Research Article

GC-MS Profiling and *in silico* Pass Prediction of Phytoconstituents Present in Ethanolic Stem Extract of Medicinally Important Plant *Vincetoxicum subramanii*

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ABSTRACT

Introduction: Traditional herbal medicine endures the cornerstone of healthcare across many Asian and Africannations, withfolk remedies playing an increasingly crucial role, especially in regions with limited medical access.However, lacking scientific validationfor medicinal plants may carry significant risks. Within the southern WesternGhats of Tamil Nadu, *Vincetoxicum subramanii* has been traditionally used to treat diverse health conditions. Thisstudy aims to analyze the phytochemical composition of *V. subramanii* stem ethanolic extract using GC-MS andassess its impending therapeuticprospects through in silico predictions. **Materials and Methods**: The stemportions of *V. subramanii* were extracted with ethanol, and the active chemical entities were ascertained via GC-MS study. The identified constituents were parallelled and examined for their biological activity using the PASS prediction method. **Result**: A total of 4 phytochemical constituents were detected through GC-MS analysis of V.subramanii stem ethanolic extract. The *in silico* predictions indicated that *V. subramanii* has the potentialanesthetic effect and couldbe applicable in treating phobic syndromes. **Conclusion**: This study underlines that thestem ethanolic extract of *V. subramanii* contains subtle phytoconstituents with capable biological activities.Further research is warranted to explore its pharmaceutical standpoints and drug development applications.

Keywords: Anesthetic, Antiarrhythmic, Antiseborrheic, GC-MS, Pass prediction, *V. subramanii*.

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INTRODUCTION

Herbal plants are a valuable treasure from nature, offering humanity an alternative solution to the limitations of current antibiotics, especially as these plants contain bioflavonoids, catechins and polyphenols, which may inhibit the growth of certain bacteria.[1] They are also recognized as vital sources of lead compounds in drug development, holding promise for discovering novel therapeutic agents.[2] Plants produce a variety of compounds that can be classified as primary or secondary metabolites, depending on their chemical structure, biosynthetic pathways and functional groups. Understanding the chemical composition of plants is necessary, not only for medical advancements but also for discovering contemporary supplies of biologically active ingredients.[3]

Chromatographic and spectral fingerprints are decisive for the purity control of multifaceted herbal medicine, as they deliver all-inclusive profiles that denote the chemical configuration and alterability of plant-derived compounds. These diagnostic procedures safeguard uniformity, legitimacy and effectiveness by enabling detailed identification and quantification of phytochemicals within medicinal plant species.[4,5] GC-MS (Gas Chromatography-Mass Spectrometry) is an extensively beneficial analytical approach for appraising bioactive plant components comprising lipids, essential oils, steroids, fatty acids, terpenoids

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and alkaloids.[6,7] Over recent years, GC-MS has become a valuable tool for profiling secondary metabolites.[8,9]

The genus *Vincetoxicum* of the family Apocynaceae is scattered throughout numerous territories, principally in Europe, Asia and parts of North America, where particular species display acclimatization to temperate and subtropical microclimates.[10,11] *Vincetoxicum* species are established for their abundant phytochemical variety, embodying alkaloids, flavonoids, flavonols, phenols, sterols and terpenoids, which add to their bioactive effects.[11,12] Therapeutically, these metabolic derivatives have established activities such as free radical scavenging, anticancer, anti-inflammatory, antimicrobial and antidiabetic effects, positioning *Vincetoxicum* as a promising candidate for vital applications.[13,14]

Vincetoxicum subramanii, commonly known as broad petal ipecac or subramani's ipecac, is a medicinal plant from the family Apocynaceae with immense remedial functions.[15,16] Traditional healers have used parts of this plant as expectorants, rheumatic analgesics and treatment for diabetes and cancer.[17,18] The entire plant exhibits natural antioxidant properties,[15] while raw leaf juice is traditionally used to treat jaundice.[19] It is well known that medicinal plants contain a diverse array of secondary metabolites, some of which are key contributors to their curative potentials. As a result, the objective of the present investigation was to distinguish diverse secondary metabolites in *V. subramanii* through GC-MS analysis and their biological activities using *in silico* technique.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh stem portions of *V. subramanii* were collected from Meghamalai, Theni District, Tamil Nadu in August 2022. The specimens were taxonomically validated from the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu (BSI/SRC/2022-05- 23/Tech/398). The collected plant materials were thoroughly rinsed with running tap water to remove surface debris, shade-dried and stored as air-tight bundles for future experiments.

