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Research Article

Phytochemical Profiles of Methanolic Seeds Extract of *Cuminum cyminum* using GC-MS Technique

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ABSTRACT
The aims of this study were identify the chemical components as well as anti-microbial activities of the methanolic seeds extract of *Cuminum cyminum*. GC-MS analysis revealed the existence of the Benzene , 1,1'-oxybis[4-phenoxy- , Stearyltrimethylammonium chloride , Benzenemethanol , 4-hydroxy-α-[1-methylamino)ethyl]-,(R*,S*)- , Quinolin-2(1H)-one , 3,4,5,6,7,8-hexahydro-3-dimethylaminomethyl- , 5-Hepten-2-amine , N,6-dimethyl- , 2-Pentanone , 4-amino-4-methyl- , Benzedrex , α-Pinene , 10-(dimethylaminomethyl)- , Ethanamine ,2-(2,6-dimethylphenoxy)-N-methyl- , Ephedrine , 1-(1,4-cyclohexadienyl)-2-methylaminopropane , Tapentadol , 3-Pyridinecarboxaldehyde , O-acetyloxime , (E)- , 1,2,3-Propatriol , 1-indol-4-yl(ether) , 2,2,3,4-tetramethyl-5-phenyloxazolidine , Phosphorothioic acid , S-ester with trimethylenediiminodipropanthi , Cyclopentanone ,2-(2-octenyl)- , Butyl 9-tetradecenoate , Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17-[O-(phenylmethyl) and 13-Oxabicyclo[9.3.1] pentadecane , 15-chloro.

Keywords: Bioactive compounds, *Cuminum cyminum*, GC/MS, Products.

INTRODUCTION

Natural products with their diverse biological and pharmacological activities represent a gold mine for scientists searching for lead compounds for the treatment of health disorders and infections[1,2]. *Cuminum cyminum* L., belonging to the family Apaiaceae, is one of the old cultivated medicinal food herbs in Asia, Africa and Europe. *C. cyminum* is an annual herbaceous plant which grows up to 15-50 cm height somewhat angular and tends to droop under its own weight. It has a long, white root. The leaves are 5-10 cm long, pinnate or bi pinnate, with thread-like leaflets and blue green in color and are finely divided, generally turned back at the ends. The leaves are highly dissected. Whitish-red flowers are on a compound umbel (arrangement of flowers looks like an umbrella). The fruit is an elongated, oval shaped schizocarp (an aggregate fruiting body which doesn't break open naturally and has two single seeded units called mericarps). The fruits are similar to fennel seeds, when chewed has bitter and pungent taste. The fruit are thicker in the middle, compressed laterally about 5 inch long, containing a single seed[5-8]. Aromatic plants are frequently used in traditional medicine and essential oils and volatile constituents extracted from them are widely used as antioxidants and antidiabetic agents and for the prevention and treatment of different human diseases, such as cancer, cardiovascular diseases, including atherosclerosis and thrombosis, bacterial and viral infections[9,10]. Dried ripe seeds of *C. cyminum* are usually used for medicinal or culinary purposes. In Iranian traditional medicine, Cumin seeds were used for their therapeutic effects on gastrointestinal, gynecological and respiratory disorders, and also for the treatment of toothache, diarrhea and epilepsy. The seeds were also documented as stimulant, carminative and astringent[11]. Johri has been recently reported that medicinal usage of Cumin seeds has also been widespread in diverse ethnomedical systems from Northern Europe to the Mediterranean regions, Russia, Iran, Indonesia and North America, where these have remained as an integral part of their folk medicines[12,13].

MATERIALS AND METHODS

Extraction and isolation

Cuminum cyminum were collected from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the *Cuminum cyminum* was stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use[14-22]. About twenty grams of methanolic extract of *Cuminum cyminum* powdered were soaked in 50 mL methanol for ten hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture[23-34].

