

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND FTIR ANALYSIS OF ETHANOLIC STEM EXTRACT OF VINCETOXICUM SUBRAMANII (A.N.HENRY) MAVÉ & LIEDE

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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 stem of *Vincetoxicum subramanii*. The phytochemical screening of stem extracts revealed the presence of steroids, saponins, alkaloids, flavonoids, glycosides, phenolic compounds, tannins and terpenoids in ethanolic extracts. 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.

Keywords: *Vincetoxicum subramanii*, *Tylophora subramanii*, Functional group, FTIR, Stem extract.

1. INTRODUCTION

Phytomedicine, the art of using plants to treat diseases is known from time immemorial. Right from the first health problem encountered by early human and until the recent corona viral infections that occurred as a pandemic, plants are one of the important resources humans seek for medicine and treatment, the plants have provided many renowned drugs that act as life saviour at various situations to encounter the health problems. Apparently, there is a growing concern in the field of phytomedicine to develop the insights of the therapeutic potential of traditionally used and economically underused plants. Herbal interventions are much demanded in the globalized world since the existing conventional therapies and their synthetic drugs are of high cost and provide only symptomatic reliefs associated with

detrimental side effects (Srivastava *et al.*, 2019). Consequently, the need and utility of the plant-based drugs and phytotherapeutics have increased considerably.

The current study focuses on evaluating the phytochemical constituents and exploring the FTIR profile of *Vincetoxicum subramanii* stem extract. The plant *Vincetoxicum subramanii* belongs to the family Apocynaceae. The genus *Vincetoxicum* N.M. Wolf. (Apocynaceae) comprises nearly 100 species which are distributed throughout Asia, Europe, Japan and North America. Leaves, rhizome and dry seeds of *Vincetoxicum* species have various usages in folk medicine due to medicinal purposes.

Tylophora subramanii is the synonym name for that plant. It 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). The plant have been used for treating various diseases like asthma, leukorrhea, dysentery, fever and headache (Vimalpriya *et al.*, 2022). 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 phytochemicals present in the ethanol stem extract of such a medicinally important herb *Vincetoxicum subramanii*

1. PLANT DESCRIPTION

Habit: Climbing Undershrub

Leaves: Leaves simple, opposite-decussate; lamina ovate or ovate oblong (10-14 cm L and 8-10 cm b)

Flower: Monoecious, Flowers in axillary or lateral, umbellate cymes;



Fig. 1. Habit of *Vincetoxicum subramanii*
a) Flowering twig, b) Inflorescence,
c) Folicle fruit

Calyx : 5- lobed, lobes lanceolate, glandular at base.

Corolla: - reddish-brown, ovate-deltoid, rounded at base, shorter than the staminal column.

Anthers: erect, with small inflexed membranous appendages.

Ovary : bicarpellate, many-ovuled;

Style : apex pentagonal, flat.

Fruit: Follicles

Habitat: Rare; in edges and openings of evergreen forests.

Flowering: July - October.

Distribution: India (Kerala & Tamil Nadu)-
Endemic.

Classification

Kingdom: Plantae

Class: Dicotyledonae

Subclass: Gamopetale

Order: Gentianales

Family: Apocynaceae

Genus: Vincetoxicum

Species: *Vincetoxicum subramanii*

Common name: Subramani's Ipecac

Synonym: *Tylophora subramanii*

3. MATERIALS AND METHODS

3.1. Collection of Plant Material

Vincetoxicum subramanii was collected from Megamalai Wildlife Sanctuary of Theni district, Tamil Nadu, 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 leaves 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 dried using a rotary evaporator at 55°C. Dried extracts were stored in labelled sterile flasks at 5°C in the refrigerator, until when required for use (Karthika *et al.*, 2021).

3.3. Qualitative screening of Phytochemical Compounds

Plants are the resource of primary and secondary metabolites namely alkaloids, terpenoids, flavonoids, 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 Praveen., *et al.*, 2022). Here Preliminary qualitative phytochemical screening was carried out with the following methods.

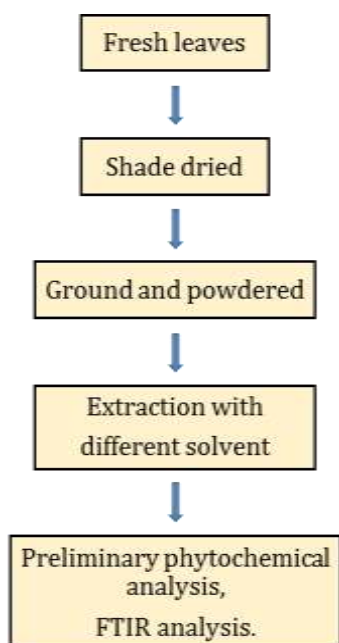


Fig. 2. Schematic representation of extraction processes

3.3.1 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.

3.3.2 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).

3.3.3 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.

3.3.4 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).

3.3.5 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.

3.3.6 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.

3.3.7 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 1 mL of extract, few drops of copper acetate were added. Formation of green colour indicates the presence of terpenoids.

3.3.8 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).