Preparation of *V.subramanii* Extract

The *V. subramanii* stem ethanolic extract was prepared as follows: 25 g of powdered *V. subramanii* stem was subjected to hot extraction with 250 mL of ethanol using Soxhlet apparatus at a temperature range of 60-65ºC for 24 hr, yielding a viscous extract. The solvent was evaporated using a rotary vacuum evaporator, producing a semi-solid mass, which was stored in a vial at 4ºC for subsequent analysis.[20]

GC-MS Analysis

For GC-MS analysis, an Agilent DB 5ms fused silica capillary column (30 0.999%) was used at a steady flow rate of 1 mL/min, with an injection volume of 1 µL and a split ratio of 10:1. The injection and mass transfer line temperature were set to 230ºC and 250ºC, respectively. The oven temperature was programmed to rise 70ºC (isothermal for 8 min) to 300ºC (isothermal for 9 min) at a rate of 10ºC per min. Compounds were separated by GC-MS, eluted from the column and detected electronically, producing chromatograms. Molecules were then directed to the electron bombardment, producing mass-charged ions. The resulting Mass-To-Charge (m/z) ratios, known as mass spectra, were recorded as histograms and molecular fingerprints.[21]

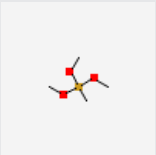
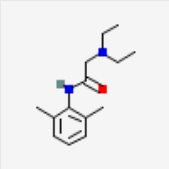
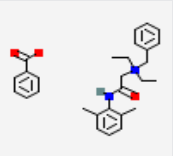
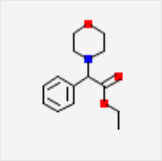
In silico prediction of biological activity

The biological activities of the identified plant metabolites were estimated using the online server Way2Drug Online. The compounds were methodically converted into SMILES format through PubChem database and they were utilized to run the server effectively. The PASS online helps in envisaging potential therapeutic aspects of the compounds by calculating the Probability Ratio of Being Active (Pa) to Being Inactive (Pi). A high Pa value signifies a greater likelihood of a compound exhibiting a specific biological effect.[22]

RESULTS

The GC-MS result of *V. subramanii* stem ethanolic extract offered a comprehensive chemical profile, divulging four metabolic constituents that underwrite its potential pharmacotherapeutic activities (Table 1; Figures 1-4). Trimethoxy methyl silane presented distinctive *m/z* peaks around 76, 104 and 121, suggestive of its silane structure, which may contribute to protective and stabilizing properties within the extract. The presence of lidocaine, recognized through *m/z* values of 58, 72 and 86, proposes the stem's potential analgesic properties, possibly correlating with traditional uses of the plant for pain relief. Furthermore, benzyl diethyl-(2,6-Xylylcarbamoylmethyl)-ammonium benzoate was perceived, with unique *m/z* peaks including 175, 232 and 91, signifying its structure as an ammonium benzoate derivative, which may interject to the extract's general bioactivity. Last of all, Acetic acid, 2-(4-Morpholyl)-2-Phenyl-, Ethyl ester unveiled discrete *m/z* values at 176, 232 and 91, coherent with the disintegration pattern of morpholine esters. This compound's occurrence aims to conceivable improvement of the clinical potential of the extract. Among all the compounds identified, lidocaine

Table 1: Component detected in the stem ethanolic extract of *V. subramanii*.

Sl. No.	Compound Name	ST	MF	MW (g/mol)	RT (mins)	Peak area (%)
1.	Trimethoxy methyl silane		$C_4H_{12}O_3Si$	136.22	22.83	53.7
2.	Lidocaine		$C_{14}H_{22}N_2O$	234.34	26.58	83.3
3.	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate		$C_{28}H_{34}N_2O_3$	446.6	33.33	70.2
4.	Acetic acid, 2-(4-morpholyl)-2- phenyl-, ethyl ester		$C_{14}H_{19}NO_3$	249.3	33.34	63.3

*(ST-Structure, MF-Molecular Formula, MW-Molecular weight) the structure is retrieved from the PubChemwebsite.

had a match factor of 85.9% pointing to its confirmed presence in the studied sample. In general, the GC-MS profile provides an inclusive chemical fingerprint of *V. subramanii* stem, clarifying its chemical makeup.

