

GC-MS analysis of bioactive compounds in ethanolic leaf extract of *Hellenia speciosa* (J.Koenig) S.R.Dutta

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Research Article

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Abstract

Hellenia speciosa (J.Koenig) S.R.Dutta, is a plant species belonging to the family Costaceae. It is widely distributed in China, India, Malaysia, Indonesia, tropical and subtropical Asia. In Ayurveda, the rhizome of the plant has been extensively used to treat fever, rash, asthma, bronchitis and, intestinal worms. The objective of the present study was to investigate the phytochemical constituents of the leaf of *Hellenia speciosa* using Gas Chromatography and Mass Spectroscopy analysis (GC-MS). The GC-MS analysis revealed the presence of 17 phytochemical components in the ethanolic leaf extract of *Hellenia speciosa*. The prevailing bioactive compounds present in *Hellenia speciosa* were Thymol (RT-10.019;3.59%), Caryophyllene (RT-11.854;0.62%), Caryophyllene oxide (RT-13.919;1.34%), Artumerone (RT-14.795;1.35%), Hexadecanoic acid methyl ester (RT-17.536; 2.77%), 9,12-Octadecanoic acid methyl ester (RT-19.163;1.35%), Squalene (RT- 24.980;1.19%), Piperine (RT-25.745;3.11%), Beta Tocopherol (RT-26.681;2.88%) Vitamin E (RT- 27.290;2.64%), Progesterone (RT-29.608;3.18%), Caparratriene (RT-29.861;9.72%), and Testosterone (RT-30.73;5.81%). The compounds were identified by comparing their retention time and peak area with that of the literature and by interpretation of mass spectra. The results and findings of the present study suggest that the plant can be used as a valuable source in the field of herbal drug discovery. The presence of bioactive compounds justifies the use of plant leaves for treating various diseases with fewer side effects and recommended the plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

Introduction

Medicinal plants are the traditional source of chemical compounds in the field of biotechnology for the discovery of herbal medicine. Most of the pharmaceutical industries depend on these plants for the secondary metabolites for the development of health care products. The secondary metabolites are the phytochemical constituents present in the crude extracts of the plants [1]. In India, there is an increasing demand for natural products from plant sources due to their medicinal properties and safety issues. According to World Health Organization, about 80% of the population follow plant-based traditional medicines for primary healthcare [2]. Traditional systems of medicines are prepared from a single plant species or combinations of several plants species. The bioactive component of the plant may be derived from any parts of the plant like leaves, roots, bark, flowers, fruits, and seeds [3]. Plant-based medicines that are derived from crude leaf extracts contain different phytochemicals. These phytochemicals are the bioactive principles having a unique and complex structure to treat various ailments. Screening of plants by chromatographic methods provides information on its pharmacological activities which help to select the plant of medicinal property [4]. Gas chromatography-mass spectrometry (GC-MS) is the accurate technique employed for the detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants [5], [6]. Hence in the present study, the GC-MS technique was adopted for the detection and identification of phytochemical compounds present in the medicinal plant, *Hellenia speciosa* belonging to the Costaceae family.

Hellenia speciosa is one such plant with medicinal importance. It is a perennial herb mainly cultivated in the rainy season and grows well in clayey loam soil [7]. It can be propagated by different methods such as stem cutting, division of clumps, or rhizome [8]. The plant has many medicinal uses, juice of rhizome is applied to the head for cooling and relief from headache, bruised leaves are applied in fever, a decoction of the stem is used in fever and dysentery. The leaf infusion or decoction from leaves, young stems are used against diarrhoea, cough, cuts, wounds, scabies, the antidote for snakebite, jaundice, arthritis [9], burning sensation, constipation, leprosy, skin diseases, asthma, bronchitis, inflammations, anaemia, intestinal worm infection [10]. The leaves and rhizomes of *Hellenia speciosa* have been reported to possess steroid –diosgenin, which is anti-diabetic in nature. The Leaves also possess hypoglycaemic properties and insulin potentiating action in addition to decreasing blood glucose [11]. In Indian traditional medicine, *Hellenia speciosa* rhizome was found to possess anthelmintic, anti-inflammatory, antidiabetic, hepatoprotective, antihyperlipidemic, antispasmodic, and antimicrobial activities [12],[13]. In this paper, the GC-MS analysis of leaf extract has been studied as part of the exploration for bioactive compounds.

