983).The voucher specimen is deposited in the herbarium of the Department of Botany, Kongunadu Arts and Science college, Coimbatore, Tamil Nādu.

(Figure 1: Habit of *Tylophora subramanii* a) Flowering twig, b) Inflorescence, c) Folicle fruit)

2.2. PREPARATION OF PLANT EXTRACT

After collection of the plant material the stem and leaf sample were dried at room temperature until they were free from moisture. The plant material was subjected size reduction to get coarse powder, 200 gm of shade dried plant material were grinded to powder then stored in clean ait tight container (Karthika et al., 2021). It was separately subjected to Soxhlet extraction with the following solvent Hexane, Chloroform, Acetone, Ethanol, Water in order of their increasing polarity. The solvents were then evaporated under reduced pressure and died using a rotary evaporator at 55℃. Dried extracts were stored in labelled sterile flasks at 5℃ in the refrigerator, until when required for use.

2.3. PHYTOCHEMICAL ANALYSIS

Plants are the resource of primary and secondary metabolites namely alkaloids, terpinoids, flavanoids, saponins, coumarins, glycosides, phenolics, carboxylic acids, amino acids, sugars, proteins etc. these phytochemicals have significant biological functions and also which contribute specific characteristic and property of the plant (R. Shyam Praveen., et al., 2022). Here Preliminary qualitative phytochemical screening was carried out with the following methods

2.3.1 Test for alkaloids

a) **Dragendorff test**: To 2-3mL of each extract, add few drops of Dragendorff reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

b) **Mayer's test:** To 2-3mL of each extract was added with few drops of Mayer˝s reagent. Formation of white precipitate indicates the presence of alkaloids.

2.3.2 Test for flavonoids

Shinoda test: To 2-3mL of each extract, few fragments of magnesium metal were added in a separate test tube followed by dropwise addition of conc. HCl. Formation of magenta colour indicated the presence of flavonoids (George et al., 2015).

2.3.3 Test for glycosides –

a) Keller-Kiliani Test: To 2mL of extract, glacial acetic acid, one drop of 5% FeCl3 and conc. H2SO4 was added. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green colour indicates the presence of glycosides. 5.2.3.4. Test for phenols

b**)** Ferric chloride test: When 0.5mL of FeCl3 solution was added to 2mL of test solution, formation of a dark violet colour indicated the presence of phenols.

2.3.4 Test for saponins

a) Foam test: 1mL of each extract was taken in separate test tubes and to this 5mL of distilled water was added. Then this mixture was shaken vigorously. A persistent froth that lasted for at least 15min indicates the presence of saponins (Kalawole et al., 2006)

2.3.5 Test for steroids

a) Liebermann-Burchard Test: 2mL of each extract was mixed with chloroform. Added 1-2mL of acetic anhydride and 2 drops of conc. H2SO4 from the side of the test tube. Formation of brown ring at the interface of the two supernatant layers indicates the presence of steroids.

2.3.5 Test for tannins

a**)** Braemer's test: 2mL of each extract was diluted with distilled water followed by the addition of 2-3 drops of 5% ferric chloride solution. Indication of green-black or blueblack coloration showed the presence of tannins.

2.3.6 Test for terpenoids

a) Salkowski test: 2mL of chloroform and conc. H2SO4 were added to 1mL of each extract. Appearance of reddish-brown colour indicates the presence of terpenoids.

b) Copper acetate test: To 1mL of extract, few drops of copper acetate were added. Formation of green colour indicates the presence of terpenoids

2.3.7 Test for Triterpenoids:

2 ml of test solution, added a piece of tin and 2 drops of thionyl chloride. The result was observed (Kolawole et al., 2006).

2.3.8 Test for Phenols:

2 ml of aqueous extract is added to 2 ml of 2N Hcl and ammonia. The appearance of pink-red turns blue-violet indicates the presence of phenols (Harborne, 1973)

2.3.9 Test for Coumarins:

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates the presence of coumarins (Harborne, 1973).

