Journal of Applied and Natural Science
13(4), 1225 - 1230 (2021)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Gas chromatography-Mass spectrometry analysis of bioactive compounds in chloroform extract of *Psoralea corylifolia* L.

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Article Info
https://doi.org/10.31018/jans.v13i4.2884
Received: August 5, 2021
Revised: October 23, 2021
Accepted: October 29, 2021

How to Cite
Ranjith Kumar, S. *et al.* (2021). Gas chromatography-Mass spectrometry analysis of bioactive compounds in chloroform extract of *Psoralea corylifolia* L.. *Journal of Applied and Natural Science*, 13(4), 1225 - 1230. https://doi.org/10.31018/jans.v13i4.2884

Abstract
Psoralea corylifolia is the most important medicinal plant which has various secondary metabolites and its leaves are broadly used in many sectors due to its antimicrobial activity. This study aimed to find the bio-active compounds of chloroform extract of *P. corylifolia* leaves using Perkin-Elmer Gas Chromatography – Mass Spectrometry (GC-MS). The results of GC-MS compounds in the chloroform extract was appropriate to the database of National Institute of Standards and Technology (NIST). GC -MS analysis of chloroform extract of *P. corylifolia* leaves reveal the presence of bioactive compounds as Hexadecanoic acid, 3-hydroxy-, methyl ester , Hydroxylamine, O-decyl- , 2,4-Di-tert-butylphenol) Cubenol , Neophytadiene , Phytol , Linoleic acid ethyl esterand 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-) . These bio-active compounds are mainly responsible for various biological activities. Hence, this study will make a good way for the production of various products for curing various disease-causing pathogens by using *P. corylifolia* leaves.

Keywords: *Psoralea corylifolia,* GC-MS, antimicrobial, bio-active compounds

INTRODUCTION

The importance of therapeutic herbs is well-known even in ancient times. There were no synthetic medicines available at the time, thus people relied solely on natural treatments to treat any illnesses. As a result, it was assumed that all plants possess a diverse set of medical properties and are immensely useful to human health and well-being. To discover more about the medicinal properties of plants, biological study is required (Sofowora *et al*. 2013). However, many therapeutic plants and their medical characteristics have yet to be discovered. Only 6% of the 4,00,000 plant species have been researched for biological function, and only a few have been investigated phytochemically (International Union for Conservation of Nature and Natural Resources, 2013). This demonstrates that many medicinal plants require research on their activity and pharmacological qualities. Additionally, plant medicines' recent return can be attributed to a number of factors, including their effectiveness and lack of adverse effects when compared to contemporary pharmaceuticals. *Psoralea corylifolia*, often known as babchi, is a prominent herb that has been utilized in traditional Ayurvedic and Chinese medicine for its miraculous benefits in the treatment of numerous skin disorders for a long time (Siva

et al., 2015). Chemoprotective, antioxidant, antibacterial, and anti-inflammatory effects of this plant are also being researched pharmacologically (Zhang *et al.,* 2016). Apart from the fact that key constituents of *P. corylifolia* performed a wide spectrum of biological functions, the precise biological actions of the plant chemical constituents have remained elusive, and just a few chemicals identified from this plant have been used for medications. With the medicinal properties of *P. corylifolia* in mind, the present study aimed to identify bioactive compounds of the leaves of this plant, categorizing each specific compound with its concentration using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, and elucidating their chemical composition and biological activity.

MATERIALS AND METHODS

Collection of plant materials

P. corylifolia (Fabaceae) leaves were taken from medicinal plants block which was maintained by the Department of Sericulture, Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam, (Tamil Nadu). It is located at 11.20° North Latitude and 76.56° East Longitude at an altitude of 320 m above mean sea level.

The height of the plant was about 30-170 cm grown under the warm condition in sandy loam soil. The flowers are small in size with purple colour. The leaves are the simple, broad and blunt end with white hairs on the upper and lower surfaces of leaves and its arrangement is racemes. The collected leaves were shade dried, powdered and used for the study.

