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Original Research Article

Metabolite Profiling Using FTIR and GC-MS Techniques and Bioactivities of Fennel (*Foeniculum vulgare*) Flowers a Traditional Iraqi Medicinal Plant

Ali Abed Gatea[1*]

[1]AL-Qasim Green University, Faculty of Biotechnology, Department of Medical Biotechnology, Iraq

***Corresponding Author:** Ali Abed Gatea
AL-Qasim Green University, Faculty of Biotechnology, Department of Medical Biotechnology, Iraq

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Abstract: The Apiaceae species Foeniculum vulgare exists as a well-established therapeutic plant which people frequently use to treat digestive problems, promote milk production, aid as a carminative and acts as a diuretic and respiratory treatment. This study employed gas-chromatography mass spectrometry (GC–MS) method to identify different phytoconstituents present in Foeniculum vulgare methanolic extract. Standards-based analysis according to FT-IR scans the molecular bond interactions of both polymers and all other types of compounds with IR light. The analytical method detected specific absorption bands which existed in organic molecules. Management of bioactive compounds in Foeniculum vulgare was confirmed by the study at different spectral ranges after FT-IR analysis. Fourier-transform infrared spectroscopic profile solid analysis of Fennel (Foeniculum vulgare) Peak (Wave number cm-^{l}), Intensity, Corr. Intensity, Area, Corr. Area, Bond, Functional group assignment (669.30, 59.416, 3.865, 2.859, 0.176, C-Cl and alkyl halides), (684.73, 63.115, 0.860, 2.285, 0.038, C-Cl, and alkyl halides). (827.46, 74.505, 1.152, 2.387, 0.081, =C–H and Alkenes), (873.75, 72.300, 2.593, 2.555, 0.131, =C–H and Alkenes), (927.76, 69.360, 0.661, 4.511, 0.180, =C–H and Alkenes), (1010.70, 48.730, 0.709, 18.575, 0.677, C-F and alkyl halides), (1236.37, 79.328, 0.352, 2.182, 0.052, C-F and alkyl halides), (1313.52, 79.285, 1.798, 2.296, 0.008, C-F and alkyl halides), (1417.68, 73.681, 0.430, 2.204, 0.073, C=C and Aromatic), (1604.77, 77.448, 9.752, 2.817, 0.060, N-H and Amide), (2918.30, 81.850, 1.636, 2.468, 0.286, C-H and Alkane), (3269.34, 74.844, 0.747, 2.381, 0.034, N-H and Amide). GC-MS detected bioactive compounds 2-Hydroxytetrahydropyran, Pentan-2-One, 3-Carene, Acetic acid-1-13C, L-Fenchone, 2-Aminobicyclohexyl, 1-Methyl-2-methylene-4-isopropyl, L-histidine, (-)-Sabinene, 2-Methyl-1-undecanol, 2-Methyl-1-undecanol, (E)-Anethole, 4-Pentyloxy-2,3-dicyanophenyl, Pentane, 1,2-epoxy-2-methyl, 1,3,8-P-menthatriene, 16-Methylheptadecan-1-ol, Methyl oxophenylacetate, Trans-p-mentha-2,8-dienol, Diphenylmethanone, Fenchyl acetate.

Keywords: *FTIR, GC-MS, Fennel (Foeniculum vulgare), Bioactivities, Medicinal Plant*.

INTRODUCTION

Through the ages humans have relied on plants as their principal medicine source. Traditional medicines together with beneficial plants function as treatment agents which contribute to good health maintenance throughout most developing nations. According to WHO estimates traditional medicines that primarily use plant drugs provide healthcare bases for 80 percent of developing country populations. Most plants across the globe are currently applied for medical purposes. The plants belonging to this category possess natural healing potential that medical science recognizes as medicinal properties. Natural products make up the majority of plants that are known or considered to be herbs and dietary supplements. The substances responsible for drug properties in medicines primarily come from secondary metabolites.