Gas chromatography – mass spectrum analysis

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. The combination of a principle separation

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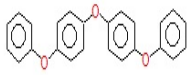
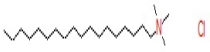
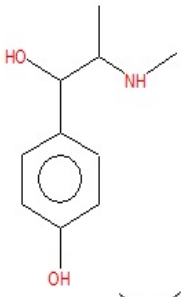
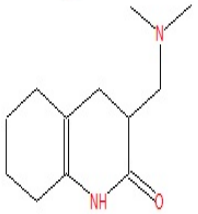
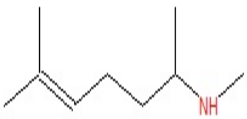
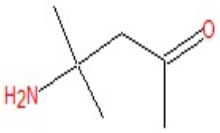
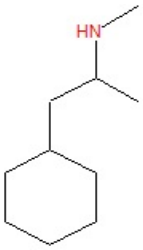
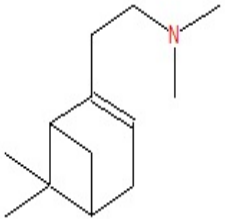
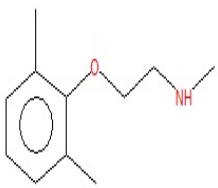
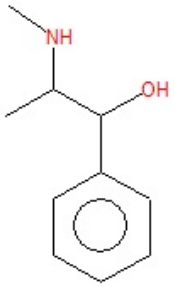
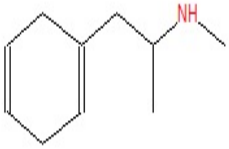
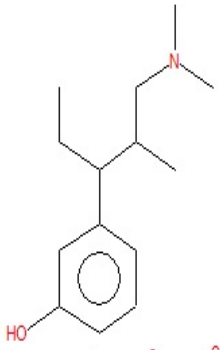
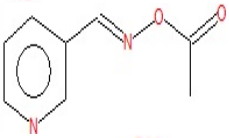
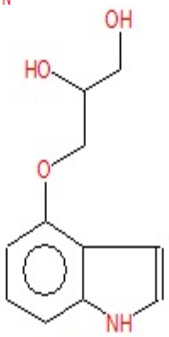
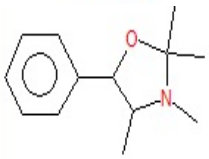
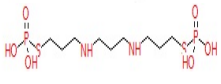
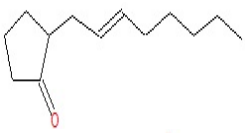
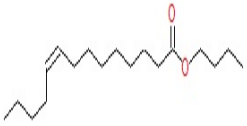
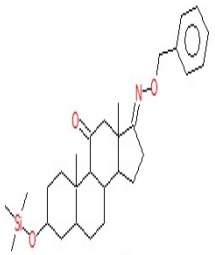
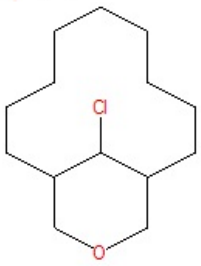
Table 1: Major phytochemical compounds identified in methanolic extract of *Cuminum cyminum*.

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1.	Benzene, 1,1'-oxybis[4-phenoxy-	3.150	354	354.125595		51,77,92, 168,184,215, 233,337	anti-androgenic activity
2.	Stearyltrimethylammonium chloride	3.613	347	347.331879		58,69,97, 153,182,240, 297	antibacterial activity
3.	Benzenemethanol, 4-hydroxy-α-[1-methylamino)ethyl]-,(R*,S*)-	3.722	181	181.110279		58,65,77, 95,121,147,164,182	Unknown
4.	Quinolin-2(1H)-one, 3,4,5,6,7,8-hexahydro-3-dimethylaminomethyl-	4.466	208	208.157563		58,65,77, 91,103, 117,134,148, 163,208	New chemical compounds
5.	5-Hepten-2-amine, N,6-dimethyl-	4.792	141	141.15175		8,71,84, 5,110,126,1 1	Unknown
6.	2-Pentanone, 4-amino-4-methyl-	4.958	115	115.0997143		58,71,84,100, 114	anti-inflammatory effects by inhibiting cyclooxygenase (COX)
7.	Benzedrex	5.284	155	155.167399		58,67,83,95,140,155	Unknown
8.	α-Pinene, 10-(dimethylaminomethyl)-	5.725	193	193.18305		58,65,77,91,105,119,178,193	anti-inflammatory via PGE1
9.	Ethanamine ,2-(2,6-dimethylphenoxy)-N-methyl-	5.805	179	179.131014		51,58,65,77,91,105,121,133,146,160,179	Anti-Arrhythmia Agent

