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GC-MS and FT-IR analysis of a coastal medicinal plant-*Hyptis suaveolens* (L.) Poit

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ABSTRACT

Objective: To investigate the bioactive components of a coastal medicinal plant, *Hyptis suaveolens* (L.) Poit. (*H. suaveolens*) leaves using fourier transform-infrared spectroscopy and gas chromatography-mass spectrometer (GC-MS).
Methods: The chemical compositions of the ethanol extract of whole plant of *H. suaveolens* was investigated using PerkinElmer GC-MS, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standard and Technology library.
Results: The results of fourier transform-infrared spectroscopy analysis confirmed the presence of secondary alcohols, phenols, alkanes, alkynes, aromatics, nitro compounds and aliphatic compounds. GC-MS analysis of the ethanolic extract revealed the existence of 30 phytochemical compounds. 5,5-Dimethylimidazolidin-2,4-diamine (20.35%) was found to be the major compound.
Conclusions: The results of this study offer a platform to use *H. suaveolens* leaves as herbal alternative for various diseases.

1. Introduction

Plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in all organisms. In higher plants, such compounds are often concentrated in seeds and vegetative storage organs. Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicides, pesticides and pharmaceutical drugs[1-6]. For long medicinal plants, their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases[7].

Secondary metabolites (compounds) have no apparent function in a plant's primary metabolism, but often have ecological roles, as pollinator attractants and represent chemical adaptations to environmental stresses or serve as chemical defense against microorganisms, insects and higher predators and even other plants (allelochemicals). Secondary metabolites are frequently accumulated by plants in smaller quantities than primary metabolites[8,9].

A survey of current pharmaceutical use revealed that, of the total prescription drugs dispensed, 25% are plant-derived[10]. Plant compounds are highly varied in structure: many are aromatic substances and most of which are phenols or their oxygen-substituted derivatives. However, there is an increasing attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance that pathogenic microorganisms build against antibiotics[11]. New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants[12]. Of the various pharmaceuticals used in modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through the observations of indigenous medical practices[13]. Cheng *et al*. stated that antimicrobial compounds derived from plants may inhibit bacteria by a different mechanism than the presently used antibiotics and may have a clinical value in the treatment of antibiotic-resistant microbial strains[14].

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Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or may serve as models for pharmacologically active compounds in drug synthesis. The general research methods include proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemopharmacological investigations, toxicological and clinical studies, and standardization and use of active moiety as the lead molecule for drug design[7].

Within a decade, there were a number of dramatic advances in analytical techniques including fourier transform-infrared spectroscopy (FT-IR) and gas chromatography-mass spectrometer (GC-MS) that were used in the determination of phytochemicals. The aim of this study is to determine the bioactive compounds present in the *Hyptis suaveolens* (*H. suaveolens*) leaves which may provide an insight into the use of traditional medicine.

2. Materials and methods

The dried powdered sample (100 g) was extracted with 1000 mL of solvent in a Soxhlet apparatus. The resultant filtrate was concentrated into powdered form through complete evaporation of the solvent extraction using rotary evaporator. The solid residue of greenish-brown colour obtained was designated as the extract, which was stored in a refrigerator until further analyses.

2.1. Preliminary phytochemical screening

Qualitative chemical tests were conducted to gain a general idea regarding the nature of constituents present in the extract. Aqueous, petroleum ether, chloroform, ethanol and acetone extracts were subjected to preliminary phytochemical investigations for detection of specific compounds as per the standard methods prescribed by Harborne[15].

2.2. FT-IR analysis

Oven-dried leaf samples (60 ˚C) were ground into fine powder using a mortar and pestle. About 2 mg of the sample was mixed with 100 mg potassium bromide (FT-IR Grade) and then compressed to prepare a salt-disc (3 mm diameter). The disc was immediately kept in the sample holder and FT-IR spectra were recorded in the range of absorption between 400 and 4000 cm^{-1}. All investigations were carried out with a Shimadzu FT-IR spectrometer.

2.3. GC-MS analysis

Ethanolic extract of *H. suaveolens* leaves were subjected to GC-MS analysis. Extracts were dissolved in high-performance liquid chromatography-grade and subjected to JEOL GCMATE II GC-MS (Agilent Technologies 6890N network GC system for GC). He was used as the carrier gas at a flow rate of 1 mL/min. The temperature was programmed at 80 ˚C for 5 min, then increased to 300 ˚C at the rate of 15 ˚C/min. The temperatures of injector and electronic ignition detector (70 eV) were 280 and 300 ˚C, respectively and 2 μL of plant extract was injected with a Hamilton syringe into the GC-MS manually.

