International Journal of Scientific Research and Engineering Development-– Volume 5 Issue 2, Mar-Apr 2022

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

BIOACTIVE COMPOUND SCREENING AND FTIR ANALYSIS OF ETHANOLIC LEAF EXTRACT OF TYLOPHORA SUBRAMANII A.N.HENRY

[1]Vimal Priya, S. [2]Shyam Praveen, R.and[3]Karthika,*K.

[1&3]Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641029, Tamil Nadu, India.

[2]Department of Territory and Agro Forestry System (TESAF), University of Padova, Italy.

Corresponding Author* E.mail: karthikak_bo@kongunaducollege.ac.in

ABSTRACT

Medicinal plants have been used in the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. There is continuous and urgent need to discover new active biological compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. An active compound of the medicinal plant has become a promising acquaintance in the development of phytomedicine to combat various diseases or disorder. The present investigation was carried out to assess the qualitative phytochemical analysis of leaves of *Tylophora subramanii* A.N.Henry. The phytochemical screening of leaf extracts revealed the presence of steroids, saponins, alkaloids, flavonoids, glycosides, phenolic compounds, tannins and terpenoids in ethenolic tracts. The major functional group present in this plant was determined by FTIR analysis showed the existence of functional groups such as alkanes, aromatic compound, aromatics, carboxylic acid, phenol, aromatics ester and alkene compounds

Key words: *Tylophora subramanii,* Functional group, FTIR, Leaf extract.

1. INTRODUCTION

In Indian traditional medicine system plants play a significant role, concerning its cultural and economic values. The compounds from plants are called as phytocompounds, which has properties of protection or disease prevention. More than 50% of plant compounds and their derivatives are used as natural drugs in clinical use. Recently people have shown interest in natural products as it is cheap, safe and natural without harmful effect (Gowtham *et al*., 2019). Identification of the chemical nature of phytochemical compounds present in the medicinal plants will provide some information on the different functional groups responsible for their medicinal properties.

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 (Vimalpriya et al., 2022). 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. Hence the present study was aimed to identify the bioactive phytocompounds present in the ethenol leaf extract of such a medicinally important herb Tylophora subramanii.

2. PLANT DESCRIPTION

Climbing Undershrub,Pubescent stout twiners. Leaves to 14 x 8 cm, ovate or elliptic, oblong, obtuse at apex, aciculate, rounded or cordate at base, rusty pubescent; nerves 6 pairs, irregular; petiole 1.5-3 cm long. Umbel axillary, solitary, 10-15-flowered; peduncle to 10 mm long. Flowers creamy, 1.5 cm across; pedicels 8 mm long, stout, pubescent; sepals 3.5 x 1.5 mm, oblong, acute, hirsute; corolla lobes 6 x 5 mm, ovate, obtuse, oblique at apex; corona 3 x 3 mm, orbicular to shortly 5-lobed; Gynostegium 3 mm high, shortly 5-lobed at apex, glabrous.The Flowering and fruiting period is March-April (Kottaimuthu et al., 2015).

Classification

Kingdom: Plantae

Class: Dicotyledonae

Subclass: Gamopetale

Order: Gentianales

Family: Asclepidaceae

Genus:Tylophora

Species: Tylophora subramanii

Common name: Subramani's Ipecac

Figure: 1 Habit of *Tylophora subramanii*

3. MATERIALS AND METHODS

3.1.Collection of Plant Material:

Tylophora subramanii was collected from Megamalai Wildlife Sanctuary of Theni district, Tamilnadu, India. Plant specimen was identified by Dr. Ravichandran. Senior preservation Assistant, Botanical Survey of India, Southern Regional Centre, Coimbatore.

3.2.Preparation of Plant Extracts

The shaded dried leafs were powdered in the medical grinder. 50 grams of leaf powder was weighed, 500 ml of different solvents (hexane, chloroform, acetone, ethanol and distilled water) used for soxhlet and Maceration extraction. 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 (Karthika et al., 2021).

