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GC-MS Profiling of different fractions of *Physalis minima* Linn., leaves

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Abstract:

Objective: To explore the volatile bioactive compounds present in different fractions of methanolic crude extract of *Physalis minima* by GC-MS analysis.

Method: The leaves of *Physalis minima* were extracted by soxhlation technique. The crude methanol extract thus obtained was fractioned with n-hexane, chloroform and ethanol. Gas chromatography Mass spectroscopy was used for the chemical profiling of different fractions of *Physalis minima*. The volatile bio-actives were further identified by their peak areas in GC-MS field. An extensive literature review has been carried out for determining the pharmacological use of the identified constituents.

Results: A total of 35, 40, and 38 components were found in n-hexane, chloroform and ethanol fractions with a total peak area of 97.35%, 97.24%, 98.16% respectively. The major phytoconstituents identified include dodecanoic acid methyl ester, 9,12-octadecadienoic acid (Z,Z)- methyl ester, 9,12,15-octadecatrienoic acid methyl ester, 2,6-Dodecadien-1-ol, 3,7,11-trimethyl- (Z,E) and 9-Octadecenoic acid (Z)- methyl ester that possess appreciable anti-oxidant, anticancer, anti-inflammatory and antimicrobial effects. Also, phytol, often used as precursor in vitamin E preparation was identified in n-hexane fraction.

Conclusion: Thus, the GC-MS analysis of the *Physalis minima* leaf fractions showed an enriched presence of various bioactive constituents possessing proven anti-oxidant, antimicrobial, anti-tumour, anti-fungal, anti-inflammatory activities.

Keywords: *Physalis minima*, Cape gooseberry, phytochemicals, GC-MS analysis, anti-cancer, anti-oxidant.

Introduction

The genus *Physalis* is a well-known genus possessing 80-100 species belonging to the Solanaceae family. *Physalis minima* Linn. is a pantropical annual herb that grows up to a height of 0.5-1.5 m tall and distributed throughout India, tropical Africa, Malaysia, Afghanistan, Australia, Singapore and Baluchistan. The plant is commonly known as Wild Cape gooseberry, Tankaari, Sodakku thakkali. *Physalis minima* Linn. grows at a great rate on porous soil that is rich in organic matter and commonly seen on wastelands, roadsides, bunds of fields and around houses.[1]

Physalis minima Linn. is considered to be one of the significant medicinal plants used traditionally. It is a herbaceous plant with short stems, yellowish flowers and yellowish green fruits having sweet and sour taste. It consists of broad leaves which are petiolate, pubescent, ovulate to cordate, and possess reticulate palmate venation. The flowers are pedicellate and mostly seen in summer. The fruits are like berry in nature, round shaped and totally enclosed in calyx. The entire part of the plant is considered to be safe as a medicinal plant, other than calyx. Malay community in Malaysia consumes the decoction of the whole plant as a treatment for the cancer and leaves are used for the treatment of cancer.[2–4]

Physalis minima has been primarily used as a diuretic, bitter tonic, laxative and appetizer. Fruits of the plant are rich in vitamin C and acts as purgative, diuretic and pain reliever. Leaves are used in the treatment of measles externally, taken orally to treat bad breath, tonsillitis, jaundice, sore throat, angina pectoris and as anthelminthic and roots as stomachic.[5,6]

Different extracts of *Physalis minima* have been found to possess numerous antioxidants and anticancer agents. The ethanolic extract of whole plant acts against erythrocytic stage in malarial treatment, inhibitory action on the lipase alpha glucosidase and amylase activity in-vitro.[7] The methanolic extract of *Physalis minima* leaves was found to possess anti-ulcer activity while that of whole plants were reported with antimicrobial, antioxidant, antipyretic, anti-inflammatory and analgesic activity Additionally, the methanolic extract of aerial parts of *Physalis minima* showed cytotoxic activity against hepatocellular carcinoma Furthermore, the ethanolic extract of *Physalis minima* showed breast cancer cell suppression.[8-10] Also, withanolides, isolated from *Physalis minima* exhibited cytotoxic activity against human melanoma cells.[11]

Eventhough, *Physalis minima* consists of different therapeutically active constituents, a detailed chemical profiling of volatile bio-actives was not found. The present study reports the different phytochemical

constituents that were present in the n-hexane, chloroform and ethanol fractions of methanol leaf extract using GC-MS analysis. All the phytochemical constituents present were expressed in the form of peaks and the compounds were identified using NIST library.