2.4. Identification of components

Interpretation of mass spectrum obtained from GC-MS was conducted using the database of National Institute Standard and Technology having more than 62000 patterns. The spectrum of the unknown component was compared with the spectra of the known components stored in the National Institute Standard and Technology library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Results

3.1. Preliminary phytochemical screening

Preliminary screening tests were useful for detecting bioactive principles from plant parts and could lead to drug discovery and development. In the present study, several phytochemical constituents of the aqueous, petroleum ether, chloroform, ethanol and acetone leaf extracts of *H. suaveolens* were evaluated qualitatively. Alkaloids, quinones, steroids, coumarins, proteins, flavonoids and terpenoids were present in all the five extracts. Carbohydrates were present only in aqueous and petroleum ether extracts, whereas glycosides were present in all the extracts except aqueous and petroleum ether extracts. Tannins were found to be present in all the extracts other than petroleum ether extract. Saponins were noticed in aqueous, petroleum ether and acetone extracts, and phytosterols were noticed in petroleum ether, chloroform and ethanol extracts (Figure 1).

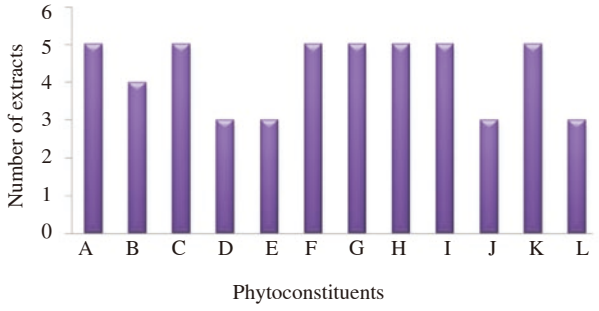

Figure 1. Phytochemical screening of *H. suaveolens* leaf extracts.
A: Alkaloids; B: Phenolic compunds; C: Flavonoids; D: Saponins; E: Glycosides; F: Terpenoids; G: Steroids; H: Coumarins; I: Quinones; J: Phytosterols; K: Proteins; L: Carbohydrates.

3.2. Determination of functional groups in H. suaveolens using FT-IR

The FT-IR spectrum of the *H. suaveolens* leaf sample was shown in Table 1. The absorption at 3313 cm^{-1} was the stretching of alkyne groups. The bands at 3193 and 2364 cm^{-1} were ammonium ion and aliphatic cyanide or nitrite groups respectively. The bands at 1668, 1454, 1400, 1334 and 1195 cm^{-1} were open-chain imino (-C=N-)

stretch, methyl C-H asymmetric or symmetric bend saturated aliphatic alkane or alkyl group frequencies, phenol or tertiary alcohol OH bend, primary or secondary alcohol OH in plane bend, and aromatic C-H in plane bend, respectively (Table 1).

Table 1
FT-IR analysis of *H. suaveolens* leaf samples.

Origin	Group frequency, wavenumber (cm^{-1})	Assignment
C-H	3313.48	Alkyne stretch
N-H	3193.90	Ammonium ion
N-H	2364.57	Aliphatic cyanide/nitrite
-C=N-	1668.31	Open-chain imino
C-H	1454.23	Methyl asymmetric or symmetric bend
O-H	1400.22	Phenol or tertiary alcohol bend
O-H	1334.65	Primary or secondary alcohol in plane bend
C-H	1195.78	Aromatic in plane bend
C-F	1114.78	Aliphatic fluoro compounds stretch
C-H	808.12	1,3-disubstitution meta aromatic ring aryl group
C-Cl	752.19	Aliphatic chloro compounds stretch
C-Br	653.82	Aliphatic bromo compounds stretch
C-Br	603.68	Aliphatic bromo compounds stretch
S-S	462.88	Aryl disulphides

3.3. GC-MS analysis of ethanolic leaf extract of H. suaveolens

The results pertaining to the GC-MS analysis were given in Figure 2 and Table 2. Thirty compounds were detected in the ethanolic extract of *H. suaveolens* leaf and 5,5-dimethylimidazolidin-2,4-diamine (20.35%) was found to be the major compound followed by 1-hexadecene (8.43%). The GC-MS spectrum of the extract showed the presence of more long-chain hydrocarbons. Various aliphatic acids, aromatic compounds and ketones were also identified.