Vanilla straw is a medicinal plant that exists as a two-year or lasting herbaceous crop which grows in the Apiaceae plant family. The main constituents in Foeniculum vulgare seed operate as flavoring components in multiple food and cosmetic and pharmaceutical products. Multiple therapeutic properties including hepatoprotection and antispasmodic effects, diuretic behavior, anti-inflammation, pain relief, antioxidant potential and antibacterial, antimicrobial, antifungal, anti-diabetic and anti-neurological and anticancer properties have been identified in essential oils from Foeniculum vulgare seeds. Essential oils of Foeniculum vulgare mainly consist of two major chemical groups: phenylpropanoid derivatives and monoterpenoids according to literary reports. The essential oil biomarkers found in Foeniculum vulgare include trans-anithole as well as fenchone combined with a-pinene and b-pinene and camphene [1-3]. Gas-chromatography mass-spectrometry (GC–MS) functions as one of the essential methods to identify plant materials' phytoconstituents according to reports. GC–MS stands as an advantageous approach for analyzing chemical compositions of plant samples when compared to alternative techniques. Fenchone represents an essential oil biomarker from Foeniculum vulgare that exists in different commercially available products. A chiral GC technique exists for analyzing fenchone compounds found in essential oil samples. The literature contains no information about fenchone biomarker analysis in diverse commercial products [4]. This research determined different phytoconstituents within Foeniculum vulgare methanolic extract using GC–MS technique [5]. The active involvement of plant natural product chemistry has led to the successful development of various drug prospect compounds within pharmacological discovery initiatives. Plants constitute the most extensive and plentiful reservoir of drugs used in both traditional remedies and modern pharmaceutical medicines as well as nutraceuticals and food supplements and folk remedies and pharmaceutical intermediates for synthetic medications. IR spectroscopy maintains the ability to obtain biochemical data from biological specimens without altering the state of the sample. Present-day research in biological spectroscopy studies cellular and tissue analysis through infrared observation to determine if the technique detects tissue and cellular degeneration accurately [6, 7]. The use of infrared spectra for biomolecule detection experienced renewed interest following the rise of Fourier transforms infrared Spectrometers due to their combination of high signal-noise ratio with precise absorbance and wave number measurements. FT-IR stands among the top spectroscopic tools for chemical constituent identification and compound structure determination since it serves as an essential method for surveys in pharmaceutical materials throughout various nations [8-10]. The extensive sample applications alongside distinctive fingerprint characteristics have allowed FT-IR to gain significance for pharmaceutical analysis during recent years [11]. The examination of biological tissues and cells for molecular structure and intermolecular interactions utilizes infrared spectroscopy as a potent analytical technique. The present research sought to investigate the bimolecular substances found in medical plants.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The team obtained Foeniculum vulgare plant flowers from Medical Consulting Office at Hillah City located in Babylon Governorate.

Preparation of the Plant Material

A domestic grinder processed the dried flowers which underwent 10 days of clean environmental air-drying at room temperature. Further analysis needed the stored powdered samples which received containment inside glass bottles at room temperature.

Sample Preparation

The powdered materials went into a lyophilizer to extract all moisture content. Fine powder required grinding the samples with an agate mortar and pestle. A complete mix of powdered flowers with paraffin liquid received FTIR spectroscopic analysis.

GC–MS analysis of Methanolic Extract of Foeniculum vulgare

The sample analysis used ‘‘Perkin Elmer GC–MS coupled with Clarus 600 T mass Spectrometer (USA)’’ as the instrument. The system possessed three primary units including auto-sampler and auto-injector that were integrated with the gas chromatograph Clarus 600 featuring a single quadrupole mass spectrometer. A software program called ‘‘TurboMass Solution Software Version 5.4’’ operated during the GC–MS analysis of samples. The sample separation occurred with an Elite 5 MS (30 m 9 0.25 mm i.d., 0.25 lm thickness) capillary CG column which functioned through Perkin Elmer USA. The analyses used helium gas as the carrier vector at 65.2 kPa constant pressure. The temperature settings of the separation procedure followed a specified gradient format. The heat control system initiated at 40 C during the first 2 minutes then it progressed to 100 C during the following 2 minutes and the temperature later rose at 5 C/ min until achieving 300 C before resting at this heat for 5 minutes [12, 13]. The complete analytical operation lasted sixty-one minutes. The conditions for the interface temperature stood at 280 C while the ion source temperature used 240 C and the injector operated at 220 C. The instrument operated with an electron energy of 70 eV. The analysis used both ‘‘National

Institute of Standard and Technology (NIST, 2005) Library'' and ''WILEY, 2006, Library'' to identify the unknown components.