10.	Ephedrine	5.891	165	165.115364		58,77,91,105, 132,146	Unknown
11.	1-(1,4-cyclohexadienyl)-2-methylaminopropane	6.600	151	151.1361		58,77,91,105, 132,150	Antiviral
12.	Tapentadol	7.888	221	221.177964		58,77,107,22 1	anti-inflammatory
13.	3-Pyridinecarboxaldehyde , O-acetyloxime , (E)-	8.769	164	164.058578		50,67,77,91,1 04,122,141	Unknown
14.	1,2,3-Propatriol , 1-indol-4-yl(ether)	9.043	207	207.089543		57,77,104,11 6,133,146,17 6,207	Anti-Bacterial and Anti-Fungal Activity
15.	2,2,3,4-tetramethyl-5-phenyloxazolidine	9.478	205	205.146665		51,56,65,71,9 1,99,117,133, 148,174,190, 205	Anti-Candida
16.	Phosphorothioic acid , S-ester with trimethylenediimin odipropanthi	10.005	382	382.055101		58,70,84,102, 118,130,149, 168,189,197, 223,245	New chemical compounds
17.	Cyclopentanone ,2-(2-octenyl)-	14.502	194	194.167066		55,67,84,97,1 10,123,137,1 94	anti-tumor activity, and antiviral
18.	Butyl 9-tetradecenoate	15.458	282	282.25588		55,69,83,97,1 11.166.189.2 09,227,282	New chemical compounds

19.	Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17-[O-(phenylmethyl)	15.652	481	481.30122		55,73,91,105, 135,147,207, 281,295,360	New chemical compounds
20.	13-Oxabicyclo[9.3.1] pentadecane , 15-chloro-	16.144	244	244.159393		55,67,81,95,109,135,149,177,208,244	New chemical compounds

technique (GC) with the best identification technique (MS) made GC–MS an ideal for qualitative and quantitative analysis for volatile and semi-volatile compounds[35-39]. The GC-MS analysis of the plant extract was made in a (Agilent 789 A) instrument under computer control at 70 eV. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred referred to as the Retention time (RT). While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector[40-49]. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The M/Z (mass / charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries[50-54].

RESULTS AND DISCUSSION

Identification of phytochemical compounds

The medicinal applications of cumin include use as a stimulant, carminative, an astringent, against indigestion, flatulence and diarrhea[55-59]. Sawi *et al.,* reported that in the herb and seed oils, 21 constituents were identified, representing 90.2%and 95.6% of the total amounts, respectively[60]. Borges *et al.,* reported chemical composition of the oil was determined by chromatography spectrometric methods and physicochemical indexes[61]. Some major components of Chinese cumin oil (c-terpinene, *q*-cymene and *b*-pinene) were previously found in cumin oils obtained from Turkey[62], Pakistan[63] and Iran[64]. It is well known that cuminal and cuminic alcohol show very strong antimicrobial and antioxidative activities. In another study performed by Derakhshan *et al.*,[65], the main constituents of *C. cyminum* essential oil were found to be cumin aldehyde, r-mentha-1,3-dien-7-al, r-mentha-1,4-dien-7-al, terpinene, *p*-cymene and *b*-pinene. In fact, the composition of the essential oil of *C. cyminum* depends on many factors, such as plant part, harvest-time, extraction- method, type of cultivar, geographic origin and storage conditions. In general, cumin aldehyde, menthane derivatives, cterpinene, p-cymene and b-pinene are major components of many essential cumin oils and are mainly responsible for the aroma and biological effects. Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Adiantum capillus-veneris*, shown in Table 1. The GC-MS chromatogram of the 20 peaks of the compounds detected was shown in Figure 1-20. Chromatogram GC-MS analysis of the methanol extract of *Adiantum capillus-veneris* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be Benzene, 1,1'-oxybis[4-phenoxy-, Stearyltrimethylammonium chloride,

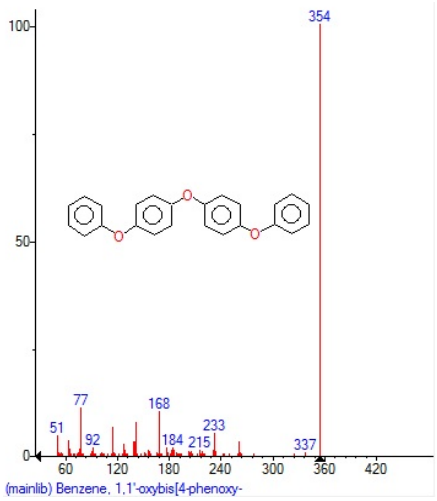


Figure 1: Mass spectrum of Benzene , 1,1'-oxybis[4-phenoxy- with Retention Time (RT)= 3.150