Materials and Methods

Reagents

The chemicals and reagents used for the preliminary phytochemical screening were of high purity. The solvents used for the extraction including methanol, n-hexane, chloroform and ethanol were procured from the Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Plant material collection and authentication

The leaves of *Physalis minima*, were collected from Kallakurchi, Tamil Nadu, India at 11° 44′ 16.8′′ N latitude and 78° 57′ 43.2′′ E longitude. The plant was identified and authenticated by Prof. P. Jayaraman, M.Sc., Ph.D., at Plant Anatomy Research Centre, Chennai, No. PARC/2021/4567.

Preparation of Extract

The fresh, healthy leaves of *Physalis minima* were separated and collected. The leaves were allowed to dry at room temperature for 12 days. The dried leaves were grinded into powdered form using pulveriser and extracted by Soxhlation technique using methanol.[12]

Fractionation of crude Methanolic extract

The concentrated crude methanolic extract was further partitioned with n-hexane, chloroform and ethanol for three times each and the extracted n-hexane, chloroform and ethanol fractions were collected and evaporated to dryness.[12]

Phytochemical Analysis:

All the three fractions of leaf extract of the *Physalis minima* were subjected to the preliminary phytochemical screening for the presence of secondary metabolites like alkaloids, carbohydrates, cardiac glycosides, steroids, proteins, flavonoids, tannins, quinones and lipids.[13]

GC-MS analysis of phytochemical compounds

The phytochemical constituents extracted from the leaves of *Physalis minima* were analyzed by using gas chromatography-mass spectrometry (GC-MS). GC-MS was carried out in Shimadzu 17A GC combined with Shimadzu QP2010PLUS MS, with Class VP Chromatography Data System version 4.3 operating system. SPb-5 capillary column is used for GC and helium gas as carrier. 1 µL sample is injected in split mode and injector and detector temperature is set at 250 and 280 °C, respectively. Ion source temperature se at 180 °C, ionization voltage at 70 eV, and electron multiplier at 900 V. The peaks present in the GC chromatogram were identified using the flame ionization detector. All the peaks were analysed by using the NIST version 2.0, and the results were combined in a single peak table.[14]

Results

Phytochemical Screening

The n-hexane, chloroform and ethanol fractions of *Physalis minima* have shown a wide range of secondary metabolites. The presence of phytoconstituents in the fractions of *Physalis minima* are shown in table 1.

able-1- Phytochemical screening of *Physalis minima* Linn.

S. No	Phytoconstituents	n-hexane fraction	Chloroform fraction	Ethanol fraction
1	Alkaloids	-	+	+

2	Carbohydrates	-	-	+
3	Cardiac glycosides	-	-	-
4	Steroids	-	+	-
5	Proteins	-	+	+
6	Flavonoids	-	+	+
7	Tannins	-	+	+
8	Quinones	-	-	-
9	Lipids	+	-	-

GC-MS analysis

The GC-MS analysis of the n-hexane, chloroform, and ethanol fractions of methanol extract of leaves of *Physalis minima* were found to contain a large number of volatile bioactives. The major constituents identified in n-hexane fraction of leaves were Oxirane[(hexyloxy)methyl], hexadecanoic acid methyl ester, dibutyl phthalate, 9,12-octadecadienoic acid (Z,Z)-methyl ester, 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z), phytol, methyl stearate, 2,6-dodecadien-1-ol, 3,7,11-trimethyl-(Z,E), squalene, and Vitamin E.

Table-2- Major phytocompounds identified in n-hexane fraction of the leaves of *Physalis minima* Linn., by GC-MS analysis

S.No	Retention Time (Rt)	Name Of The Component	Molecular Formula	Molecular Weight	Peak Area %
1	2.53	Oxirane[(hexyloxy)methyl]	$C_9H_{18}O_2$	158	16.82
2	32.57	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	897	12.42
3	35.78	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	944	8.06
4	35.96	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	925	16.38
5	36.20	Phytol	$C_{20}H_{40}O$	296	14.88
6	36.33	Methyl stearate	$C_{19}H_{38}O_2$	298	4.21
7	43.35	2,6-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z,E)	$C_{15}H_{26}O$	771	2.76
8	47.49	Squalene	$C_{30}H_{50}$	949	2.47
9	54.90	Vitamin E	$C_{29}H_{50}O_2$	430	4.35

The chloroform fraction was found to possess decanoic acid methyl ester, dodecanoic acid methyl ester, Methyl tetra decanoate, hexadecanoic acid methyl ester, Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester, dibutyl phthalate, 9-octadecenoic acid (Z)-methyl ester, methyl stearate, 3,7-dimethyloct-6-enyl isobutyl carbonate, and succinic acid, di(3,7-dimethyloct-6-en-1-yl) ester.

