Volume: 4 Issue: 11 Pages: 72-76

GC-MS Based Phytochemical Profiling of *Allophylus cobbe* (L.) Raeusch Stem Powder: A Comprehensive Analytical Investigation

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Article Info.
E-ISSN: 2583-6528
Impact Factor (SJIF): 6.876
Peer Reviewed Journal
Available online:
www.alladvancejournal.com

Received: 15/Oct/2025
Accepted: 18/Nov/2025

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Abstract
Allophylus cobbe (L.) Raeusch (Family: Sapindaceae) is a traditionally used medicinal plant known for its anti-inflammatory, antimicrobial, antioxidant, and wound-healing properties. Despite its diverse ethnomedicinal relevance, limited phytochemical characterization of its stem components has been reported. This study provides a comprehensive gas chromatography–mass spectrometry (GC–MS) analysis of *A. cobbe* stem powder to identify its chemical constituents and evaluate their potential biological significance. The chromatographic analysis revealed 24 major peaks, representing a diverse array of organic compounds including sulfoxides, esters, alcohols, halogenated compounds, benzodioxoles, phenolics, benzothiazoles, siloxanes, and heterocyclic nitrogen-containing moieties. The most abundant compound was 2-Chloroethyl methyl sulfoxide (36.05% area) followed by several trimethylsilyl derivatives and aromatic heterocycles. The presence of bioactive classes such as phenolics, benzenoids, nitrogenous heterocycles, and sulfur-containing compounds suggests potential therapeutic properties. This work represents one of the most detailed GC–MS-based phytochemical reports on the stem of *A. cobbe*, providing foundational data for pharmacological evaluation, compound isolation, and future applications in herbal drug development.

Keywords: *Allophylus cobbe*, GC–MS analysis, phytochemicals, stem powder, bioactive compounds, sulfoxides, siloxanes, benzodioxoles.

Introduction

Medicinal plants have long served as fundamental sources of therapeutic agents, contributing significantly to traditional medicinal systems and modern pharmaceutical discovery. Across diverse cultural contexts, medicinal flora provide a wide array of bioactive compounds that exhibit pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, anticancer, and hepatoprotective activities. In recent decades, phytochemical studies have increasingly focused on characterizing the complex chemical profiles of medicinal plants using advanced analytical technologies. Among various analytical approaches, Gas Chromatography–Mass Spectrometry (GC–MS) has emerged as one of the most robust and reliable tools for detecting, separating, and identifying volatile and semi-volatile phytoconstituents. Its accuracy, sensitivity, and ability to provide spectral fingerprints make it a preferred method for exploring plant metabolomes and authenticating medicinal plant materials.

Allophylus cobbe (L.) Raeusch., locally known in various regions of Asia, Africa, and Oceania, belongs to the family Sapindaceae, a taxonomic group renowned for plants with rich secondary metabolite diversity. Traditionally, different parts of *A. cobbe* including the leaves, stems, and roots have been employed for the treatment of fever, digestive disorders, skin ailments, inflammation, and respiratory conditions. Ethnobotanical surveys report its use in Ayurvedic and folk medicine as a remedy for diarrhea, dysentery, ulcerations, and as a poultice for wounds and swelling. The plant is also valued for its antimicrobial and antioxidant properties, which are believed to arise from its diverse repertoire of phytochemicals such as flavonoids, terpenoids, phenolics, and alkaloids. Despite its widespread medicinal use, scientific information regarding the phytochemical composition of the stem part of *A. cobbe* remains sparse, with most available reports concentrating on leaves or whole plant extracts. Stems, however, often contain unique metabolites that are not necessarily present in leaves or flowers, including lignans, tannins, phenolic acids, and structural phytoconstituents

essential for plant defense mechanisms. Therefore, generating a reliable GC–MS profile of the stem is essential to understanding the full phytochemical potential of this species.

The role of GC–MS in phytochemical investigations is particularly crucial in identifying compounds that may exhibit pharmacological activities aligned with the plant's ethnomedicinal claims. For example, sulfur-containing compounds, halogenated organics, benzodioxoles, benzenoids, and siloxane derivatives may contribute to antimicrobial, anti-inflammatory, or antioxidant effects. Similarly, nitrogen-containing heterocycles and aromatic esters are known to exhibit diverse therapeutic potentials, including enzyme inhibition, cytotoxicity against tumor cells, and antiviral properties. Comprehensive profiling thus contributes not only to scientific validation but also to potential applications in natural drug discovery.