2.3.10 Test for fixed oils and fats

a) Small quantity of the extracts was separately pressed between two filter papers oil stain on the paper indicates the presence of fixed oil.

b) The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of 1 cm layer of foam.

2.3.11 Test for gums and mucilage's

About 10 ml of the extracts was added to 25 ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates

2.4.12 Test for volatile oil

0.1 ml of NaOH was added to 2ml of extract then add diluted Hcl shaken the formation of white precipitate indicate the presence of volatile oil.

2.3.13 Test for protein and amino acid

a) Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of aqueous filtrate. A characteristic of purple colour indicates the presence of amino acid

b) Millon's test: To 2 mL of filtrate, few drops of Millon's reagent were added. A yellow precipitate indicates the presence of amino acid

c) Biuret test: 2ml of extract with few drops of 2% of copper sulphate solution, add 1 ml of ethanol followed by excess of potassium hydroxide pellets, formation of pink colour in the extract layer indicates the presence of protein.

2.3.14 Test for carbohydrates

a) Molisch's test To 2 mL of filtrate, two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1 mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. Appearance of a violet colour ring indicates the presence of carbohydrates.

b) Barfoed's test To 1 mL of filtrate, 1 mL of Barfoed's reagent (copper acetate in glacial acetic acid) was added and heated on a boiling water bath for 2 min, formation of red precipitate indicates the presence of carbohydrates.

2.4. THIN LAYER CHROMATOGRAPHY (TLC PROFILE)

TLC can be used to identify compound present in a given substance and is less time consuming, low cost and can be performed with less complicated technique (Karthika et al., 2015). It has a wide application and significantly used in pharmaceutical purposes (Nazar et al., 2015; Talukdar AD et al., 2010). The mobile phase should be prepared freshly at the time of experiment (Figure 2 and 3).

The purity of the solvents and quality of the solvent's mixture should be strictly followed. Thin layer chromatography was carried out with the various extract of stem and leaf of *Tylophora subramanii* and maximum spots been separated on precoated silica gel G TLC plate with trial-and-error methods.

For TLC analysis plate with Silica gel 60 F254 TLC (Merck, Germany), 15X9 cm was cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-µl of sample by using capillary at distance of 1 cm at 5 tracks, by the different solvent system Hexane: Acetic acid (9:1) solvent system-1 and In the solvent system-2, Ethyl acetate: Methanol: Glacial acetic acid: water (9:2:1:2) used.

After pre saturation with mobile phase for 20 min for development were used. Freshly prepared iodine spray reagents were used to detect the bands on the TLC plates. The movement of the analyte was expressed by its retention factor (Rf), values were calculated for different samples.

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by solvent front}}$$

3. RESULTS AND DISCUSSION

3.1. PERCENTAGE OF YIELD EXTRACT

The yield of sequential extracts (%) is shown in [Table 1].

Table-1: Extractive value of different extracts of *Tylophora subramanii* stem and leaf

NAME OF THE SOLVENT USED	STEM		LEAF	
	Colour of extract	Percentage yield (%w/w)	Colour of extract	Percentage yield (%w/w)
Hexane	Light Green	4.04%	Green	9.68%
Chloroform	Green	2.44%	Dark green	3.77%
Acetone	Dark green	1.96%	Dark green	3.095%
Ethanol	Dark yellowish Brown	7.36%	Yellowish Brown	14.74%
Water	Brown	3.57%	Light brown	5.01%

3.2. PHYTOCHEMICAL ANALYSIS

Phytochemical screening of the sequential extract of *Tylophora subramanii* revealed the presence of various bioactive components of which phenolics, saponins, alkaloids, tannin, Glycosides, Proteins, Carbohydrates, and Amino acids are the most prominent components and the result of phytochemical test given in the [Table 2]. Among these phytochemical tests, Alkaloids, were present in all solvent extracts. whereas most of the active compound are alkaloid, flavonoid, Glycosides, Tannin, Phenols are present in the ethanolic extract of plant material.