Materials And Methods

Collection of Plant Material

Hellenia speciosa was collected from Thrissur district of Kerala, taxonomically identified and confirmed by the botanist Dr. S. Ravikumar, PG and Research Department of Plant Biology and Biotechnology, Presidency College, Chennai. [Voucher specimen no. PCMRDRM2017001] (Fig. 1, Fig. 2). The leaves were washed with water, shade dried at room temperature, and powdered in an electric blender as shown in Fig. 3. The powdered leaves were stored in an opaque container at room temperature for further analysis.

Preparation of Plant Extract

Preparation of plant extracts was done according to the combination of the methods described by Pizzale and Lu and Foo [14],[15]. About 1g of fleshy dried powder of *Hellenia speciosa* plant materials was extracted with 20 mL ethanol for 1 min using an Ultra Truax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18°C until use.

GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample [16]. GC-MS analysis was carried out to identify some of the potent volatile and semi-volatile constituents present in the ethanol extract of *Hellenia speciosa*. GC-MS analysis was carried out on the GC-MS-5975C Agilent system comprising an autosampler and gas chromatograph interfaced to a mass spectrometer

employing the following condition. The sample was injected into the injected port of the Gas chromatography (GC) device. The GC instrument vaporizes the sample and then separates and analyses the various components. Each component produces a specific spectral peak that may be recorded on a paper chart electronically. The time elapsed between elution and injection is called the "retention time". Differentiate between some compounds was identified using the Retention time. The peak is measured from the base to the tip of the peak [17].

COLUMN

Column Elite – 1 fused silica capillary column (30x 0.25mm ID x1 EM df, composed of 100% dimethyl polysiloxane), operating in an electron impact mode at 70eV; helium (99.999%) was used as a carrier gas at a constant flow of 1.51ml/minutes.

CONDITION

Injection volume-1µL through autosampler split ratio- 10:1; injection temperature 2400°C; ion source temperature 2000°C; Oven temperature was programmed from 700°C (isothermal for 2 minutes), with an increase of 100°C/minutes to 3000°C/minutes, ending with a 9minutes isothermal at 3000°C. Mass spectra were taken at 70eV; with a scan range of 40-1000m/z. Solvent cut time was 5 minutes; MS start time being 5minutes; MS end time being 35 minutes; Ion source temperature was set to 2000°C and interface temperature being 2400°C [18].

Identification of bioactive compounds

Bioactive compounds from the plant extract were identified based on retention time. Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST). The compound name, molecular weight, molecular formula, and structure were ascertained. The peak in GCMS of ethanol extract of leaf of *Hellenia speciosa* represents the presence of the secondary phytochemical compounds like lipid, steroid, alkaloid, and fatty acids and its esters.

Results

GC-MS is the best technique to identify the bioactive constituents of long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, etc. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become sophisticated means for analysis of various compounds [19]. The GC-MS chromatogram of the ethanolic extract of *Hellenia speciosa* showed the peaks indicating the presence of 17 bioactive compounds in Fig. 4. The compounds with their Retention time (RT), Molecular formula, Molecular weight, and Area percentage were presented in Table 1.