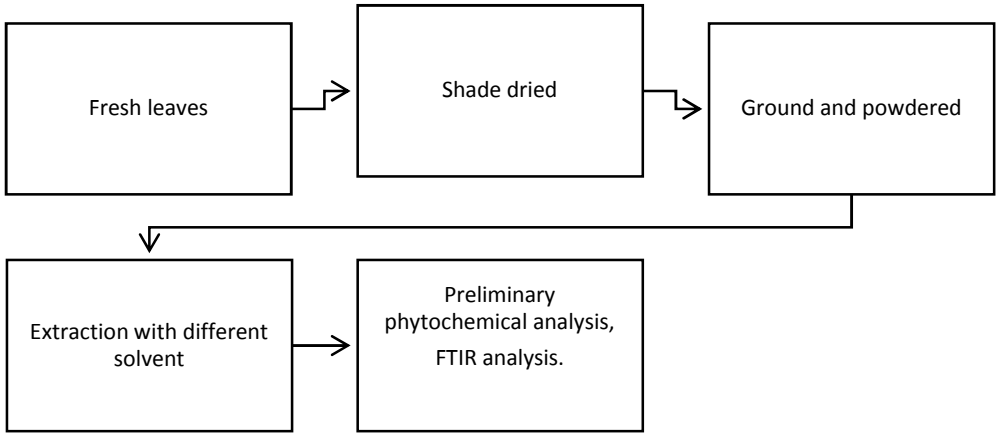

Fig. 2: Schematic representation of extraction processes

3.3. Qualitative screening of Phytochemical Compounds:

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 (shyam *et al*., 2022). Here Preliminary qualitative phytochemical screening was carried out with the following methods Allthequalitativetests[Table-1] weredonetoassessthepresenceoftheactivephytochemical constituents in the defatted leaf materials according to (Harborne 1984);(Wagner*et al*. 1984).

Table-1: Preliminary phytochemical screening

S.No	Phytocompounds	Nameofthetests	Combinations ofsolutions	Results to beobserved
1	**Alkaloids**	a)Mayer'stest	2ml of extract + fewdrops of 1HCl. Take1ml of this mixture andadd6dropsofMayer'sreagent.	Yellow-creamishprecipitate.
		b)Wagner'stest	2ml of extract + fewdropsof1HCl.Take1mlofthismixtureandadd6dropsofWagner'sreagent.	Brownish-redprecipitate.
		c)Dragendroff's test	2ml of extract + fewdrops of 1HCl. Take1ml of this mixture +add6dropsofWagner'sreagent.	Orangeprecipitate.
2	**Flavonoids**	a)Shinodatest	1mlofextract+threepiecesofmagnesium chips andaddfewdropsofconcentratedHCl.	Appearanceofan orange, pinkor red to purplecolour.
		b)Sulphuric acidtest	1 ml of extract + fewdrops of concentratedsulphuric acid.	Appearanceofdirtybrowncolour.
		c)Ferric chloridetest	1ml of extract + twodropsoffreshlyprepared ferric chloridesolution.	Appearanceofgreen,blueorvioletcolour.
3	**Steroid**	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.	Appearance of brown ring at the interface of the two supernatant layers indicates the presence of steroids.

4	**Terpenoids**	Salkowski test	5ml of extract + 2mlof chloroform + 3mlof concentrated sulphuricacid.	Appearanceofreddishbrownlayer.
5	**Triterpenoid**	Salkowski's test	Filtrate + few drops of conc. H2SO4 (Shaken well and allowed to stand)	Golden yellow layer (at the bottom)
6	**Tannin**	Braymer's test	1mL filtrated + 3mL distilled water + 3 drops 10% Ferric chloride solution	Blue-green colour
7.	**Phenol**	Ferric chloride test	Extract aqueous solution + few drops 5% ferric chloride sol	Dark green/bluish black colour
8.	**Coumarins**	NaOH paper test	0.5gm moistened extract is taken in test tube, mouth of test tube is covered with 1N NaOH treated filter paper, heated for few min. in water bath	Yellow fluorescence from paper under the UV light
9	**Glycosides**	Keller-killanitest	5mlofextract+2mlofglacialaceticacidcontainingonedropofferricchloridesolution.	Appearanceofbrown ring.
10.	**Saponin**	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
11.	**Gums and Mucilage**	Alcohol test	Dissolve 100mg extract in 10mL distilled water + 25mL absolute alcohol (constant stirring)	White or cloudy precipitate

12.	**Volatile oil**	Fluorescence test	l0 mL of extract, filtered till saturation, exposed to UV light	Bright pinkish fluorescence
13.	**Fixed oil**	Spot test/ Stain test	Little quantity of plant extract is pressed in between to filter papers	Oil stain on the paper
14.	**Carbo-hydrate**	Molish's test	2mL filtrate+ 2 drops of alcoholic α–naphthol + 1mL conc. H2SO4 (along the sides of test tube)	A violet ring
15.	**Protein**	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.
16.	**Aminoacid**	Ninhydrin test	2 drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of aqueous filtrate.	purple colour indicates the presence of amino acid

3.3.FTIR analysis

It is a valuable device for the identification and characterization of functional groups (chemical bonds) present in the compound. Besides, FTIR spectra are unique that they are like a molecular "fingerprint". (Rakhi *et al*., 2018)The drop forms a thin film between the cells. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet using a hydraulic press, which was then used for the analysis (Rani,*et al*., 2021). The samples of *Tylophora subramanii* ethanolic leaf extract was treated for FTIR spectroscopy IR-Affinity (Shimadzu, Japan). The samples were run at an infrared region between 1000 nm and 4000 nm and standard DLATGS detector was used at 2.8 mm/sec mirror speed.