Preparation of plant extract

25 g of the powdered leaves of medicinal plant *P. corylifolia* were weighed separately in chloroform and percolated overnight. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples, sealed with another filter disc and compressed. This was fitted to an electric heating mantle with Soxhlet unit, filled with 250 ml chloroform, and a temperature of 60 ºC was maintained for 6 hours. The residual extract was collected in a flask and transferred to a rotary flask vacuum evaporator for evaporation of the solvent. The residue thus obtained was stored at 4^0C in airtight bottles for future use.

GC-MS analysis

The GC-MS (GC-7890A, MS 5975C) with Fused silica 15m x 0.2 mm ID x 1m capillary column was utilized for the GC-MS study. The instrument was set to an initial temperature of 110 °C and kept at that temperature for 2 minutes. The oven temperature was raised to 280 °C at a rate of 5 °C/min after this time and sustained for 9 minutes. The injection port temperature was kept at 250°C, while the flow rate of helium was kept at 1 ml/min. 70 eV was used as the ionization voltage. The sample was injected in a 10:1 split mode. The range of mass spectral scans was chosen to 30-450 (m/z). The substances included in the plant's sample were identified using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained using GC-MS (Fig . 1).

The National Institute of Standards and Technology (NIST) database, which contains over 62,000 patterns was used to interpret the mass-spectrum GC-MS results. The unknown components' spectra were compared to the spectrum of known components recorded in the NIST library. The components of the test materials were identified by their name, molecular weight, and structure.

RESULTS AND DISCUSSION

Identified bio-active compounds in P*. corylifolia* leaves

The phytochemical substances were confirmed using peak area, retention time, and molecular formula. The various active compounds in a chloroform extract of P*. corylifolia* leaves viz.Hexadecanoic acid, 3-hydroxy-, methyl ester (3.02), Hydroxylamine, O-decyl- (4.92), 2,4-Di-tert-butylphenol (13.05), Cubenol (15.12), Neophytadiene (20.08), Phytol (25.30), Linoleic acid ethyl ester (25.02), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (26.37) along with their retention time (RT), molecular formula, molecular weight (MW), and peak area are listed in Table 1,In respect of identified compunds, Neophytadiene (20.08), Phytol (25.30), Linoleic acid ethyl ester (25.02), 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (26.37) had higher peak area with maximum retention time compared to others due to presence of higher amount of these compounds in the *P.corylifolia* leaves. Compared to other medicinal plants, *P. corylifolia* has significant medicinal plant with a long history of therapeutic use and most of the research done on *P. corylifolia seeds* and fruits. This is the first report on identification of bioactive compounds in the *P. corylifolia* leaves. The presence of twenty chemicals (phytochemical ingredients) was found by GC-MS analysis of *P.corylifolia* leaves, indicating the plant's therapeutic properties. The compounds have a wide range of biological characteristics. The biological activities of the plants can be determined by GC-MS analysis of phyto-constituents. Earlier, Sathish *et al*. (2012) identifed the compounds-as tetradecanoic acid, n-hexadecanoic acid, 12- octadecanoic acid, dodecanoic acid, dibutyl ester, ethyl ester, and 9, 12 – octadecadienoic (Z,Z) in the *Vitex altissima* plant. Similarly, Kumar and Manimegalai (2008) found phytol in the leaves of *Lantana camara*, and Sridharan *et al*. (2011) found it in the leaves of *Mimosa pudica*.