Fourier Transform Infrared Spectroscopy

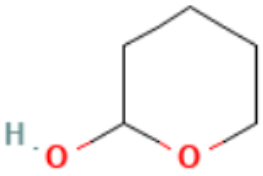
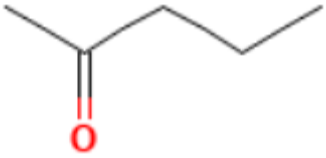
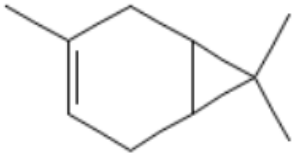
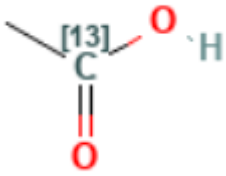
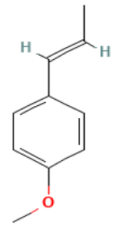
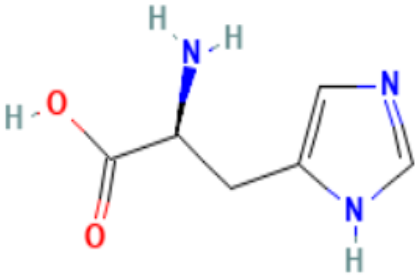
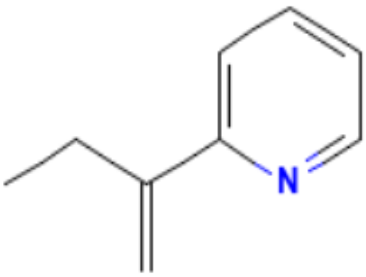
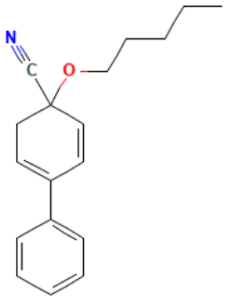
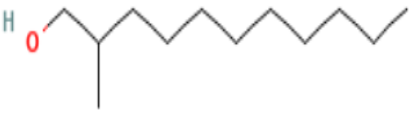
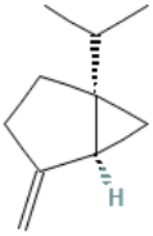
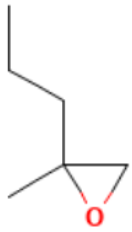
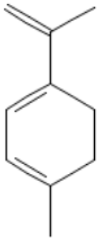
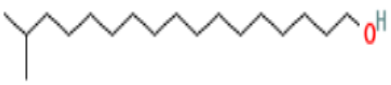
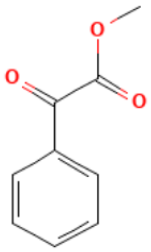
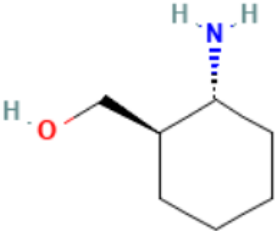
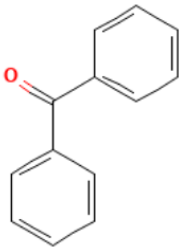
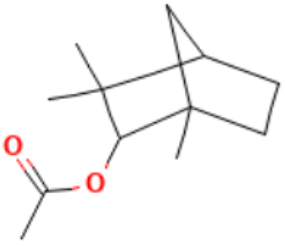
FTIR operates as a physicochemical analytical tool to generate clear data about metabolic compositions. Secondary conformation determination through FTIR spectrum analysis consists of two fundamental approaches which include spectrum treatment and band attribution steps. This section studies various methods used during different processes while analyzing their potential outcomes on the final result. Several proposals documented HMEC research to explore extrudate plant protein texturization through secondary conformations by focusing on β-sheets effects, α-helix balance with β-sheets and aggregation [14, 15] state with their process-related pH changes.