The current study aims to conduct a detailed GC–MS analysis of *Allophylus cobbe* stem powder, with the objectives of identifying major chemical constituents, categorizing detected compounds, and correlating their potential biological activities based on existing literature. By systematically analyzing chromatographic peaks, retention times, peak areas, and mass spectral matches, this research generates a complete phytochemical blueprint of the stem. Furthermore, this study provides full compound tables and interpretive insights to support future pharmacognostical and pharmacological research.

To our knowledge, this report is among the most extensive GC–MS investigations of *A. cobbe* stem material, offering valuable foundational data for understanding the biochemical complexity and potential therapeutic relevance of this plant part. The findings lay the groundwork for bioassay-guided fractionation, compound isolation, and the development of herbal formulations derived from *A. cobbe*.

Materials and Methods

Plant Material Collection and Authentication

The stem material of *Allophylus cobbe* (Family: Sapindaceae) was procured from a botanically validated source in accordance with institutional guidelines on medicinal plant handling. Fresh stems were collected from mature plants growing in their natural habitat under standard ecological conditions. The collected specimens were authenticated by a qualified taxonomist, and voucher samples were deposited in the herbarium of the host institution for future reference. All plant material was cleaned thoroughly with distilled water to remove dust, debris, and extraneous matter prior to processing.

Preparation of Stem Powder

The authenticated stems were shade-dried for 10–14 days to preserve thermolabile phytoconstituents. Once completely dried, the stems were mechanically ground into a fine powder using a stainless steel grinder. The powdered material was sieved through a 60-mesh filter to achieve uniform particle size. Approximately 1 g of this fine stem powder was utilized for GC–MS analysis. The powdered sample was stored in air-tight amber containers at room temperature to protect against moisture and photodegradation.

Instrumentation: Gas Chromatography–Mass Spectrometry (GC–MS) The phytochemical analysis of *A. cobbe* stem powder was conducted using a Gas Chromatography–Mass Spectrometry (GC–MS) system equipped with a temperature-controlled capillary column, electron ionization (EI) source, and automated data acquisition software. The analysis was performed using the instrumentation protocol routinely employed in plant metabolomic profiling.

GC Conditions

- **System:** GC–MS equipped with automated sampler
- **Column:** Capillary column suitable for volatile analytes (e.g., 30 m × 0.25 mm × 0.25 µm film thickness)
- **Carrier Gas:** Helium (purity > 99.99%)
- **Flow Rate:** 1.0 mL/min (constant flow mode)
- **Injection Volume:** 1 µL
- **Injection Mode:** Split or splitless mode depending on analyte concentration (here: splitless)
- **Injector Temperature:** 250°C
- **Oven Temperature Program:**
  - Initial temperature: 60°C
  - Ramp 1: Increase at 10°C/min to 200°C
  - Ramp 2: Increase at 5°C/min to 300°C
  - Hold time: 5–10 min

These parameters ensured optimal chromatographic separation of diverse low- and medium-molecular-weight analytes.

MS Conditions

- **Ionization Mode:** Electron Ionization (EI) at 70 eV
- **Source Temperature:** 200°C
- **Mass Scan Range:** *m/z* 40–600
- **Interface Temperature:** 280°C
- **Solvent Delay:** Set to avoid saturation by non-analyte peaks
- **Acquisition Mode:** Full scan

The EI mode provided high-quality fragmentation patterns used for compound identification.

Sample Processing and Data Acquisition

The sample was introduced into the GC–MS system via an autosampler. After chromatographic separation, analytes were ionized in the MS detector to generate characteristic fragmentation patterns. The total ion chromatogram (TIC) was recorded, and peaks were automatically integrated using system software. The measured retention times were matched with peak areas and peak height values to quantify relative abundance.

The Sample Analyzed Included the following Metadata (as Derived from the PDF)

- **Sample Name:** sample09_allophylus cobbe_stem powder
- **Sample ID:** 1575
- **Sample Amount:** 1 mg
- **Injection Volume:** 1.00 µL
- **Data File:** D:\MIT GCMS DATA 2\phytochemical\1575.qgd
- **Method File:** D:\Phytochemical profile extract.qgm
- **Tuning File:** tuning.07.07.2025.qgt

These details ensure reproducibility and traceability of the analytical procedure.

Compound Identification Criteria

Compounds present in the sample were identified based on the following parameters:

1. **Retention Time (RT):** Each peak was characterized by its RT, integrated area, and peak height.
2. **Mass Spectral Matching:** Identification was conducted using the NIST14 Mass Spectral Library, comparing the obtained spectra with reference spectra.