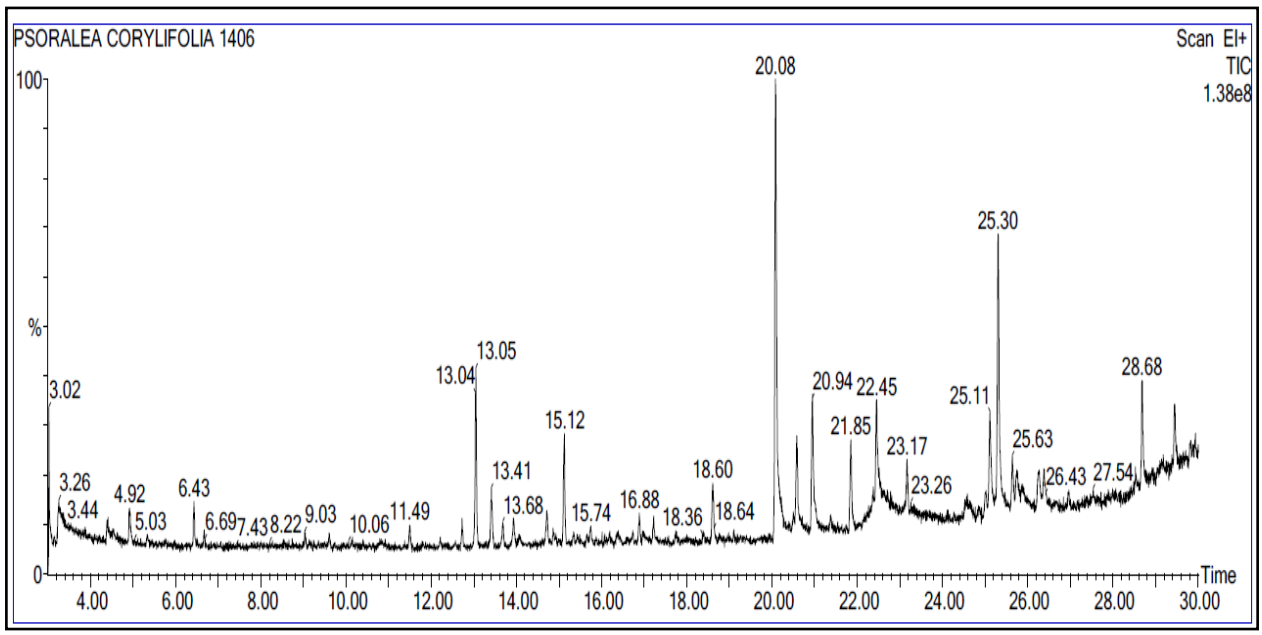
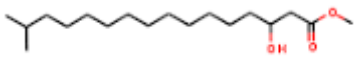
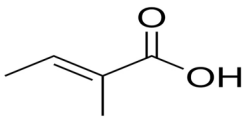
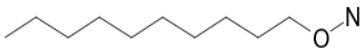
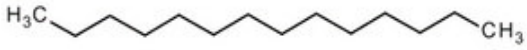
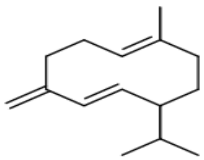
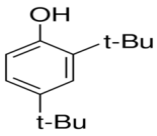
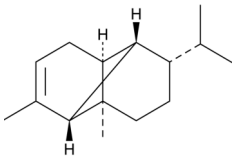
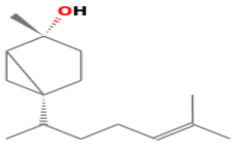
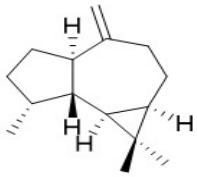
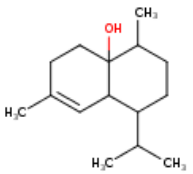
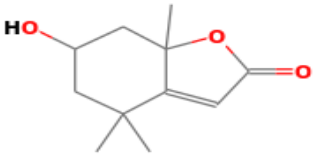


Fig. 1. *GC-MS chromatogram of Psoralea corylifolia leaf extract showing the peaks of bio-active compounds*

Table 1. Bioactive compounds present in the chloroform extract of *Psoralea corylifolia*leaves as revealed by GC-MS analysis

