ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR | Vol.15 | Issue 1 | Jan - Mar -2026

www.ijpar.com

DOI: https://doi.org/10.61096/ijpar.v15.iss1.2026.332-339

Gas Chromatography - Mass Spectrometry Analysis of Hydroalcoholic Extract of *Senna Siamea Lam* Leaves

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Published by: 17.03.2026

Abstract: The present study was undertaken to identify the phytochemical constituents present in the hydroalcoholic extract of Senna siamea Lam. leaves using Gas Chromatography–Mass Spectrometry (GC–MS) analysis. Senna siamea, belonging to the family Fabaceae, is widely used in traditional medicine for the treatment of various ailments such as fever, microbial infections, diabetes, and inflammatory disorders. In this study, the leaves were collected, shade dried, powdered, and extracted using a hydroalcoholic solvent through the maceration method. The prepared extract was subjected to GC–MS analysis to determine the presence of volatile and semi-volatile bioactive compounds. The chromatographic results revealed the presence of six major phytochemical constituents including aromatic compounds, steroid derivatives, heterocyclic compounds, and phenolic acid derivatives. These compounds are known to exhibit important biological activities such as antimicrobial, antioxidant, anti-inflammatory, and hormonal effects. The findings support the medicinal significance of Senna siamea and indicate that the plant could be a promising source of bioactive molecules for future pharmacological and pharmaceutical research.

Keywords: Gas chromatography – Mass spectrometry (GC-MS), Hydroalcoholic extract, Senna siamea.

1. INTRODUCTION

Medicinal plants have been used for centuries as an important source of therapeutic agents and continue to play a significant role in traditional and modern medicine due to the presence of diverse bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds[1,2]. Natural products derived from plants remain a major source of new drugs and pharmaceutical compounds because of their structural diversity and biological activities [3]. The investigation of phytochemical constituents of medicinal

plants is therefore essential to understand their pharmacological properties and therapeutic potential [4]. *Senna siamea Lam.* Irwin and Barneby, belonging to the family Fabaceae, is a tropical evergreen tree widely distributed in South and Southeast Asia as well as in several tropical regions of Africa [5]. The plant is commonly known as *Siamese cassia* and has been widely used in traditional medicine for the treatment of various ailments including fever, malaria, constipation, diabetes, hypertension, and microbial infections [1]. Different parts of the plant such as leaves, bark, roots, and flowers have been reported to possess various medicinal properties and are used in ethnomedicine in many countries [6]. Phytochemical investigations of *Senna siamea* have revealed the presence of several important secondary metabolites including flavonoids, phenolics, tannins, alkaloids, saponins, and glycosides which contribute to its biological activities [7]. These bioactive compounds have been associated with various pharmacological activities such as antimicrobial,antioxidant, anti-inflammatory, antidiabetic, and hepatoprotective effects [8]. Previous studies have also reported the presence of compounds such as quercetin, kaempferol, catechin, epicatechin, chrysophanol, and emodin in *S. siamea,* which are known for their therapeutic potential [9]. Modern analytical techniques are widely used for the identification and characterization of phytochemical compounds present in medicinal plants [2]. Among these techniques, Gas Chromatography–Mass Spectrometry (GC–MS) is considered one of the most effective analytical methods for identifying volatile and semi-volatile compounds present in plant extracts [4]. GC–MS combines the separation capability of gas chromatography with the detection power of mass spectrometry, enabling accurate identification of chemical constituents based on their retention time and mass spectra [3]. Several studies have utilized GC–MS analysis to identify bioactive compounds present in plant extracts, which helps in understanding their medicinal value and potential pharmaceutical applications [8]. Previous GC–MS studies on *Senna siamea* extracts have reported the presence of various compounds including fatty acids, phenolic derivatives, terpenoids, sterols, and other biologically active molecules [10]. The identification of these compounds provides scientific evidence for the traditional uses of the plant and highlights its potential as a source of novel therapeutic agents [11]. Therefore, the present study aims to analyze the phytochemical constituents *of Senna siamea* using Gas Chromatography–Mass Spectrometry (GC–MS) in order to identify the major bioactive compounds present in the plant extract and to support its medicinal importance.

2. Sample Collection and Identification

Leaves of the plant Senna Siamea selected for our study was collected from RKP College of Pharmacy, Krishnagiri, Tamil Nadu, India during the month of October 2025 and was authenticated by Dr. Ravi, Department of Botany, Government Arts College for Men, Krishnagiri.

3. Preparation Of Hydroalcoholic Extract Of Senna Siamea(Haess)

The collected leaves were washed with water; shade dried and powdered, extracted with hydroalcohol (70%) by maceration technique until the complete extraction of material and filtered. The extract was concentrated under reduced pressure to obtain a residue.

3.1. Gas chromatography - mass spectroscopy analysis

Gas chromatography – Mass spectrometry (GC-MS) (Shimadzu QP 2020) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. It is a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants. GC-MS analysis was carried out to identify some of the potent volatile and semi-volatile constitutes present in the hydroalcoholic extract of Senna siamea Lam.