Results and Discussion

Foeniculum vulgare provides medicinal value that our nation uses. Foeniculum vulgare contains diverse phytochemical compounds that produce multiple therapeutic outcomes of fennel. The work identified Foeniculum vulgare plant extract phytoconstituents through GC–MS method using methanolic extraction. This GC-MS procedure served to evaluate fenchone levels both in plant extracts and different commercial preparations. The results of GC–MS profiling of methanolic extract of Foeniculum vulgare are listed in Table 1. 2-Hydroxytetrahydropyran, Pentan-2-One, 3-Carene, Acetic acid-1-13C, L-Fenchone, 2-Aminobicyclohexyl, 1-Methyl-2-methylene-4-isopropyl, L-histidine, (-)-Sabinene, 2-Methyl-1-undecanol, 2-Methyl-1-undecanol, (E)-Anethole, 4-Pentyloxy-2,3-dicyanophenyl, Pentane, 1,2-epoxy-2-methyl, 1,3,8-P-menthatriene, 16-Methylheptadecan-1-ol, Methyl oxophenylacetate, Trans-p-mentha-2,8-dienol, Diphenylmethanone, Fenchyl acetate. The report lists different compounds and their identified percentages together with their retention times. A GC–MS analysis of unknown mixtures enables compound identification through reference to NIST and WILEY library databases. The libraries match different compounds to their GC–MS response patterns and proper retention times. The calculation of compound amounts in percentage can be performed by analyzing GC–MS responses according to established literature methods. The weight estimations of compounds cannot be computed through NIST and WILEY spectral databases. A meaningful quantity of vital species present in Foeniculum vulgare methanolic extract was detected during analysis. Fourier-transform infrared spectroscopic profile solid analysis of Fennel (*Foeniculum vulgare*) Peak (Wave number cm^{-1}), Intensity, Corr. Intensity, Area, Corr. Area, Bond, Functional group assignment (669.30, 59.416, 3.865, 2.859, 0.176, C-Cl and alkyl halides), (684.73, 63.115, 0.860, 2.285, 0.038, C-Cl, and alkyl halides). (827.46, 74.505, 1.152, 2.387, 0.081, =C–H and Alkenes), (873.75, 72.300, 2.593, 2.555, 0.131, =C–H and Alkenes), (927.76, 69.360, 0.661, 4.511, 0.180, =C–H and Alkenes), (1010.70, 48.730, 0.709, 18.575, 0.677, C-F and alkyl halides), (1236.37, 79.328, 0.352, 2.182, 0.052, C-F and alkyl halides), (1313.52, 79.285, 1.798, 2.296, 0.008, C-F and alkyl halides), (1417.68, 73.681, 0.430, 2.204, 0.073, C=C and Aromatic), (1604.77, 77.448, 9.752, 2.817, 0.060, N-H and Amide), (2918.30, 81.850, 1.636, 2.468, 0.286, C-H and Alkane), (3269.34, 74.844, 0.747, 2.381, 0.034, N-H and Amide).

Table 1: GC–MS profiling of methanolic extract of Foeniculum vulgare

No.	Compound	Formula	M.W.
1	2-Hydroxytetrahydropyran	$C_5H_{10}O_2$	102.13 g/mol
2	Pentan-2-One	$C_5H_{10}O$	86.13 g/mol
3	3-Carene	$C_{10}H_{16}$	136.23 g/mol
4	Acetic acid-1-13C	$C_2H_4O_2$	61.045 g/mol
5	L-Fenchone	$C_{10}H_{16}O$	152.23 g/mol
6	2-Aminobicyclohexyl	$C_{12}H_{23}N$	181.32 g/mol
7	1-Methyl-2-methylene-4-isopropyl	$C_9H_{11}N$	133.19 g/mol
8	L-histidine	$C_6H_9N_3O_2$	155.15 g/mol
9	(-)-Sabinene	$C_{10}H_{16}$	136.23 g/mol
10	2-Methyl-1-undecanol	$C_{12}H_{26}O$	186.33 g/mol
11	(E)-Anethole	$C_{10}H_{12}O$	148.20 g/mol
12	4-Pentyloxy-2,3-dicyanophenyl	$C_{18}H_{21}NO$	267.4 g/mol
13	Pentane, 1,2-epoxy-2-methyl	$C_6H_{12}O$	100.16 g/mol
14	1,3,8-P-menthatriene	$C_{10}H_{14}$	134.22 g/mol
15	16-Methylheptadecan-1-ol	$C_{18}H_{38}O$	270.5 g/mol
16	Methyl oxophenylacetate	$C_9H_8O_3$	164.16 g/mol
17	Trans-p-mentha-2,8-dienol	$C_7H_{15}NO$	129.20 g/mol
18	Diphenylmethanone	$C_{13}H_{10}O$	182.22 g/mol
19	Fenchyl acetate	$C_{12}H_{20}O_2$	C12H20O2