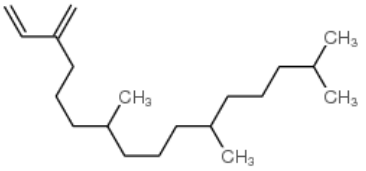
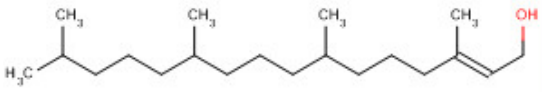
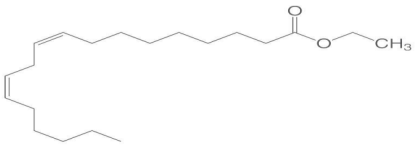
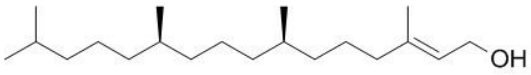
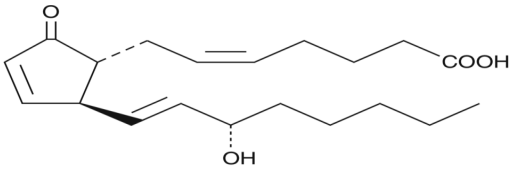
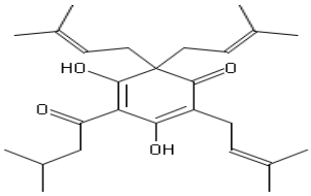
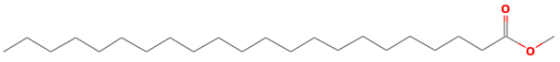
S.No.	Retention	Area (%)	Name of the compound	Molecular	Molecular	Functional
1.	3.02	1.25	Hexadecanoic acid, 3-hydroxy-, methyl ester	$C_{17}H_{34}O_3$	286.45	Ester of fatty acid
2.	3.25	1.79	Tiglic acid	$C_5H_8O_2$	100.11	Fatty acid
3.	4.92	0.71	Hydroxylamine, O-decyl-	$C_{10}H_{23}NO$	173.29	Amines
4.	6.43	0.65	Tetradecane	$C_{14}H_{30}$	198.39	Alkanes
5.	12.72	0.42	Germacrene D	$C_{15}H_{24}$	204.35	Terpenoids
6.	13.05	2.56	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	Phenols
7.	13.41	0.95	á-copaene	$C_{15}H_{24}$	204.35	Terpenoids
8.	13.92	0.56	7-epi-trans-sesquisabinene hydrate	$C_{15}H_{26}O$	222.37	Aromatic
9.	14.70	0.67	Aromandendrene	$C_{15}H_{24}$	204.35	Aromatic
10.	15.12	1.63	Cubenol	$C_{15}H_{26}O$	222.36	Terpenoids
11.	18.60	1.12	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2 (4H)-one	$C_{11}H_{16}O_3$	196.24	Phenols
12.	20.08	9.92	Neophytadiene	$C_{20}H_{38}$	278.51	Alkenes
13.	20.94	2.92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	Fatty acids
14.	22.45	3.36	Eicosanoic acid	$C_{20}H_{40}O$	312.53	Fatty acids
15.	25.02	0.48	Linoleic acid ethyl ester	$C_{20}H_{34}O_2$	308.49	Esters
16.	25.30	4.75	Phytol	$C_{20}H_{40}O$	128.17	Alcohol
17.	26.37	1.00	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.48	Steroid
18.	28.68	1.96	Prostaglandin A2	$C_{20}H_{30}O_4$	334.41	Lipids
19.	29.44	1.32	Lupulon	$C_{26}H_{38}O_4$	414.60	Fatty acid
20.	29.82	0.48	Docosanoic acid, butyl ester	$C_{23}H_{46}O_2$	354.61	Esters

Table 2. Chemical structure and its biological properties of the identified bioactive compounds

S.No.	Name of the compound	Biological activity	Chemical Structure
1.	Hexadecanoic acid, 3-hydroxy-, methyl ester	Anti-inflammatory, Antioxidant	
2.	Tiglic acid	Antimicrobial	
3.	Hydroxylamine, O-decyl-	Antimicrobial	
4.	Tetradecane	Antimicrobial	
5.	Germacrene D	Antimicrobial, anti-inflammatory, and antioxidant	
6.	2,4-Di-tert-butylphenol	Antimicrobial	
7.	á-copaene	Antimicrobial	
8.	7-epi-trans-sesquisabinene hydrate	Antimicrobial	
9.	Aromandendrene	Flavoring agent	
10.	Cubenol	Membrane stabilizer, antioxidant	
11.	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	No activity reported	

Contd……...