Table 1
Compounds identified in the leaf extract of *Hellenia speciosa*

S.No	Name of the compound	RT	Area %	Molecular Formula
1	Thymol	10.019	3.59	C ₁₀ H ₁₄ O
2	Caryophyllene	11.854	0.62	C ₁₅ H ₂₄
3	Caryophyllene oxide	13.919	1.34	C ₁₅ H ₂₄
4	Beta Asarone	14.142	9.38	C ₁₂ H ₁₆ O ₃
5	Artumerone	14.795	1.35	C ₁₅ H ₂₀ O
6	Bicyclo[3.1.1]heptane,2,6,6, trimethyl	14.795	1.35	C ₁₀ H ₁₈
7	Hexadecanoicacid,methyl ester	17.536	2.77	C ₁₇ H ₃₄ O ₂
8	Dibutylphthalate	17.848	1.98	C ₁₆ H ₂₂ O ₄
9	9,12-Octadecanoic acid methyl ester	19.163	1.81	C ₁₉ H ₃₄ O ₂
10	Squalene	24.980	1.19	C ₃₀ H ₅₀
11	Piperine	25.745	3.11	C ₁₇ H ₁₉ NO ₃
12	Beta Tocopherol	26.681	2.88	C ₂₈ H ₄₈ O ₂
13	Vitamin E	27.290	2.64	C ₂₉ H ₅₀ O ₂
14	Progesterone	29.608	3.18	C ₂₁ H ₃₀ O ₂
15	Caparratriene	29.861	9.72	C ₁₅ H ₂₆
16	Testosterone	30.730	5.81	C ₁₉ H ₂₈ O ₂
17	2-Bromo 4,5- dimethoxycinnamic acid	33.181	9.44	C ₁₁ H ₁₁ BrO ₄

The major bioactive compounds identified were Thymol (RT-10.019;3.59%), Caryophyllene oxide (RT-13.919;1.34%), Caryophyllene (RT-11.854;0.62%), Artumerone (RT-14.795;1.35%), Hexadecanoic acid methyl ester (RT-17.536;2.77%), 9,12-Octadecanoic acid methyl ester (RT-19.163;1.35%), Squalene (RT-24.980;1.19%), Piperine (RT-25.745;3.11%), Beta Tocopherol (RT-26.681;2.88%), Vitamin E (RT-27.290;2.64%), Caparratriene (RT-29.861;9.72%), Progesterone (RT-29.608;3.18%) and Testosterone (RT-30.73;5.81%). The name of the major compounds and their bioactivity were highlighted in Table 2.

Table 2
Bioactivity of compounds identified in *Hellenia speciosa* leaf extract

Name of the compound	Molecular weight	Nature of compound	Activity
Thymol	150	Monoterpene	Antifungal, antiseptic, antibacterial
Caryophyllene	204	Bicyclic sesquiterpene	Anti-inflammatory, anticancer, Antioxidant, antimicrobial, analgesic
Caryophyllene oxide	220	Bicyclic sesquiterpenes	Anti-inflammatory, anticancer, antifungal, analgesic
Artumerone	216	Aromatic	Anticancer, Antivenom. Antidepresser, Anti-inflammatory, Neuroprotection activities
Hexadecanoic acid methyl ester	270	Methyl Palmitate	Antifungal, antibacterial
9,12-Octadecanoic acid methyl ester	294	Linoleic acid methyl ester	Anticancer
Squalene	410	Lipid	Antioxidant, Antitumor
Piperine	285	Alkaloid	Antihypertensive, antioxidant, antitumor, anticonvulsant, anti-inflammatory, antibacterial, antifungal, anti-thyroid, hepato-protective, insecticidal, larvicidal activities
Vitamin E	430	Fat soluble vitamin	Anti-oxidant, anti –inflammatory, inhibition of platelet aggregation, immune enhancing activity
Progesterone	314	Steroid	Regulate blood pressure, reproduction
Caparratriene	206	Sesquiterpene	Cytotoxicity (CEM leukemic cell – IC ₅₀ 3.0x10 ⁻⁶ M)
Testosterone	288	Steroid	Reproduction