Table-2: Quanlitative phytochemical analysis of the different extracts of *Tylophora subramanii* stem and leaf

S. No	PHYTOCHEMICALS	STEM EXTRACT					LEAF EXTRACT				
		HEX	CHL	ACE	ETH	WAT	HEX	CHL	ACE	ETH	WAT
1.	ALKALOID	+	++	++	+++	+	-	+	+	+++	+
2.	FLAVANOID	-	+	+	+++	+	-	-	-	++	+
3.	STEROID	-	-	-	+	+	-	-	-	-	-
4.	TERPENOID	++	-	+	-	-	+	-	-	+	-
5.	TRITERPENOID	+	-	-	-	-	+	-	-	-	-
6.	TANNIN	-	++	++	+++	++	-	+	+	+	-
7.	PHENOL	-	+	+	++	+	-	+	+	+++	++
8.	COUMARIN	-	-	-	-	-	-	+	-	-	-
9.	GLYCOSIDES	+	+	-	+++	+	+	+	+	+++	+
10.	SAPONIN	-	-	-	-	+	-	-	-	++	+
11.	GUMS AND MUCILAGE	+++	+	-	-	-	+++	+	-	-	-
12.	VOLATILE OIL	+	+	-	-	-	-	-	+	++	+
13.	FIXED OIL	+	-	-	-	-	+	+	-	-	-
14.	CARBOHYDRATE	+	+	+	++	++	-	-	+	++	+
15.	PROTEIN	-	-	+	++	+	-	-	-	+	+
16.	AMINO ACID	-	+	+	++	++	-	-	+	++	+

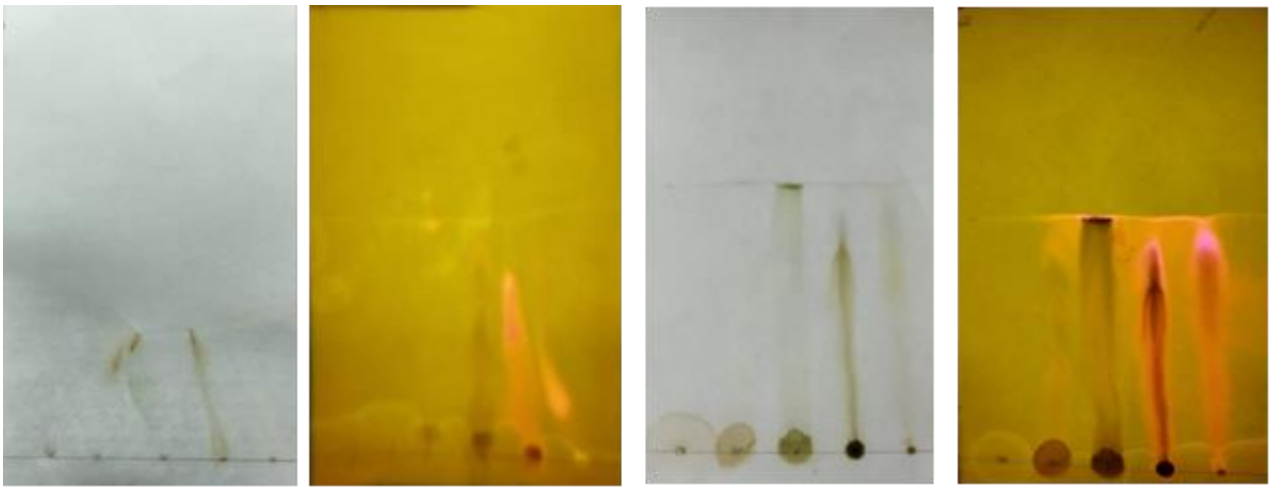
(+++ abundant; ++ moderately present: +weakly present; -----absent, HEX-Hexane, CHL-Chloroform, ACE-Acetone, ETH-Ethenol, WAT-Water).

3.4. THIN LAYER CHROMATOGRAPHY (TLC PROFILE)

Thin layer chromatography was performed with various crude extracts such as such as hexane, chloroform, acetone, ethanol and water of *Tylophora subramani* stem and leaf. For each extract, two different solvent systems were used to analysed the presence of secondary metabolites. The retention factor (Rf) for each of the extracts of *Tylophora subramani in* different solvent system were observed (Table 3and Figure 3and 4). This result will help us to select solvent system for further isolation of bioactive compounds from this plant.