Table 2. Contd……...

12.	Neophytadiene	Analgesic, antipyretic, anti-inflammatory, antimicrobial, and anti-oxidant	
13.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	No activity reported	
14.	Eicosanoic acid	Anti-inflammatory	
15.	Linoleic acid ethyl ester	Anti-inflammatory	
16.	Phytol	Antioxidant	
17.	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	Antiarthritic, Anti-cancer, Hepatoprotective, Antimicrobial, Antiasthma	
18.	Prostaglandin A2	Carbon metabolism	
19.	Lupulon	Antibiotic	
20.	Docosanoic acid, butyl ester	Antimicrobial	

Activity source: Dr. Duke's phytochemical and ethnobotanical databases (1992-2016)

Chemical structure and its biological properties

As reported in Dr. Duke's Phytochemical and Ethnobotanical Databases (1992-2016), twenty identified compounds from the extract of *P.corylifolia* leaves have different chemical structures and various biological properties; and are presented in Table 2. This, the activities of the compounds screened during GC-MS analysis justify the traditional medicinal uses. Accordingly, various biological activities of identified bioactive compounds areantimicrobial, antioxidant, anti-inflammatory, anticancer activities, membrane stabilizer, flavouring agent, analgesic, antipyretic, hepatoprotective, anti-asthmatic, and carbon metabolism. According to Rukshana *et al*. (2017), the identified compounds as Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-methyl ester, Ethyl 9,12,15-octadecatrienoate and 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1 H-1,2,3-triazole are present

in the leaf sample of *Pergularia daemia*, which were found to be an antiasthmatic and antimicrobial compounds. Apart form the pharmaceutical uses, *P. corylifolia* leaves are also used to control bacterial diseases of silkworm, *Bomyx mori*. Manimegalai *et al*. (2010) have revealed that 800 ppm hexane leaf extracts of *P. corylifolia*, *P. Amboinicus*, and the gold standard, gentamycin (50 ppm) were effective against BmNPV infecting three different silkworm breeds, namely cross breed, PM X CSR 2 which showed the antimicrobial activity of *P. corylifolia*.

In the present study, GC analysis revealed all of the compounds in the leaf extract through specific qualitative and quantitative patterns. *P. corylifolia* has a wide range of biological qualities that can be utilized to cure a variety of ailments (Table 2) . Among the identified compounds, phytol has been discovered to have antibacterial activity against *Staphylococcus aureus* by generating cell membrane disruption. The compound 9,12,15- Octadecatrienoic acid, ethyl ester (Z, Z, Z) is a polyenoic fatty acid molecule has been reported to be anti-inflammatory, hypocholesterolemic, cancer preventative, hepatoprotective, nematicides, insectifuge, antihistaminic, anti-acne, 5-alpha reductase inhibitor, and anti-androgenic properties (Vohra and Kaur, 2011). The ethyl ester of linoleic acid is an unsaturated fatty acid with anti-inflammatory and cancer-preventive properties (Tulika and Mala, 2017).

Conclusion

As a result, this form of GCMS analysis is the first step toward understanding the nature of active ingredients in medicinal plants, and it will be useful for subsequent research. In the current study, the presence of valuable bioactive components such as phytol, Hexadecanoic acid- 3- hydroxy methyl ester, Neophytadiene, and Cubenolas revealed by GCMS analysis in the chloroform extract of *P. corylifolia* leaves have various biological activities. For further research,isolating particular phytochemical constituents and exposing them to biological action, on the other hand, it will almost certainly yield positive outcomes. Hence, it was concluded that the biological values of *P. corylifolia* leaves contain pharmacologically active substances that could improve their usage as a conventional drug.

Conflict of interest

The authors declare that they have no conflict of interest.

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