4. RESULT AND DISCUSSION

4.1.Extractive Yield Percentage

The yield of sequential extracts (%) is shown in [Figure-3].

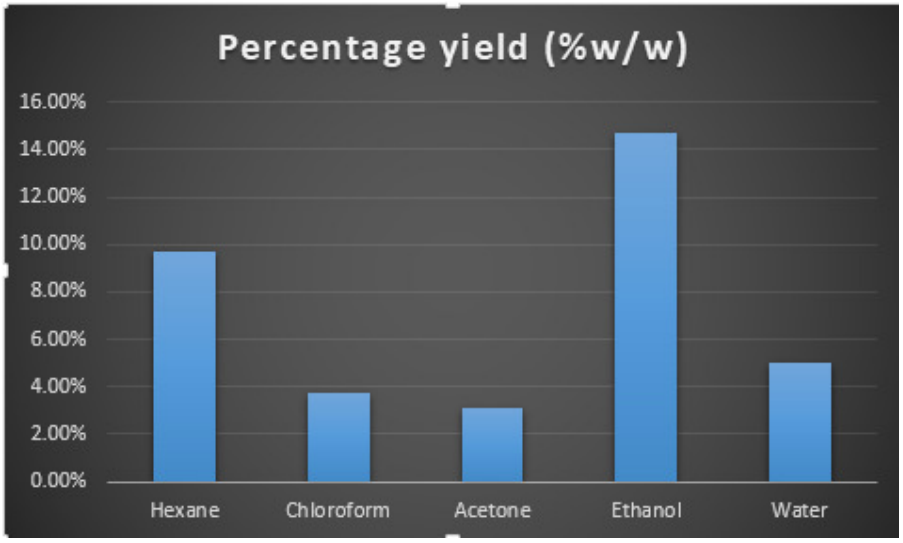

Figure 3: Extractive Yield Percentage

4.2.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* leaf

S. No	PHYTOCHEMICALS	LEAF EXTRACT				
		HEXANE	CHLOROFORM	ACETONE	ETHENOL	WATER
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.	CARBOHYDRAE	-	-	+	++	+
15.	PROTEIN	-	-	-	+	+
16.	AMINO ACID	-	-	+	++	+

4.3.FT-IR ANALYSIS

The FTIR spectrum was used to identify the functional groups of the active components present in the extract based on the peak values in the region of IR radiation. When the extracts were passed into the FTIR, the functional groups of the components were separated based on its peak ratio. Figure 4 and Table-3 reveals its functional groups present in Ethanol extracts of *Tylophora subramanii*and its peak are separated based on the IR absorption. The results are confirmed by the presence of the amine, alcohol, alkene, tertiary alcohol and halogen compounds.

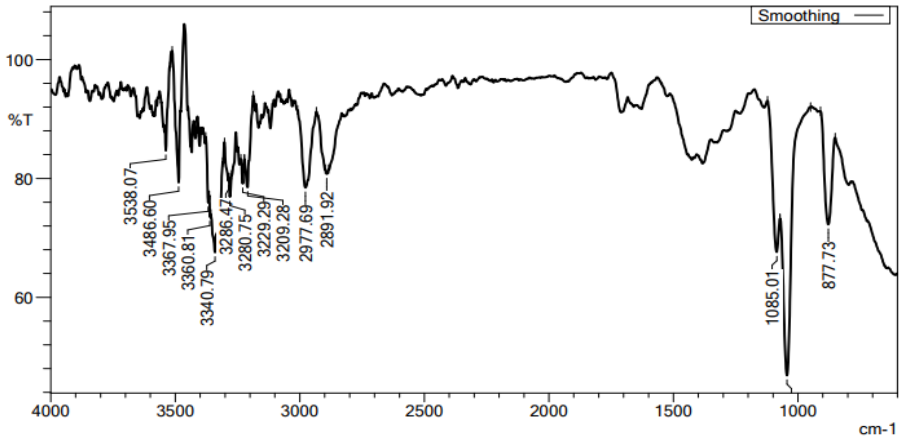

Figure 4: FTIR spectrum of Ethanol extracts of Tylophora subramanii leaf

Ethanolic leaf extratcs functional group is analyzed to identify its structure (organic and in organic compound) Figure 4 shows the result of functional group analysis. The absorption band were seen at 3538.07, 3486.60, 3367.95, 3360.81, 3340.79, 3286.4, 3280.75, 3229.29, 3209.28, 2977.69,