 **2-Hydroxytetrahydropyran**	 **Pentan-2-One**	 **3-Carene**
 **Acetic acid-1-13C**	**2-Aminobicyclohexyl**	 **(E)-Anethole**
 **L-histidine**	 **1-Methyl-2-methylene-4-isopropyl**	 **4-Pentyloxy-2,3-dicyanophenyl**
 **2-Methyl-1-undecanol**	 **(-)-Sabinene**	 **Pentane, 1,2-epoxy-2-methyl**
 **1,3,8-P-menthatriene**	 **16-Methylheptadecan-1-ol**	 **Methyl oxophenylacetate**
 **Trans-p-mentha-2,8-dienol**	 **Diphenylmethanone**	 **Fenchyl acetate**

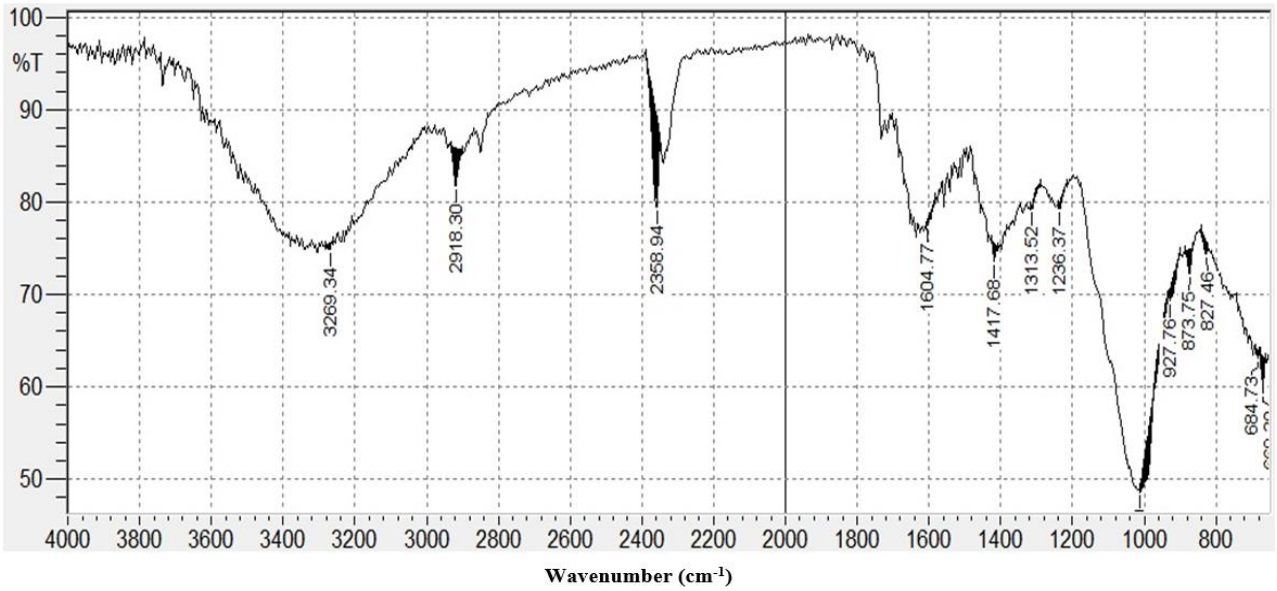


Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of Fennel (*Foeniculum vulgare*).

Table 2. Fourier-transform infrared spectroscopic profile solid analysis of Fennel (*Foeniculum vulgare*).

No.	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	669.30	59.416	3.865	677.01	663.51	2.859	0.176	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	684.73	63.115	0.860	690.52	678.94	2.285	0.038	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	827.46	74.505	1.152	840.96	821.68	2.387	0.081	Strong	=C–H	Bending	Alkenes	650-1000
4.	873.75	72.300	2.593	885.33	866.04	2.555	0.131	Strong	=C–H	Bending	Alkenes	650-1000
5.	927.76	69.360	0.661	931.62	900.76	4.511	0.180	Strong	=C–H	Bending	Alkenes	650-1000
6.	1010. 70	48.730	0.709	1012.63	933.55	18.575	0.677	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1236.37	79.328	0.352	1240.23	1217.08	2.182	0.052	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1313.52	79.285	1.798	1315.45	1290.38	2.296	0.008	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1417.68	73.681	0.430	1425.40	1408.04	2.204	0.073	Medium	C=C	Stretch	Aromatic	1400-1600
10.	1604.77	77.448	9.752	1608.63	1581.63	2.817	0.060	Bending	N-H	Stretch	Amide	1550-1640
11.	2358.94	79. 466	3.998	2389.80	2349.30	2.488	0.880	-	-	-	-	-
12.	2918.30	81.850	1.636	2931.80	2899.01	2.468	0.286	Strong	C-H	Stretch	Alkane	2850-3000
13.	3269.34	74.844	0.747	3280.92	3261.63	2.381	0.034	Bending	N-H	Stretch	Amide	3100-3500