Table 2
Compounds identified from the ethanolic leaf extract of *H. suaveolens* (L.) Poit.

Peak No.	Retention time	Compound name	Molecular formula	Molecular weight (KDa)	Area %
1	3.02	5,5-Dimethylimidazolidin-2,4-diimine	$C_5H_{10}N_4$	126	20.35
2	5.95	1,8-Cineole	$C_{10}H_{18}O$	154	3.21
3	9.25	(+)-(1aR, 2R, 5S, 5As)-2,5-Diisopropylperhydroxireno[2,3-d][1,2] dioxine	$C_{10}H_{18}O_3$	186	2.00
4	10.03	2-Propenoic acid, 2-ethylhexyl ester	$C_{11}H_{20}O_2$	184	3.14
5	10.41	Bicyclo(2.2.1)heptan-1-ol	$C_7H_{12}O$	112	1.00
6	12.83	1,4-bis(p-tolylsulphinyl)piperazine	$C_{18}H_{22}N_2O_2S_2$	362	0.85
7	13.19	3-Isopropylisoxazole	C_6H_9NO	111	2.32
8	13.71	1-Tetradecanol	$C_{14}H_{30}O$	214	5.55
9	14.45	α-elemene	$C_{15}H_{24}$	204	3.78
10	14.96	cis 3-Hexenyl tiglate	$C_{11}H_{18}O_2$	182	0.94
11	17.27	3-Trifluoroacetyl-4,5-dihydrofuran	$C_6H_5F_3O_2$	166	1.25
12	17.82	1-Hexadecene	$C_{16}H_{32}$	224	8.43
13	22.26	1-Nonadecanol	$C_{19}H_{40}O$	284	6.68
14	23.23	Phytol acetate	$C_{22}H_{42}O_2$	338	1.23
15	24.20	Neroloxide	$C_{10}H_{16}O$	152	0.98
16	26.70	1-Octadecanol	$C_{18}H_{38}O$	270	6.51
17	28.00	2-Isopropyl-4b,8,8-trimethyl-4b,5,6,7,8a,9,10-heptahydro-phenanthrane	$C_{20}H_{30}$	270	0.90
18	28.34	Isopropyl(trans-2-methylcyclopentyl) isopropoxyborane	$C_{12}H_{25}BO$	196	0.82
19	29.01	4-Tert-butyl-3,5,5-trimethyl-3-hexane	$C_{13}H_{26}$	182	1.74
20	30.97	Ethyl N-cyano-N-pentylcarbamate	$C_9H_{16}N_2O_2$	184	3.77
21	33.29	5-Bromo-1,2-dimethyl-4-nitroimidazole	$C_5H_6BrN_3O_2$	219	0.71
22	33.58	(+-)-cis-3,4,6,9-tetrahydro-10-hydroxy-7-methoxy-1,3-dimethyl-1H-naphtho[2,3-c]pyran-6,9-dione	$C_{16}H_{16}O_5$	288	1.84
23	34.45	(1rs, 2rs)-2-(di-o-tolylphosphinoyl)-1-phenyl-1-pentanol	$C_{25}H_{29}O_2P$	392	3.19
24	35.01	1-Heptacosanol	$C_{27}H_{56}O$	396	2.13
25	37.67	Bis(3-formyl-4-hydroxyphenyl) disulfide	$C_{14}H_{10}O_4S_2$	306	2.74
26	38.95	Heptyl 2-hydroperfluoroheptanoate	$C_{14}H_{16}F_{12}O_2$	444	0.81
27	39.64	1-(Benzothiazol-2-yl)-3-phenylpenta-3,4-dien-2-ol	$C_{18}H_{15}NOS$	293	1.20
28	41.29	2-Iodo-2-phenylethanol	$C_8H_9I_0$	248	1.16
29	42.91	(E)-4-Methoxy-2,2-dimethyl-5-phenylhex-4-en-3-ol	$C_{15}H_{22}O_2$	234	5.06
30	44.15	5-Chloro-2-furancarbaldeyde oxime	$C_5H_4C_1NO_2$	145	5.70