3.2. Coloumn

Column is fused silica, packed with SH-Rxi-5 Sil MS (30 m x 0.25 mm ID x 250 µm df) and the components were separated using helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 280º C.

3.3. Condition

1µL of hydroalcoholic extract sample injected into the instrument, oven temperature was as follows: 50º C (3 min) followed by 180º C at the rate of 15º C min-1.

3.4. Mass detector

The mass detector conditions were: transfer line temperature 290º C; ion source temperature 230º C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 50 to 600 Da. The spectrums of the components were compared with database of spectrum of known components stored in the GC-MS NIST (2017) library.

4. RESULTS AND DISCUSSION

Gas chromatography – Mass spectrometry (GC-MS) identified the presence of Six bioactive constituents are shown in the Table 1 and its biological activity are shown in Table 2 and the chromatogram was displayed in Fig 1.

Table 1. *Identification of bioactive compounds in HAESS by GC-MS*

Peak#	R.Time	Area	Area%	Height	Height %	Name of the Compound
1	30.074	27909	3.63	6507	4.34	3-(5-{[(3-CYANO-4,5,6,7-TETRAHYDRO-1-BENZ
2	30.834	28293	3.68	5815	3.88	(13E)-10-Hydroxy-7,7,13,17,18-pentamethyl-20-(2-m
3	33.725	38753	5.04	7544	5.03	4,7(1H,8H)-PTERIDINEDIONE, 2-AMINO-, TRIS(T
4	34.344	59503	7.74	10354	6.91	5.beta.-Androsta-1,3-diene-3,17.beta.-diol,

						bis(trimeth
5	34.496	37263	4.85	6179	4.12	Benzenepropanoic acid, TBDMS derivative
6	34.645	31095	4.05	8719	5.82	Tibolone, bis(tert.-butyldimethylsilyl) derivative

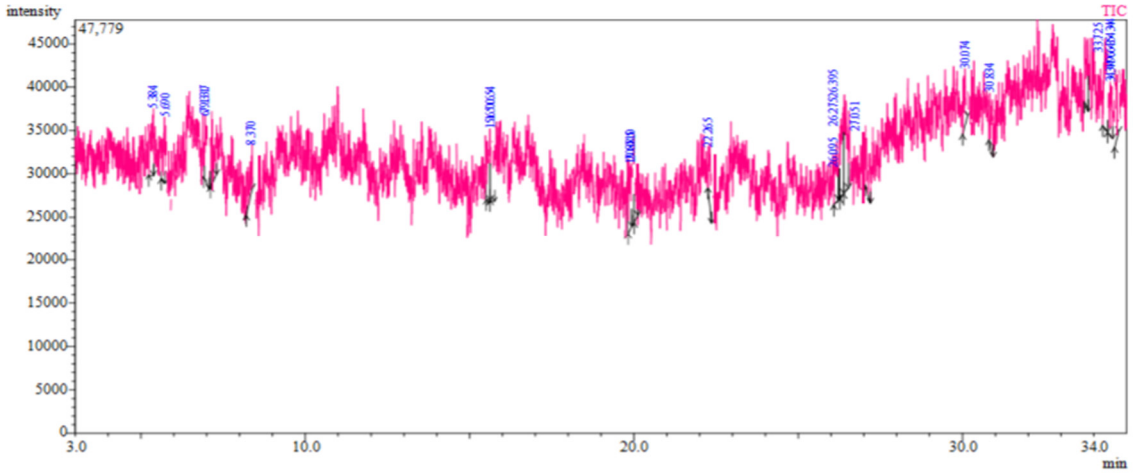

Fig 1: *Gas Chromatography - Mass Spectrometry of HAESS*

The compound 3-(5-((3-cyano-4,5,6,7-tetrahydro-1-benz…)) was detected at a retention time of 30.074 min with an area percentage of 3.63% and a height percentage of 4.34%. The presence of nitrile-containing aromatic compounds in plant extracts has been associated with several biological activities including antimicrobial and antioxidant properties. Such compounds may contribute to the medicinal value of plant extracts and are frequently identified in phytochemical investigations (12,13). Another compound identified in the chromatogram was (13E)-10-Hydroxy-7,7,13,17,18-pentamethyl-20…, which appeared at a retention time of 30.834 min with an area percentage of 3.68%. Hydroxyl-containing compounds are widely distributed in plant metabolites and are known to possess significant antioxidant and anti-inflammatory properties. The presence of hydroxyl functional groups often enhances the biological activity of natural compounds due to their ability to scavenge free radicals (14,15). The compound 4,7(1H,8H)-Pteridinedione, 2-amino… was detected at a retention time of 33.725 min with an area percentage of 5.04%, indicating a relatively higher abundance compared to some of the other compounds identified. Pteridine derivatives are important heterocyclic compounds that have been reported to exhibit various pharmacological activities such as antimicrobial, anticancer, and enzyme inhibitory effects. The detection of such compounds in plant extracts suggests potential pharmaceutical applications (16,17).