3.3.9 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)

3.3.10 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).

3.3.11 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.

3.3.12 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

3.4.13 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.

3.3.14 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.

3.3.15 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.

3.4. 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 *Vincetoxicum 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 Table-1.

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

4.2. Phytochemical analysis

Phytochemical screening of the sequential extract of *Vincetoxicum 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. Qualitative phytochemical analysis of the different extracts of *Vincetoxicum subramanii* stem

S. No	PHYTOCHEMICALS	STEM EXTRACT					
		HEX	CHL	ACE	ETH	WAT	HEX
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-Ethanol, WAT-Water).

4.3. FTIR 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 3 and Table-3 reveals its functional groups present in Ethanolic stem extracts of *Vincetoxicum 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

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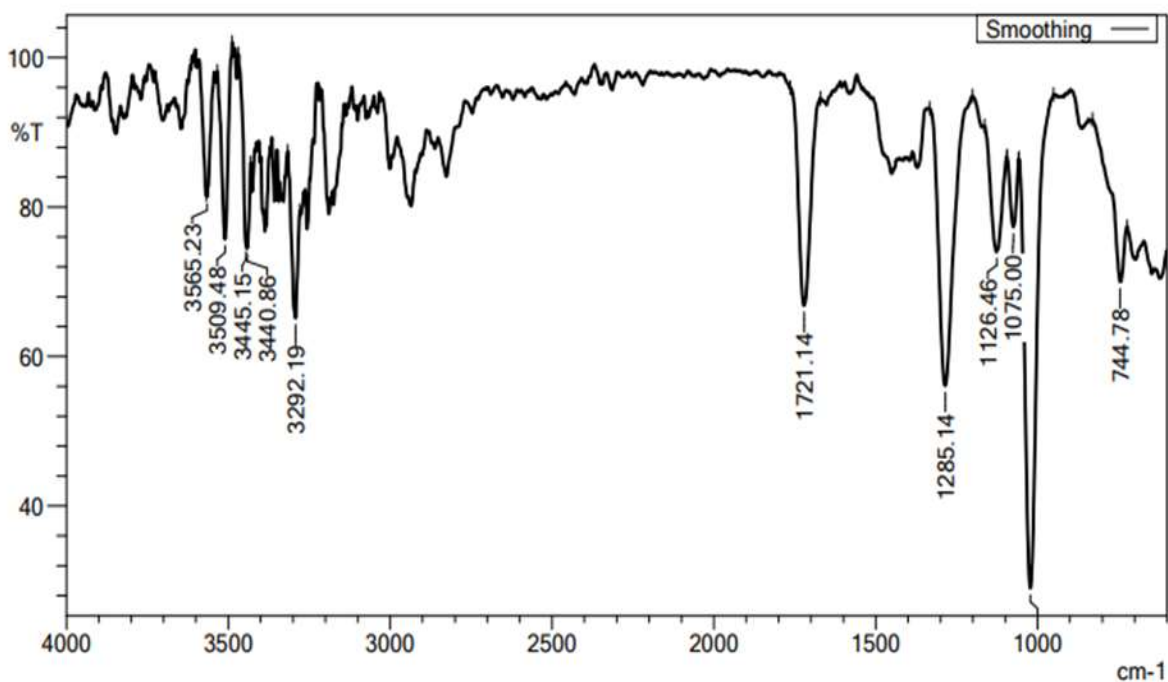


Fig. 3. FTIR spectrum of Ethanolic extracts of *Vincetoxicum subramanii* stem

The FTIR analysis revealed the presence of polyphenols and flavonoids due to O-H stretching, terpenes due to C-H group. The functional groups present in test plant are aldehydes, alkenes, amine, 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, C-H, C-H and 'Oop' stretching it confirms the presence of Aromatic compounds. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, iso nitriles, organic halogens and carbohydrate could be responsible for the various medicinal properties of *Vincetoxicum subramani*.

Table 3. Ethanolic stem extract FTIR interpretation of compounds

S. No	Standard (nm)	Wave number (cm ²)	Bond	Functional group	Phytocompound Identified
1	-	3565.23 3569.48	-	-	Unknown
2	3550-3200	3445.15 3440.86 3292.19	Single bond stretching	O-H stretching	Alcohol
3	2000-1650	1721.14	Triple bonds	C-H stretching	Aromatic compound
4	1310-1250	1285.14	Fingerprint region skeletal vibration	C-O stretching	Aromatic ester
5	1205-1124	1126.46	Fingerprint region skeletal vibration	C-O stretching	Tertiary alcohol
6	1085-1050	1075-00	Fingerprint region skeletal vibration	C-O stretching	Primary alcohol
7.	900-675	744.78	Fingerprint region skeletal vibration	C-H 'Oop"	Aromatics

5. 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 *Vincetoxicum subramanii* as a rich source of secondary metabolites. The active compound detection of ethanolic extracts of stem was done under FTIR will act as Pharmacognostic marker to distinguish the medicinally important *Vincetoxicum subramanii* species this spectroscopic technique is relatively simple, cost effective and can be use full 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 *Vincetoxicum 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.

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