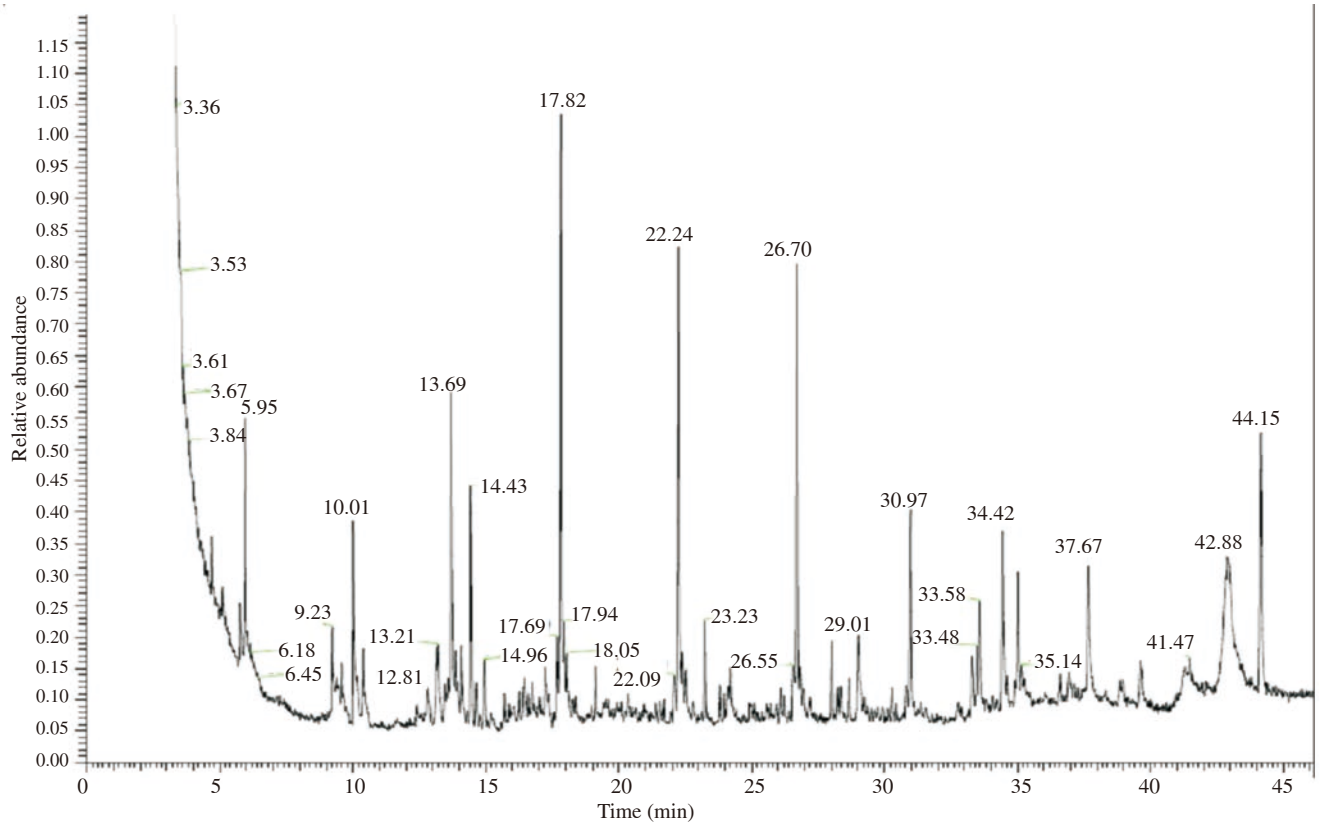

Figure 2. GC-MS chromatogram of ethanolic extract of *H. suaveolens*.

4. Discussion

Phytochemicals possess various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. The medicinal value of plants depends on the chemical substances that have a definite physiological action on the human body. The most important of the bioactive constituents of plants are alkaloids, tannins, saponins, terpenoids, steroids, glycosides, flavonoids and phenolic compounds[16-19]. In the present investigation, qualitative analysis of five different extracts (aqueous, petroleum ether, chloroform, ethanol and acetone) of *H. suaveolens* leaves were analysed for phytoconstituents. Different solvents have various degrees of solubility for different phytochemicals[20]. Among the various solvents tested, Boeing *et al*. observed maximum separation of phytochemicals with the ethanolic extract[21]. This is because ethanol is much polar than chloroform and acetone, hence extracting many of the active ingredients[22]. There are more reports available indicating the maximum extraction of phytochemicals in the ethanolic extract[23,24]. The results obtained by Agarwal and Varma regarding the methanolic extract of *H. suaveolens* revealed the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, glycosides, tannins, phenolic compounds, proteins, aminoacids, terpenoids and steroids, which is in accordance with the present result[25]. Pachkore and Dhale who analysed *H. suaveolens* by phytochemical screening revealed the presence of volatile oil, starch, proteins, tannins, saponins, fats, alkaloids and glycosides in leaves, and the absence of saponins in stem and root of the plants[26].