The prediction of biological activity through PASS analysis for the identified key chemical compounds from *V. subramanii* stem ethanolic extract disclosed significant biopharmaceutical uses (Table 2). Trimethoxy methyl

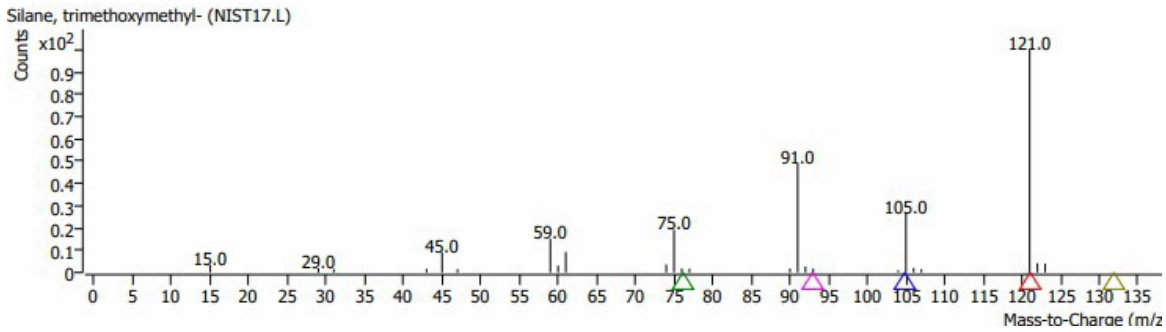


Figure 1: GC-MS spectra of Trimethoxymethylsilane from ***V.subramanii*** ethanolic stem extract.

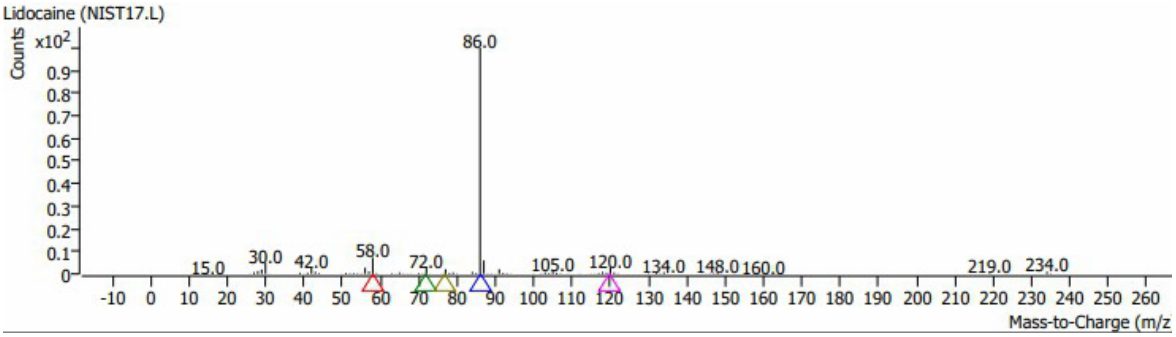


Figure 2: GC-MS spectra of Lidocaine from ***V. subramanii***e thanolic stem extract.

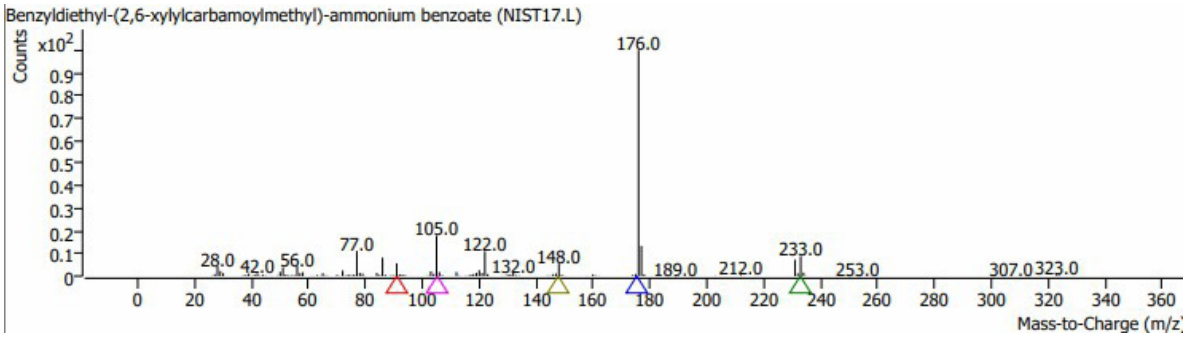


Figure 3: GC-MS spectra of Benzyl diethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate from ***V. subramaniie*** thanolic stem extract.