Discussion

GC-MS analysis of phytoconstituents in the ethanolic leaf extracts of *Hellenia speciosa* highlights the pharmaceutical importance of the plant. The identified bioactive compounds occupy many biological properties. The presence of fatty acid esters such as hexadecanoic acid methyl ester and 9,12-octadecanoic acid methyl ester have various bioactivities including antifungal, antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, hemolytic, 5-alpha reductase inhibitor, and antimicrobial activity [20]. The results are by previous studies carried out by Sudha in aerial parts of *Fluggea lucoyurus* in 2013 [21]. The 9, 12 octadecadienoic acid methyl ester was also found to have potential cancer-preventive, anti-inflammatory, and antiarthritic activities [22]. A similar result was

observed in the study carried out in *Croton tiglium* seed by Mangunwidjaja *et al.*, in 2006 [23]. Squalene is a triterpene with anticancer, antioxidant, chemopreventive, gastroprotective, hepatoprotective effects, anti-tumor, and sunscreen properties [1]. Alkaloids provide the underlying structure for the development of several antibiotics with a diverse range of action [24]. Piperine is an alkaloid used in the field of traditional medicine to treat various illnesses. Piperine may help reduce inflammation, the reduction of pain [25], improving digestion, and relief of Asthma. It has also been extensively used as an antidepressant [26], antioxidant [27],[28]. Ar-tumerone is one of the major bioactive compounds present in the plant extract. It inhibits microglia activation a property that may be useful in treating neurodegenerative disease [29]. Ar-turmerone also possesses anti-inflammatory properties as reported by [30].

Caryophyllene and caryophyllene oxide were also identified as major compounds in leaf extract and found to possess anticancer and analgesic properties [31]. Caryophyllene oxide shows potent antimicrobial against various pathogens causing damage to the cell membrane [32],[33],[34]. In addition to this, the antifungal property has also been reported by Sagbo and Mbeng in 2019 [35]. Several biological activities were attributed to caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic, and local anaesthetic. Similar studies have been reported by different researchers in different plant extracts [25],[31]. Caryophyllene, Caryophyllene oxide, and Squalene can induce apoptosis and cell cycle arrest in the G1 phase of the cells [36]. Progesterone and testosterone are the major steroid hormones that play an important role in reproduction. In the present study presence of these compounds have been reported. Vitamin E is one of the major compounds detected in the ethanol extract has been found to exhibit antioxidant, anti-inflammatory, antimicrobial [36]. Vitamin E prevents lipid peroxidation and function as an antioxidant, its role in anti-inflammatory processes, its inhibition of platelet aggregation, and its immune-enhancing activity [37]. An article by Radhakrishnan *et al.* proved that daily supplementation of Vitamin E can enhance the immune response to a specific antigen [38].

Conclusion

GC-MS analysis of ethanolic leaf extract of *Hellenia speciosa* revealed the presence of bioactive compounds like fatty acid, heterocyclic compounds, steroids, alkaloids, terpenoids, and vitamins. Among the identified compounds, Octadecanoic acid, n-Hexadecanoic acid, Caryophyllene, Ar-tumerone. Piperine and Squalene are the major compounds that might contribute to biological activities such as antioxidant, antimicrobial, anticancer, antidiabetic, and anti-inflammatory properties. Therefore, the present study results conclude that *Hellenia speciosa* may serve as a potent source of medicinal activity due to the presence of bioactive compounds. However further studies are essential to isolate, characterize and purify the active components responsible for therapeutic activity.

Declarations

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Conflict of Interest

The author declared that there is no conflict of interest.