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economically important materials such as tannins, oils, gums, flavonoids, saponins and essential oil precursors for the synthesis of complex chemical substances[27].

Spectroscopic technique has become a powerful tool for the qualitative and quantitative analysis of biological and pharmaceutical materials. FT-IR spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants[28]. Plant constituents involved in the reduction and capping of nanoparticles can be identified by the FT-IR technique[29]. The region between 4 000 and 400 cm^{-1} is of greatest practical use to the organic chemist[30].

Alkanes are found in the powdered leaf samples of *H. suaveolens*. Generally, alkanes are found in the cuticle and epicuticular wax of many plants.

They protect the plant against water loss, prevent leaching of important minerals by rain and protect against bacteria, fungi, and harmful insects[31]. Alkynes are highly bioactive and act as nematocides[32]. The presence of alkynes suggests that this plant can be used as a nematocidal agent in the near future.

An IR represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms and no two compounds produce the exact same IR spectrum. Therefore, an IR spectrum is a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of the material present. With modern software algorithms, IR is an excellent tool for quantitative analysis. Because all the frequencies are measured simultaneously and most measurements by FT-IR are made in a matter of seconds rather than several minutes[33,34].

The results pertaining to GC-MS analysis led to the identification of a number of compounds from the GC fractions of the ethanolic extract of *H. suaveolens*. These compounds were identified through mass spectrometry attached with GC. The GC shows the relative concentrations of various compounds eluted as a function of retention time. The heights of the peaks indicate the relative concentrations of the components present in the leaves. The mass spectrometer analyzes the compounds eluted at different times to identify their nature and structure. The large compound fragments into small compounds giving rise to peaks at different *m/z* ratios. Each mass spectrum is a fingerprint of a certain compound which can be identified from the data library. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The GC-MS spectrum showed the presence of more long-chain hydrocarbons, which have complex chemical compositions. When the number of carbon atoms increases in the molecule, hydrophilicity is reduced and lipophilicity is increased. The lipophilicity of a drug is higher due to its distribution; because once the drug is in systemic circulation, it is distributed to all the tissues at a particular rate depending on its physicochemical characteristics such as lipophilicity and charge[35,36].

H. suaveolens has been the subject of some previous studies regarding its chemical nature. From the petroleum ether extract of *H. suaveolens* aerial parts, a pentacyclic triterpene was isolated[37]. The A-ring contracted triterpene obtained is the first example of a compound presenting skeletal type outside the lupine series[38]. The compounds such as hentriacontane, friedelin, netriacontanone, lupeol acetate and lupeol were isolated from the benzene extract of air-dried powdered leaves and floral parts of *H. suaveolens*[39]. Recently, Mary *et al*. isolated 11 compounds including allyloctadecanoate and octadec-9-enoic acid from the aqueous extract of *H. suaveolens*[40]. The

gas chromatography-flame ionization detector analysis of the extracted oil revealed 36 chemical compounds (99.99%), of which 72.54% are monoterpenoids, 21.96% are sesquiterpenoids and 5.49% are non-terpenoid constituents. The major constituents of the oil are sabinene (25.0%), α-terpinolene (13.64%), β-caryophyllene (12.75%), 1,8-cineole (9.11%), β-pinene (5.65%), bicyclogermacrene (5.61%) and limonene (5.40%)[41].

In the past, efforts have been directed towards studying the non-volatile components of *H. suaveolens* and a number of di- and triterpenoids and steroids have been identified[42-45]. Volatile oil isolated from *H. suaveolens* was reported to contain sabinene and 1, 8-cineole[46]. The essential oil obtained after hydrodistillation of the leaves of *H. suaveolens* showed 1,8-cineole (32%) and β-caryophyllene (29%) as the major constituents[47]. Similarly in the present study, the ethanolic extract of *H. suaveolens* showed the presence of the compound 1, 8-cineole (3.21%). The concentration of this compound is normally the highest in *Hyptis* and has been reported to be up to 47.64% in a sample from Brazil[48]. The presence of these phytochemicals has been attributed to the bioactive principles which is responsible for ethnopharmacological activities of most medicinal plants[49-51]. The presence of various bioactive compounds confirms the application of *H. suaveolens* for various ailments by traditional medical practitioners. Moreover, isolation of individual phytochemical constituents may lead to the development of novel drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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