2891.92, 1085.01 and 877.73. It indicate the presence of functional group like, OH, C=CH,C-H, cyclic ethers, aryl-O, peroxides and C-O-C

S.No	Standard (nm)	Wave number (cm^2)	Bond	Functional group	Phytocompound Identified
1	3530-3450	3538.07 3486.60	Single bond stretching	Dimeric OH-stretching Hydroxyl group (Inter molecular hydrogen bond)	Poly hydroxy compound and Hydroxy compound
2	3400-3200	3367.95 3360.81 3340.79	Single bond stretching	Normal Polymeric OH stretching and C=C H stretching	Poly hydroxy compound
3	3300-3100	3286.4 3280.75 3229.29 3209.28	Single bond stretching	C=C-H stretching	Transition metal carbonyls
4	2960-2850	2977.69 2891.92	Triple bonds	Asymmetric stretching of $–CH_2(CH_2)$ vibration and Symmetric stretching of $–CH_2(CH_2)$ vibration	Saturated aliphatic compound lipids ,lipids and protein
5	1140-1070	1085.01	Fingerprint region skeletal vibration	C-O- stretching, ether groups, aryl-O-stretching	Cyclic ethers
6	890-820	877.73	Fingerprint region skeletal vibration	Peroxides, C-O-O- stretching	Aromatic phosphates

Table 3: Ethanolic leaf extract FTIR interpretation of compounds

The FTIR analysisrevealed the presence of alkaloids due to N-H stretching, polyphenols and flavonoids due to O-H stretching, terpens due to C-H group. The functional groups present in test plant are aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers and organic halogen compounds. These were confirmed by FT-IR spectrophotometer study that predicted the presence of the groups: O-H, N-H, C-H, CCl, C=C, nitrates and silicates stretching. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles,

isonitriles, organic halogens and carbohydrate could be responsible for the various medicinal properties of *Tylophora subramanii*.

5. CONCLUSION

In the present study analysis of different extracts of leaf was done under FTIR will act as Pharmacognostic marker to distinguish the medicinally important *Tylophora subramanii*species this spectrocopic techinque is relatively simple,cost effective and can be usefull to easily detect functional groups. The results of present study is a way to predict and compare the phytoconstituents present in this plant with other bioactive medicinally important plants. The ethanolic extract of *Tylophora subramanii* contain significant amounts of phenolics and flavonoids. Phenolics and flavonoids are ubiquitously seen in most of the plant species and reported to possess a broad spectrum of biological properties.The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property. Further the bioactive compounds need to be isolated and the structure of the compounds can be determined by using advanced analytical techniques such as Mass and NMR Spectrophotometers.

6. CONFLICTS OF INTREST

The authors declare no conflicts of interest.

Refference

Gowtham, K. Thenmozhi. M. Suganthi, M. Abirami, G. and Jayanthi, M. (2019). Bioactive compound screening and Ftir Analysis in Abrus Precatorius L. Roth. *Journal of The Gujarat Research Society*, 21(9), 172-180.

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.

Karthikeyan, S., M. Sanjappa and M. Kothari 2009. Flowering Plants of India: Dicotyledons. Vol. 1. (Acanthaceae Avicenniaceae). 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.

Kottaimuthu, R., Ramasubbu, R., Irudayaraj, D. F., &Mohanraj, A. Extended Distribution of Tylophora subramanii AN Henry (Apocynaceae: Asclepiadoideae) in Western Ghats of Tamil Nadu, India.

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

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).

Raki Mandloi, Prakash Solanki, Ravin chouhan, Mahesh Baviskar (2018), Phytochemical Screening of Cassia fistula bark and leaves ethanolic extracts and FTIR analysis. *Internationl Journal for Research Trends and Innovation* (IJRTI), 3(1), 1-5.

Rani, K. S., Mumtaz, M., Priyank, R., & Chandran, M. (2021). Phytochemical screening by FTIR spectroscopic analysis of methanol leaf extract of herb Andrographis echioides. *Journal of Ayurvedic and Herbal Medicine*, *7*(4), 257-261.

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.

S.vimal priya, R. Shyam Praveen, K. Karthika (2022). Phytochemical analysis and TLC profile of Tylophora subramanii A. N. Henry (Asclepidaceae)-an endemic medicinall plant species of southern India. *International journal of Scientific development and Research* (IJSDR),7(3), 168-174.

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

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

Wagner, R., & Wagner, K. G. (1984). Determination of quinolinic acid phosphoribosyl-transferase in tobacco. *Phytochemistry*, *23*(9), 1881-1883.