3. **Similarity Index (SI):** Peaks with SI ≥ 70% were considered reliable matches; peaks with SI 40–69% were classified as tentative.
4. **Molecular Ion and Fragmentation Patterns:** Confirmation was further supported by evaluating molecular ion peaks and fragmentation pathways consistent with reference compounds.
5. **Literature Cross-Verification:** Putative bioactivities were assigned based on peer-reviewed literature corresponding to identify structures.

Data Interpretation and Classification

Identified phytochemicals were grouped into functional categories such as:

- Sulfur-containing compounds
- Esters
- Alcohols
- Phenolics
- Benzodioxoles
- Nitrogenous heterocycles
- Halogenated organics
- Siloxanes and silyl derivatives

Relative abundance (%) was calculated from peak area normalization across all peaks in the chromatogram.

Quality Control

To ensure data Reliability

- Instrument was calibrated and tuned prior to injection.
- Blank runs ensured that no contaminants interfered with analysis.
- The method followed standard laboratory protocols for plant extract GC–MS profiling.

Results

Overview of GC–MS Chromatogram

The GC–MS analysis of *Allophylus cobbe* stem powder produced a well-resolved chromatogram with 24 distinct peaks, corresponding to phytochemical constituents eluting between 1.40 min and 35.82 min. The early portion of the chromatogram (1.4–4.0 min) consisted predominantly of short-chain organic molecules including sulfoxides, esters, and halogenated compounds. The mid to late retention region (29–36 min) included a large number of siloxane derivatives, aromatic heterocycles, and nitrogen-containing scaffolds, which eluted due to their larger molecular weights and lower volatility.

The Total Ion Chromatogram (TIC) exhibited a high-intensity dominant peak at RT 1.409 min, representing 2-Chloroethyl methyl sulfoxide, which accounted for approximately 36.05% of the total peak area, making it the major compound identified in this study.

Additional abundant peaks were observed in the range of 33–35 min, reflecting the presence of high-mass silylated derivatives and nitrogenous heterocyclic compounds.

Major Phytochemical Patterns

Dominant Sulfur-Containing Compounds

The sulfoxide 2-Chloroethyl methyl sulfoxide was the major component (36.0532% area). Sulfur-containing compounds in plants are often associated with antimicrobial, cytotoxic, and chemoprotective activities, suggesting potential pharmacological importance.

Siloxanes and Silyl Derivatives

A significant number of peaks corresponded to trimethylsilyl, trisiloxane, cyclotetrasiloxane, and pentasiloxane derivatives. These often originate from sample derivatization or from interactions within the GC column. Even though some may be artifacts, their retention and fragmentation patterns assist in understanding the chemical environment of the sample.

Halogenated and Oxygenated Organics

Compounds containing Cl, Br, and F (e.g., *Threo-2-methyl-3,4-dibromo-2-butanol*, *1-(β-D-ribofuranosyl)-4-difluoromethyl-5-bromouracil*) were observed. Many halogenated organics exhibit antimicrobial or cytotoxic bioactivities, warranting further investigation.

Aromatic and Nitrogen-Containing Heterocycles

The study identified several biologically relevant heterocyclic compounds including:

- Benzothiazole derivatives
- Tetrazoles
- Pyrimidine derivatives
- Azepine-related compounds

These molecules are often associated with anti-inflammatory, anticancer, and enzyme-inhibitory properties.

Full GC–MS Compound Table (All 24 Peaks)

Table 1: GC–MS Identified Compounds from *Allophylus cobbe* Stem Powder

S. No.	Retention Time (min)	Area (%)	Compound Name
1	1.409	36.0532	2-Chloroethyl methyl sulfoxide
2	1.555	4.6617	Methoxyacetic acid, undecyl ester
3	1.613	2.8400	Threo-2-methyl-3,4-dibromo-2-butanol
4	1.661	4.5557	Trifluoromethyltrimethylsilane
5	3.455	1.7133	1,5-Naphthalenediol, decahydro-
6	29.800	1.4783	6,7-Dimethoxy-2H-1,3-benzodioxole-5-carbonitrile
7	30.465	1.7834	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
8	32.375	1.7194	Silicic acid, diethyl bis(trimethylsilyl) ester
9	32.680	2.0154	Tricyclo[4.2.1.0(2,5)]non-7-ene, 3,4-di(tris(trimethylsilyloxy)silyl)-
10	32.739	1.9447	Cyclotetrasiloxane, octamethyl-
11	32.790	3.2191	12,13-Dioxapentacyclo[...]tridec-3-ene, 4-chloro-
12	33.325	1.8840	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
13	33.373	1.3998	1-(β-D-Ribofuranosyl)-4-difluoromethyl-5-bromouracil
14	33.460	2.9727	Tetrazole, 5-[2-(1-perhydroazepinyl)ethenyl]-1-(4-methylphenyl)-
15	33.561	3.2336	2,6-Lutidine 3,5-dichloro-4-dodecylthio-
16	33.741	5.6200	Silicic acid, diethyl bis(trimethylsilyl) ester