Table-3: TLC profile of various extract of stem and leaf

TLC profile of different solvent extract	Solvent system 1 Hexane:Acetic acid (9:1)		Solvent system 2 Ethyl acetate: Methanol: Acetic acid: water (9:2:1:2)	
	Rf value of stem	Rf value of leaf	Rf value of stem	Rf value of leaf
Hexane	0.13,0.37	0.39,0.73	0.22, 0.44	0.81
Chloroform	0.26,0.46	0.62	0.52, 0.60	0.74,0.81
Acetone	0.69	0.39,0.87	0.61	0.55, 0.72,0.84
Ethanol	0.62	0.74	0.52	0.56, 0.72,0.81,
Water	0.52	0.51,0.80	0.71	0.75,0.92



(a) (b) (c) (d)

Figure 2

TLC PROFILE OF VARIOUS SOLVENT EXTRACTION OF STEM AND LEAF IN SOLVENT SYSTEM-1

TLC profile of stem and leaf solvent system 1(a-stem under visible light, b-stem under UV visible light, c-leaf under visible light, d-leaf under UV visible light)

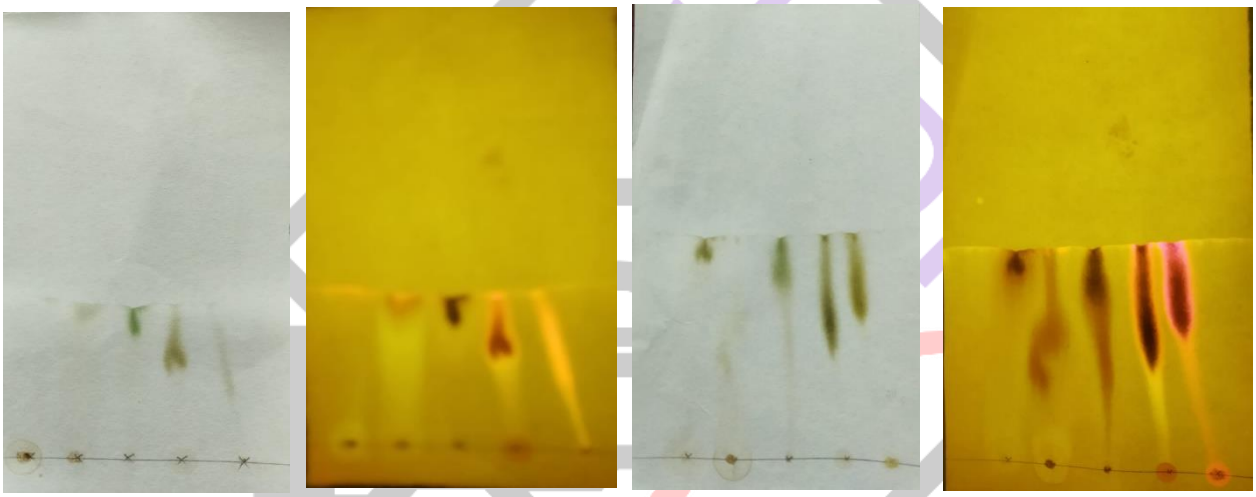


Figure-3

(e) (f) (g) (h)

TLC PROFILE OF VARIOUS SOLVENT EXTRACTION OF STEM AND LEAF IN SOLVENT SYSTEM-2

TLC profile of stem and leaf solvent system-2(e-stem under visible light, f-stem under UV visible light, g-leaf under visible light, h-leaf under UV visible light)

4. CONCLUSION

Researchers have spent tremendous amount of time and resource to find the importance of medicinal plants. Each and every plants contain special compounds which helpful in various pharmacological purposes. The investigation of our present study is a preliminary screening of *Tylophora subramanii* as a rich source of secondary metabolites. These findings suggested that *Tylophora subramanii* could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. The stem and leaf portion of the *T.subramanii* can provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by the organisms. Further purification, identification and characterization of the active compounds would be our priority in future studies.