The development of Fourier Transform Infrared (FT-IR) spectrometry occurred to solve the problems encountered with traditional dispersive instruments. The primary challenge existed in the time needed to complete the scanning procedure. The technique required for simultaneous measurement of infrared frequencies as opposed to sequential measurement needed development. Scientists developed an interferometer as a basic device to solve the measurement problem. The interferometer generates particular signals containing the entire spectrum of infrared frequencies encoded together. The fast measurement of the signal takes approximately one second for completion [16, 17]. The measurement duration for each sample decreases to few seconds instead of multiple minutes. The main quality of FT-IR detection stands above dispersive techniques due to its ability to obtain all frequencies instantaneously which results in quick analysis periods of less than one second. The phenomenon also gets labeled as the Felgett Advantage. The sensitivity of FT-IR has improved significantly due to several points. The instrument shows improved detector sensitivity as well as enhanced optical throughput (called Jacquinot Advantage) leading to reduced noise and many scans can be combined through signal averaging for achieving precise noise reduction [18-20]. The interferometer features its one and only moving component as a mirror which continuously shifts throughout its operation. Mechanical failure stands as an extremely unlikely event because of the instrument design. The devices utilize an internal HeNe laser system to function as wavelength calibration standard (referred to as Connes Advantage). User calibration is not needed since these instruments perform self-calibration

[21, 22]. FT-IR provides measurements which demonstrate exceptional accuracy through multiple advantages as well as multiple others. Thus, it a very reliable technique for positive identification of virtually any sample. Small contaminants become detectable because of the sensitivity advantages provided by this technique. Participants who worked as chemical technicians at General Electric Company stated that FT-IR serves as an essential instrument for quality control and quality assurance testing which enables both standard comparison analysis between batches and unknown contaminant detection. The practical application of infrared analysis for quantitative testing increased because FT-IR detectors provide both enhanced precision and improved detection capability as well as sophisticated software algorithms. Simple routines and calibrations can be developed from quantitative methods to create standardized analysis procedures. The creation of the Fourier Transform Infrared (FT-IR) technology has successfully brought practical enhancements to infrared spectroscopy [23]. The development of new sampling techniques became feasible because older technology systems succeeded through this advanced method. Infrared analysis now has no practical limitations because of this advancement [24, 25]. The plant species Foeniculum vulgare exists within the single genus Foeniculum (most botanists define as consisting of only this species). Fennel stands as a strongly aromatic vegetable with multiple dietary and medicinal purposes because it shares similar tastes with anise and forms part of absinthe's essential ingredients. The selection of Florence fennel features bulbed enlargement on its stem base which people use as a vegetable type. The leaves of fennel serve as food for Lepidoptera larvae, particularly the anise swallowtail and mouse moth species. Fennel serves as a perennial herb through its scientific name Foeniculum vulgare. From its base to its top it grows to reach 2.5 meters in height while displaying glaucous green color in its erect shape through hollow stems. Its leaves develop up to 40 cm length and contain finely dissected segments that become threadlike structures about 0.5 millimeters wide [26]. The plant leaves bear a resemblance to dill leaves although they remain slimmer in appearance. The terminal compound umbels reach 5–15 cm in width by producing small yellow flowers that appear in sections containing 20 to 50 flowers each. Each flower stem stands short. Traditional medicine describes fennel plant uses for digestional purposes while also helping people lose weight and detoxify their bodies and enhance metabolism and minimize stomach cramps and heart burn symptoms for pregnant women experiencing morning sickness and battling bloating and kidney flushing alongside promoting healing after radiation therapy and chemotherapy treatments.

CONCLUSION

IR spectroscopy functions as a vibration-based spectrum. This detection technique stands as the main value of the method through its ability to detect organic molecule bands. The detection of IR spectrum absorption bands corresponds to specific frequencies which each different bond exhibits. Different medicinal properties of Foeniculum vulgare stem from the characteristic functional groups including Carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, and halogens. Additional scientific research will support the discovery of novel bioactive compounds throughout medicinal plants.

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