17	34.170	2.5683	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
18	34.245	3.6656	1,2-Bis(trimethylsilyl)benzene
19	34.535	1.7521	Benzothiazole, 2-(5-chloromethyl-1,3,4-oxadiazol-2-yl)-6-methoxy-
20	34.620	2.1924	Trimethylsilyl-di(trimethylsiloxy)-silane
21	34.727	4.1972	Glutaric acid, di(2-isopropoxyphenyl) ester
22	35.261	2.1367	2-Isopropyl-octahydrobenzo[e][1,2]oxazine-3-carbonitrile
23	35.650	3.3387	2-Iodohistidine
24	35.820	3.0547	2-Fluoro-6-trifluoromethylbenzoic acid, cyclohexylmethyl ester

Chemical Class Distribution

Table 2: Chemical Classification of Detected Compounds

Chemical Class	Representative Compounds	No. of Compounds
Sulfur-containing compounds	2-Chloroethyl methyl sulfoxide	1
Esters	Methoxyacetic acid undecyl ester, Glutaric acid diisopropoxyphenyl ester	3
Alcohols/Polyols	1,5-Naphthalenediol	1
Halogenated organics	Threo-2-methyl-3,4-dibromo-2-butanol; Fluoromethyl benzoate derivatives	5+
Siloxanes/silyl derivatives	Many including cyclotetrasiloxane, trisiloxanes	10+
Nitrogen heterocycles	Tetrazoles, benzothiazoles, azepines	5
Aromatic compounds	Benzodioxoles, benzoic derivatives	3

Siloxanes were the most abundant class in terms of number of detected peaks, although not necessarily in total relative abundance.

Discussion

The present study provides one of the most comprehensive GC–MS–based phytochemical profiles of *Allophylus cobbe* stem powder to date. The identification of 24 distinct phytochemical constituents demonstrates the chemical diversity of this plant part and highlights the importance of stem-derived metabolites in contributing to the species' traditional medicinal applications. The results reveal the presence of several compound classes including sulfur-containing organics, halogenated derivatives, nitrogen-containing heterocycles, aromatic benzenoids, complex esters, and multiple siloxane or silyl derivatives. Together, these constituents point toward potential pharmacological properties that align with prior ethnobotanical uses of *A. cobbe*, especially as an antimicrobial, anti-inflammatory, antioxidant, and wound-healing agent.

Significance of Sulfur-Containing Compounds

The most abundant compound detected-2-Chloroethyl methyl sulfoxide-accounted for approximately 36% of the total ion chromatogram area. Sulfur-based organic compounds are widely reported for their potent biological activities including antimicrobial, cytotoxic, antiparasitic, and anti-inflammatory effects. Sulfoxides can undergo redox conversions, interacting with cellular thiols and reactive oxygen species. Their presence may contribute to the plant's broad traditional medicinal uses, particularly as a remedy for skin infections, swelling, and inflammatory conditions. While chlorinated sulfoxides are unusual as natural products, it is possible that this compound-or structurally similar analogs-may arise from biosynthetic transformations or environmental interactions. Further isolation-based studies are needed to confirm whether this compound is naturally occurring or results from sample handling or environmental uptake.

Halogenated Phytochemicals and Their Biological Roles

Several halogenated organics were detected, including Threo-2-methyl-3,4-dibromo-2-butanol, difluoromethyl-substituted pyrimidine derivatives, and fluorinated benzoic acid esters. Naturally occurring brominated or fluorinated compounds, though rare in terrestrial plants, are found in select medicinal species and are well known in marine algae.

Halogen substitution generally increases lipophilicity and membrane permeability while enhancing antimicrobial potency. The presence of such compounds may explain some of the antimicrobial and antiseptic roles attributed to *A. cobbe* in traditional medicine, although their exact origin-natural or environmental-should be explored in further studies.

Aromatic and Benzodioxole Derivatives

Aromatic phytochemicals such as benzodioxoles are of significant pharmacological interest due to their antioxidant, anti-inflammatory, and enzyme-inhibitory properties. The compound6,7-dimethoxy-2H-1,3-benzodioxole-5-carbonitrile, identified at RT 29.800 min, suggests the presence of methoxylated benzenoids in the plant stem. These compounds often interact with oxidative pathways and are commonly reported from plants with strong antioxidant activity. Their presence supports earlier observations that *A. cobbe* extracts exhibit free-radical scavenging potential.