5. ACKNOWLEDGEMENT

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6. CONFLICTS OF INTREST

The authors declare no conflicts of interest.

References

Gamble, J.S. (1957). Flora of the Presidency of Madras (Rep. Ed.). Adlard & Son, Ltd. London.

George, L. B., Mathew, S., Jani, D., Patel, S., & Highland, H. (2015). Tylophora indica extracts manifest potent in vitro anti-plasmodium activity. *International Journal of Pharma Sciences*, *5*(6), 1297-1306.

Harborne, J.B. (1973). Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York.

Jagtap, A.P. and N.P. Singh, (1999). Fascicles of Flora of India, Fascicle 24, Botanical Survey of India, Calcutta, India.

Jayakumar, M., & Karuppusamy, S. (2015). Preliminary Phytochemical Investigation on Tylophora Subramanii Henry (Apocynaceae)–An Endemic Medicinal Plant Species Of Southern India. *Kongunadu Research Journal*, *2*(2), 84-87. b

Karthika, K., & Paulsamy, S. (2015). TLC and HPTLC fingerprints of various secondary metabolites in the stem of the traditional medicinal climber, solena amplexicaulis. *Indian journal of pharmaceutical sciences*, *77*(1), 111.

Karthikeyan, S., M. Sanjappa and M. Kothari 2009. Flowering Plants of India: Dicotyledons. Vol. 1. (AcanthaceaeAvicenniaceae). Botancial Survey of India, Kolkatta.

Karuppusamy S. (2007). Medicinal plants used by Paliyan tribes of Sirumalai hills of Southern India. Nat. Prod. Rad. 6: 436-442.

Kolawole, O. (2006). *Determinants of profit efficiency among small scale rice farmers in Nigeria: A profit function approach* (No. 1004-2016-78920).

Krishnamoorthy Karthika,, & Vimal priya Subramanian. (2021). A phyto pharmacological review of medicinaly important plant solena amplexcaulis (cucurbitaceae). *Kongunadu Research Journal*, *8*(2), 44-48.

Matthew, K.M. (1983) The Flora of the Tamil Nadu Carnatic, I(III). The Rapinat Herbarium, St Joseph's College, Tiruchirapalli, India.

Nayar, T.S., Rasiya Beegam, A. and Mohanan, R. (2006). Flowering plants of Kerala. Tropical Botanical Garden and Research Institute. p.1079.

Nazar, S., Hussain, M. A., Khan, A., Muhammad, G., & Bukhari, S. N. A. (2020). Alkaloid-rich plant Tylophora indica; current trends in isolation strategies, chemical profiling and medicinal applications. *Arabian Journal of Chemistry*, *13*(8), 6348-6365.

R. Shyam Praveen. S., Jamuna, S., & Venkatachalapathi, A. (2022). *In Vitro* shoot induction in the important medicinal plant *Cyclea peltata* (Lam) Hoo. F. Thoms (Menispermaceae). *International Journal of Scientific Research*, *11*(2).

Ravichandran, V., & Karuppusamy, S. (2016). Check list of Endemic flowering plants of Western Ghats from Megamalai Wildlife Sanctuary, Tamilnadu, India. *Journal of Biological Records*, *1*(2), 36-51.

Srinivasan, S. R. 1987. Asclepiadaceae. In: Henry, A. N. et al. (Eds), Flora of Tamil Nadu. Botanical Survey of India, Southern Circle, Coimbatore.

Talukdar AD, Choudhury MD, Chakraborty M, Dutta BK. "Phytochemical screening and TLC profiling of plant extracts of Cyatheagigantea (Wall. Ex. Hook.)" Haltt. And Cyatheabrunoniana. Wall. ex. Hook. (Cl. and Bak.). Assam Uni J SciTechnol Biol Env Sci 2010; 5:70-4.

Tseng, Y.H. and C.T. Chao, (2011). Tylophora lui (Apocynaceae) a new species from Taiwan. Ann. Bot. Fennici. 48: 515-518