Table-3- Major phytocompounds identified in chloroform fraction of the leaves of *Physalis minima* Linn., by GC-MS analysis

S.No	Retention Time(Rt)	Name of the Component	Molecular Formula	Molecular Weight	Peak Area %
1	18.90	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	2.03%
2	23.76	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	18.58%
3	28.33	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	10.33%
4	32.49	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.69%
5	33.37	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	6.05%
6	35.82	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	4.62%
7	36.29	Methyl stearate	$C_{19}H_{38}O_2$	298	4.07%
8	39.49	3,7-Dimethyloct-6-enyl isobutyl	$C_{15}H_{28}O_3$	266	1.69%

		carbonate			
9	45.74	Succinic acid, di(3,7-dimethyloct-6-en-1-yl) ester	$C_{24}H_{42}O_4$	364	1.19%

The GC-MS profiling of ethanol fraction of leaves were found to possess glycerine, dodecanoic acid methyl ester, dodecanoic acid, methyl tetra decanoate, hexadecanoic acid methyl ester, n-hexadecanoic acid, trans-13-octadecenoic acid methyl ester, methyl stearate, (2,3-Diphenyl cyclopropyl) methyl phenyl sulfoxide, and dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester.

Table 4: Major phytocompounds identified in ethanol fraction of the leaves of *Physalis minima* Linn., by GC-MS analysis

S.No	Retention Time (Rt)	Name Of The Component	Molecular Formula	Molecular Weight	Peak Area %
1	2.545	Glycerine	$C_3H_8O_3$	92	2.610
2	23.757	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	21.858
3	26.148	Dodecanoic acid	$C_{12}H_{24}O_2$	200	9.065
4	28.392	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	11.436
5	32.479	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	7.834
6	34.028	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.464
7	35.812	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	7.446
8	36.270	Methyl stearate	$C_{19}H_{38}O_2$	298	3.125
9	41.908	(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	$C_{22}H_{20}OS$	332	10.648
10	53.294	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	456	4.925

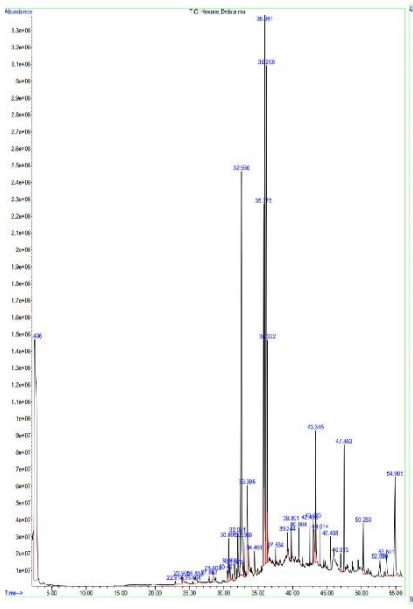

Figure 1

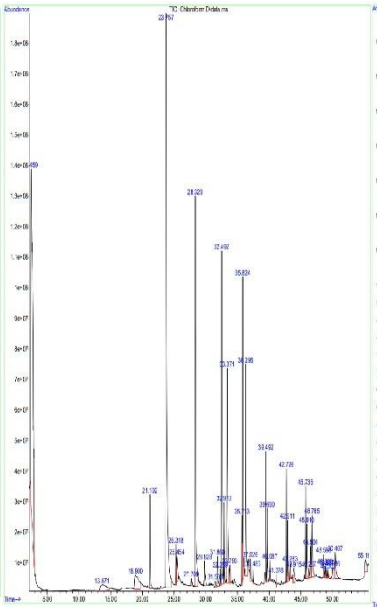

Figure 2

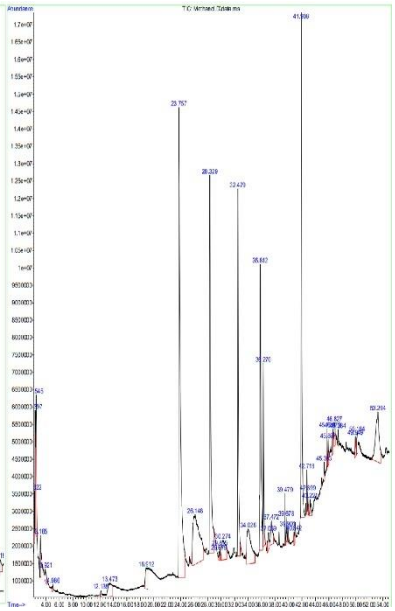

Figure 3

Figure 1: GC-MS chromatogram of the n-hexane fraction of leaves of *Physalis minima* Linn.
Figure 2: GC-MS chromatogram of the chloroform fraction of leaves of *Physalis minima* Linn.
Figure 3: GC-MS chromatogram of the ethanol fraction of leaves of *Physalis minima* Linn.