Among the compounds identified, 5-beta-Androsta-1,3-diene-3,17-beta-diol showed a comparatively higher peak with a retention time of 34.344 min and an area percentage of 7.74%, indicating that it is one of the major constituents of the extract. Steroidal compounds such as androsta derivatives are known to possess various biological activities including anti-inflammatory, hormonal, and

metabolic regulatory properties. The presence of steroidal compounds in plant extracts is often associated with important therapeutic effects (18,19). Another compound detected in the chromatogram was Benzenepropanoic acid, TBDMS derivative, which appeared at a retention time of 34.496 min with an area percentage of 4.85%. Phenylpropanoic acid derivatives are widely found in plants and are known to exhibit antioxidant, antimicrobial, and anti-inflammatory activities. These compounds also play an important role in plant defense mechanisms and contribute to the pharmacological potential of medicinal plants (20,21). The final compound identified in the GC–MS chromatogram was Tibolone, bis(tert-butyldimethylsilyl) derivative, detected at a retention time of 34.645 min with an area percentage of 4.05%. Silyl derivatives are commonly observed in GC–MS analysis as a result of derivatization processes that increase the volatility and detectability of compounds during analysis. Such derivatization techniques are frequently used in chromatographic studies to improve the identification of bioactive molecules (22,23).

Overall, the GC–MS analysis of the HAESS extract revealed the presence of several classes of phytochemicals including aromatic compounds, steroid derivatives, heterocyclic compounds, and phenolic acid derivatives. The variation in retention time and peak area indicates differences in the concentration and chemical properties of the detected compounds. The presence of these bioactive constituents suggests that the extract may possess important pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, and therapeutic activities. Similar GC–MS based phytochemical investigations of medicinal plants have also reported diverse bioactive compounds responsible for their medicinal properties and biological activities (24,25,26,27,28,29,30).

Table 2. *Biological activity of phytocompounds identified by GC-MS*

S. No.	Compound Name	Derivative	Biological Activity
1.	3-(5-{[(3-Cyano-4,5,6,7-tetrahydro-1-benzimidazol-2-yl)thio]methyl}-1,3,4-oxadiazol-2-yl)-2,3-dihydro-2-thioxo-1,3-thiazol-4-one	Trimethylsilyl (TMS) derivative	Antimicrobial; anticancer (12,13)
2.	(13E)-10-Hydroxy-7,7,13,17,18-pentamethyl-20-(2-methylpropylidene)cyclopenta[a]phenanthren-3-one	Trimethylsilyl (TMS) derivative	Anti-inflammatory; hormonal activity (14,15)
3.	2-Amino-4,7(1H,8H)-pteridinedione	Tris(trimethylsilyl) derivative	Antibacterial; enzyme inhibition (16,17)
4.	5β-Androsta-1,3-diene-3β,17β-diol	Bis(trimethylsilyl) derivative	Estrogenic; anabolic activity (18,19)
5.	3-Phenylpropanoic acid	tert-utyldimethylsilyl (TBDMS) ester	Antioxidant; antimicrobial(20,21)
6.	Tibolone	Bis(tert-butyldimethylsilyl) derivative	Hormone replacement therapy; estrogenic and progestogenic activity (22,23)

The GC–MS analysis summarized in Table 2 revealed the presence of several phytocompounds possessing significant biological activities. For instance, 2-amino-4, 7(1H,8H)-pteridinedione demonstrated antibacterial and enzyme inhibition properties, whereas 5β-androsta-1,3-diene-3β,17β-diol showed estrogenic and anabolic activity. In addition, 3-phenylpropanoic acid exhibited antioxidant and antimicrobial effects, while tibolone derivatives were associated with hormone replacement and anti-estrogenic properties. The occurrence of these compounds indicates that the extract contains bioactive constituents that may contribute to antimicrobial, anti-inflammatory, and therapeutic potential (31).

5. CONCLUSION

The present investigation demonstrated the effectiveness of GC–MS analysis in identifying bioactive phytochemical constituents present in the hydroalcoholic extract of Senna siamea leaves. The analysis revealed six major compounds belonging to different chemical groups such as steroid derivatives, aromatic compounds, heterocyclic compounds, and phenolic acid derivatives. These compounds are known to exhibit significant biological activities including antimicrobial, antioxidant, anti-inflammatory, and hormonal properties. The presence of these phytoconstituents provides scientific evidence supporting the traditional medicinal use of Senna siamea. Therefore, the plant may serve as a potential natural source of therapeutic agents. Further research involvingisolation,structural characterization, and pharmacological evaluation of these compounds is required to explore their full medicinal and pharmaceutical potential. Overall, the findings of this study indicate that Senna siamea leaves contain important bioactive molecules that may contribute to its therapeutic potential.

The results also demonstrate that GC–MS is an effective analytical technique for identifying chemical constituents in plant extracts. Further studies involving isolation, characterization, and pharmacological evaluation of these compounds are necessary to explore their potential applications in drug discovery and pharmaceutical development.

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