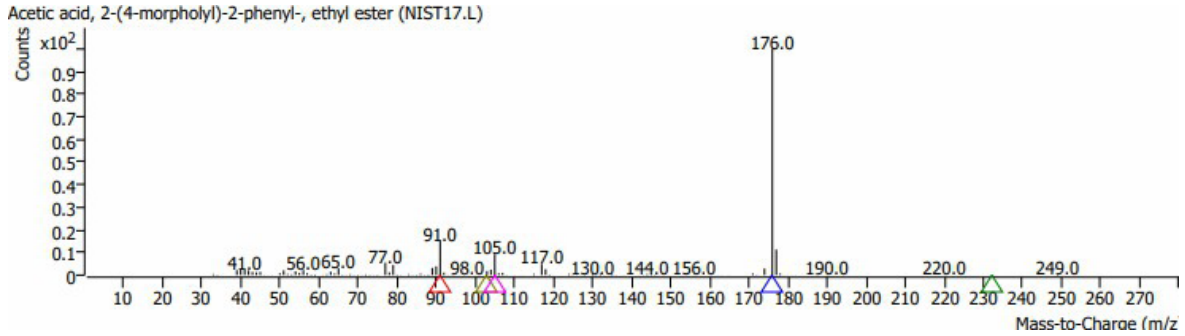


Figure 4: GC-MS spectra of AAPE (Acetic acid,2-(4-morpholyl)-2-phenyl-,ethylester) from ***V. subramanii*** ethanolic stem extract.

silane showcased Pa of 0.861 advocating a possibility as a sugar-phosphatase repressor. Lidocaine demonstrated a Pa of 0.792 and a Pi of 0.004, strengthening its conventional usage as a numbing agent. This supports the plant's traditional applications for pain reduction and elevates the prospect of its usage in local pain treatment. Benzyldiethyl-(2,6-Xylylcarbamoylmethyl)-ammonium benzoate presented a high Pa of 0.942, directing strong potential as an antiarrhythmic agent. Finally, Acetic Acid, 2-(4-Morpholyl)-2-Phenyl-, ethyl ester indicated a Pa of 0.902, recommending effectiveness in treating phobic disorders, which may reflect nervous system-modulating properties relevant to psychological health. These outcomes encourage additional clinical investigations by highlighting the therapeutic elasticity of *V. subramanii* compounds.

DISCUSSION

The metabolic diversity of *Vincetoxicum* species has been extensively explored over the years using GC-MS, providing valuable insights into their phytochemical richness. Notably, analyses of *V. rossicum* extracts have identified a variety of chemical constituents such as neophytadiene, phytol, squalene, tridecanal and palmitic acid.[23] Similarly, analyses of *V. canescens* subsp. *Canescens* and *V. canescens* subsp. *pedunculata* were found to contain oleic acid, linoleic acid, sitosterol and glutamic acid.[24] Investigations on *V. hirundinaria* also revealed the presence of unique acetophenones and glycosidic derivatives.[25] Compared to these previously studied species, *V. subramanii* stem ethanolic extract exhibits a more limited range of chemical constituents, with only subtle variability. This conclusion proposes a variation in phytochemical profiles across *Vincetoxicum* species, with *V. subramanii* showing fewer detectable compounds or possibly lower concentrations, underscoring the unique metabolic characteristics within this genus.

With PASS online, researchers may anticipate various biological activities for phytochemicals based on their structural formulae, making it a powerful tool

Table 2: Displays the Pa and Pi values of various biological activities of phytoconstituents as determined by PASS online.

Sl. No.	Compound	Pa	Pi	Activity
1.	Trimethoxy methyl silane	0.861	0.014	Sugar-phosphatase inhibitor
2.	Lidocaine	0.792	0.004	Anesthetic
3.	Benzyldiethyl-(2,6Xylylcarbamoylmethyl)-ammonium benzoate	0.942	0.003s	Antiarrhythmic
4.	AceticAcid2-(4-Morpholyl)-2-Phenyl-Ethyl Ester	0.902	0.005	Phobic disorders treatment

for phytochemical investigations.[26,27] Lidocaine has significant effectuality in reducing discomfort and side effects during nasotracheal intubation.[28] Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate is widely used in commercial products as local anesthesia due to its numbing effects.[29] AAPE or etomidate, is a short-acting intravenous hypnotic utilized for anesthesia induction and patient sedation in emergency settings, enhancing the activity of the inhibitory neurotransmitter GABA (gamma-aminobutyric acid). When administered by a skilled healthcare professional, etomidate is safe and effective for procedural sedation in emergency departments.[30]

The report on phytochemicals present in this plant extract of *V. subramanii* justifies the traditional medicinal usage of this species by the local healers of Meghamalai, the Western Ghats and it could be recommended as a plant of phytopharmaceutical importance. However, further studies are needed to ascertain its bioactivity and toxicity profile for various diseases.

CONCLUSION

This study aimed to identify bioactive phytochemicals present in *V. subramanii* stem ethanolic extract. *In silico* activity, predictions indicated that the identified phytochemicals may hold potential in various areas, including the treatment of phobic disorders, anesthetic and antiarrhythmic applications. However, detailed studies are required to determine the most effective bioactive components and elucidate their mechanisms of action.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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