Discussion

Several studies were reported on the GC-MS profiling of herbal extracts with interesting bioactive compounds.[15–24] Rajabudeen E *et al*., has studied the GC-MS analysis in methanol extract of *Tephrosia villosa* (L.) and found the presence of stigmasterol and lupenol that acts as pain relieving agents.[17] Pradhan S *et al.,* has studied the GC-MS analysis in floral essential oil of *Lantana camara* and reported the presence of gitoxygenin which is a cardiac glycoside.[20]

In the present study, we performed GC-MS profiling of different extracts of *Physalis minima.* The results of the study showed various phytochemical constituents with numerous reported pharmacological activities. Hexadecanoic acid methyl ester, a fatty acid exhibits the antifungal and antibacterial, anti-inflammatory, and anticancer properties. Literatures reported its antibacterial activity against both gram-positive and gram-negative bacteria such as *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa.*[25,26] 9,12-Octadecadienoic acid (Z,Z)- methyl ester, is a linoleic acid ester with reported anticancer and anti-inflammatory properties. 9,12,15-Octadecatrienoic acid (Z,Z,Z) methyl ester, showed anti-inflammatory activity.[27] Phytol is a diterpene acting as a precursor in the preparation of vitamin K1 and vitamin E and have found to possess biological activities including antioxidant, diuretic and anticancer.[28,29] Vitamin E, a fat soluble vitamin possess anti-oxidant and hypoglycaemic activity.[30] Squalene, identified in the n-hexane fraction is a polyunsaturated hydrocarbon reported with the anti-oxidant, anti-cancer, detoxifier and anti-infectant effects.[31]

Decanoic acid methyl ester, was found to possess antibacterial and anti-inflammatory properties. Dodecanoic acid methyl ester is an hypocholesterolemic agent that decreases the blood cholesterol level. Methyl tetradecanoate identified in chloroform and ethanol fractions was found to possess larvicidal activity.[32,33] 9-Octadecenoic acid (Z) methyl ester is a linoleic acid ester showed the anticancer and anti-oxidant activities [27]. Caryophyllene has an ability to bind to the CB_2 receptors and has anti-microbial and anti-oxidant properties. At low ambient temperatures, it promotes cold tolerance.[34] Caryophyllene oxide, is an oxygenated terpenoid that acts as an antifungal agent against dermatophytes.[35] Glycerine is helpful in skin healing and moisturizing, anti-acne and eye disorders. n-Hexadecanoic acid ($C_{16}H_{32}O_2$) is a saturated fatty acid witness antioxidant, anti-inflammatory, anti-androgenic and potent mosquito larvicide. The anti-inflammatory activity is due to the significant inhibitory activity of the enzyme PLA_2.[36,37] trans-13-Octadecenoic acid methyl ester ($C_{19}H_{36}O_2$) showed reported biological activities like antiandrogenic, anti-inflammatory, anticancer.[38] Methyl stearate is one the most available constituent in the n-hexane, chloroform and methanol fractions of the methanol extract of leaves. It is a fatty acid that shows the anti-inflammatory, anti-helminthic, GABA aminotransferase inhibitor activities.[39] (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, is a carboxylic acid consisting of sulfoxide group is helpful in treatment of bruises and skin eruption.[40] Tetradecanoic acid ($C_{14}H_{28}O_2$) is an unsaturated fatty acid that showed antioxidant, anticancer, and larvicidal activities.[41]

Conclusion

The present study used the soxhletation technique for the initial extraction of phytochemical constituents from the leaves of *Physalis minima* Linn., using methanol followed by successive fractionation yielding n-hexane, chloroform and ethanol fractions. GC-MS analysis of all the three fractions revealed the presence of several major phytochemical constituents of medicinal importance. Literatures revealed the major identified phytochemical constituents with reported sedative, anaesthetic, hypocholesterolemic, anti-inflammatory, anti-oxidant, antifungal, antimicrobial, anti-cancer, anti-helminthic activities. The phytoconstituents, phytol, vitamin E are the identified vitamin sources that are helpful for the human body therapeutically. Thus, the leaves of *Physalis minima* Linn., acts as a reservoir of several biologically active constituents which can be isolated and identified in future.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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