Availability of data and material Not applicable

Code Availability Not applicable

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Figures



Figure 1

Hellenia speciosa



Figure 2

Flower of *Hellenia speciosa*



Figure 3

Dry powder of *Hellenia speciosa*

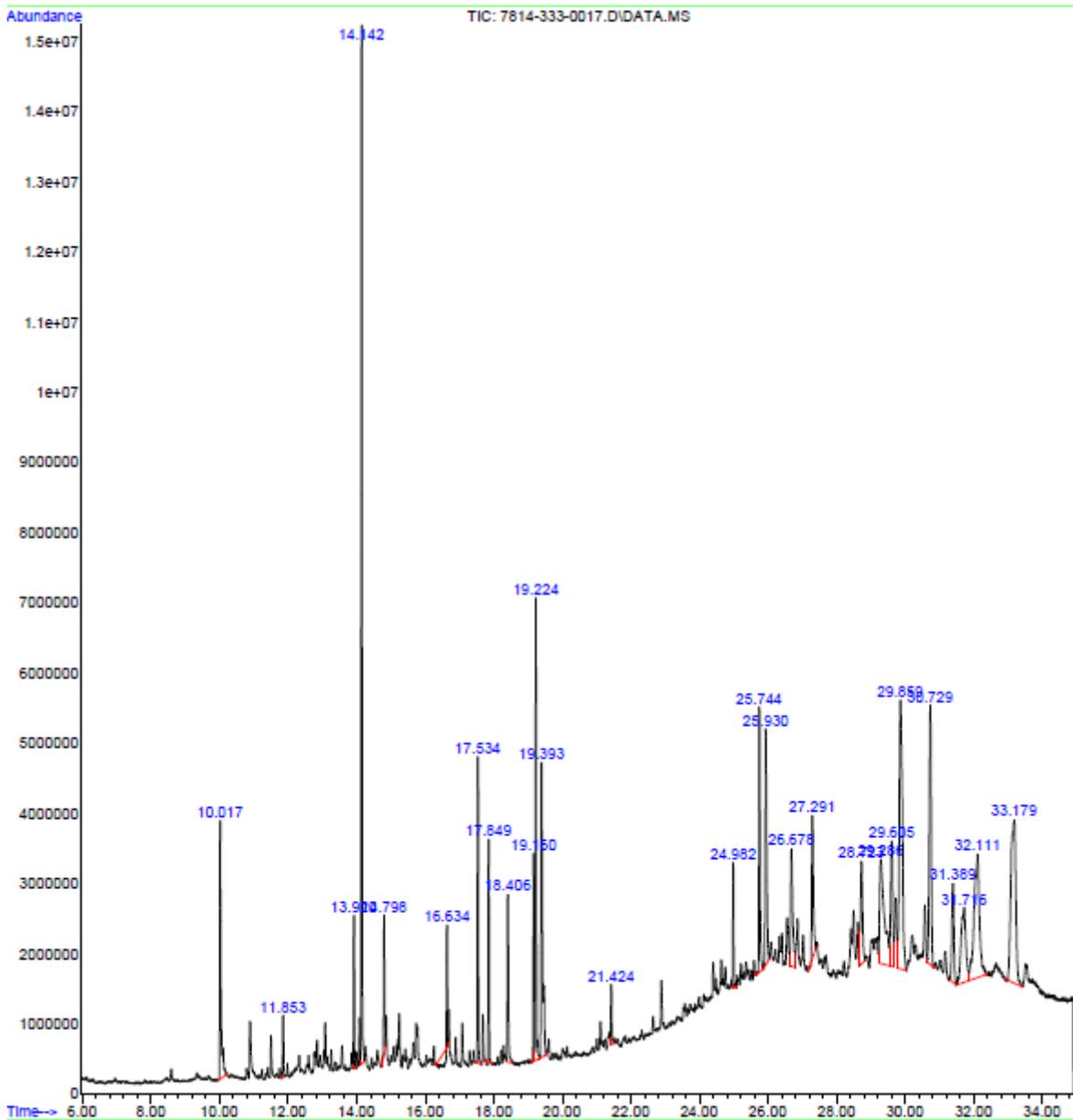


Figure 4

GC-MS Chromatogram of Ethanolic extract of *Hellenia speciosa*