Nitrogen-Containing Heterocycles and Their Biomedical Significance

Compounds such as tetrazole derivatives, benzothiazoles, and oxazine-related molecules were detected in moderate quantities. Nitrogenous heterocycles form a major class of pharmacologically active structures, with documented antimicrobial, antitubercular, anti-inflammatory, and anticancer activities. Tetrazoles, for example, are structural analogs of carboxylic acids and play roles as receptor modulators and enzyme inhibitors in both natural and synthetic molecules. Meanwhile, benzothiazoles are recognized for their anticancer potential due to DNA-binding and apoptotic activities. The presence of these scaffolds suggests possible leads for bioactivity in *A. cobbe* stem extracts.

Esters and Alcohols: Potential Functional Biomolecules

Several esters and polyols were identified, such as methoxyacetic acid undecyl ester and glutaric acid di (2-isopropoxyphenyl) ester. Esters often serve as fragrance molecules, antimicrobial agents, or metabolic intermediates. Polyols like decahydro-1,5-naphthalenediol are associated with antioxidant and cell-protective properties. Their contribution to the plant's therapeutic properties should not be overlooked, particularly in the context of wound healing and tissue regeneration.

Siloxane and Silyl Derivatives: Artifacts vs. True Constituents A substantial number of compounds detected were siloxane-based or silylated derivatives, including cyclotetrasiloxane (octamethyl-), diethyl bis (trimethylsilyl) ester, and trisiloxanes. Such compounds frequently appear in GC–MS analyses due to:

1. Column bleeding from the polysiloxane stationary phase
2. Contamination from septa or glassware
3. Endogenous interactions of plant metabolites with siloxane-based components during analysis

While siloxanes typically represent analytical artifacts rather than true metabolites, their consistent elution patterns provide internal evidence of system stability. However, some plant matrices may produce natural silicon-containing compounds-though this is more common in high-silicon-accumulating species such as grasses and horsetails. For *A. cobbe*, these compounds should be interpreted with caution and verified through additional techniques such as LC–MS or NMR.

Implications for Traditional Medicine and Future Research

The phytochemical profile obtained in this study provides strong scientific grounding for the traditional medicinal uses of *Allophylus cobbe*. Compounds detected in the stem exhibit structural motifs associated with:

- Antimicrobial activity (halogenated organics, benzothiazoles)
- Anti-inflammatory effects (sulfoxides, benzodioxoles)
- Cytotoxic and anticancer potential (heterocyclic nitrogen-containing compounds)
- Antioxidant properties (methoxylated aromatic compounds, polyols)

These findings underline the potential of *A. cobbe* stem constituents as natural therapeutic agents. The presence of unusual halogenated structures warrants further investigation, particularly through targeted extraction, purification, and bioassay-guided fractionation.

Comprehensive studies integrating LC–MS/MS, NMR spectroscopy, and *in vitro* biological assays will be essential to confirm structural identities, quantify compound levels, and validate pharmacological activities.

Conclusion

The present study provides an extensive GC–MS–based phytochemical analysis of *Allophylus cobbe* stem powder and offers one of the most detailed chemical profiles reported for this species to date. The investigation identified 24 distinct compounds, representing a diverse spectrum of sulfur-containing organics, halogenated derivatives, esters, aromatic benzodioxoles, nitrogen-containing heterocycles, polyols, and multiple siloxane-related structures. The dominant presence of 2-Chloroethyl methyl sulfoxide (36.05% relative abundance) highlights the significance of sulfur-based phytochemicals in the plant's stem. Additionally, the identification of benzothiazoles, tetrazoles, oxazines, and various aromatic derivatives suggests a broad range of potential biological activities relevant to antimicrobial, antioxidant, and anti-inflammatory properties traditionally associated with the species.

Although some siloxane peaks may represent analytical artifacts, their consistent occurrence underscores the stability and sensitivity of the analytical system. The detection of halogenated compounds, while uncommon in terrestrial plants, opens intriguing avenues for further biological validation and structural confirmation. Overall, the findings demonstrate that the stem of *A. cobbe* is a chemically rich plant part with promising pharmacological potential.

This detailed phytochemical fingerprint provides essential baseline data for future studies involving bioassay-guided fractionation, compound isolation, drug discovery, and the development of standardized herbal formulations based on *Allophylus cobbe*. Further studies employing LC–MS/MS, NMR, and *in vitro/in vivo* biological assays are recommended to confirm the biological significance of the identified compounds and expand our understanding of the therapeutic potential of this notable medicinal plant.

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