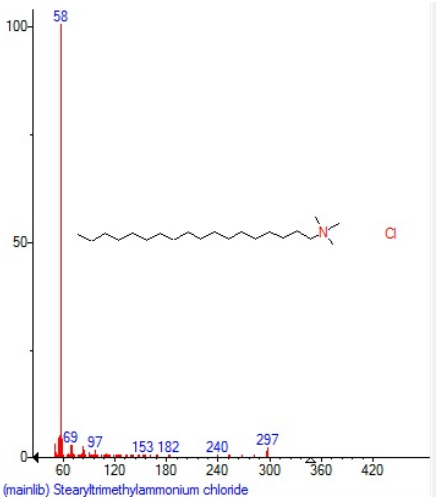


Figure 2: Mass spectrum of Stearyltrimethylammonium chloride with Retention Time (RT)= 3.613

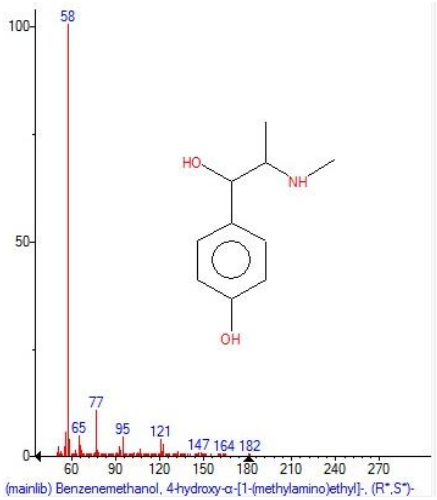


Figure 3: Mass spectrum of Benzenemethanol, 4-hydroxy-α-[1-methylamino)ethyl]-,(R*,S*)- with Retention Time (RT)= 3.722

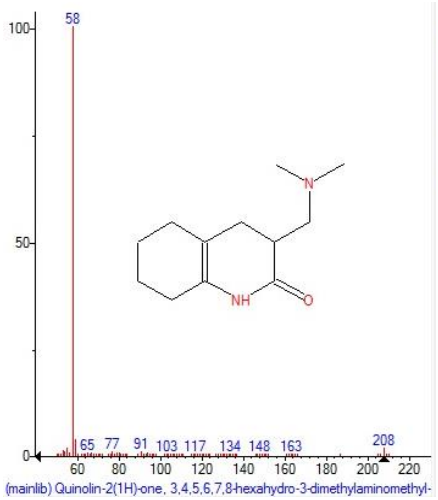


Figure 4: Mass spectrum of Quinolin-2(1H)-one, 3,4,5,6,7,8-hexahydro-3-dimethylaminomethyl- with Retention Time (RT)= 4.466

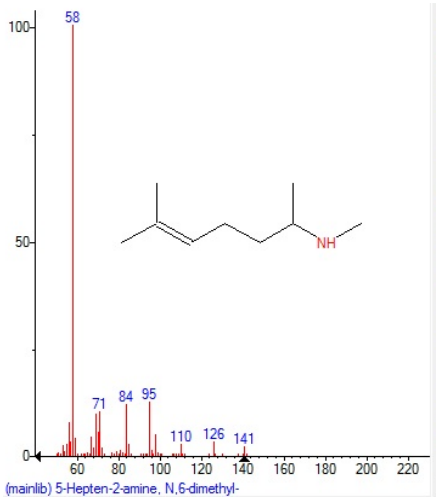


Figure 5: Mass spectrum of 5-Hepten-2-amine, N,6-dimethyl- with Retention Time (RT)= 4.792

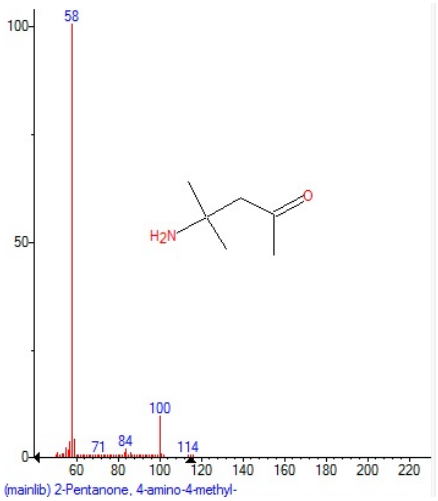


Figure 6: Mass spectrum of 2-Pentanone, 4-amino-4-methyl- with Retention Time (RT)= 4.958

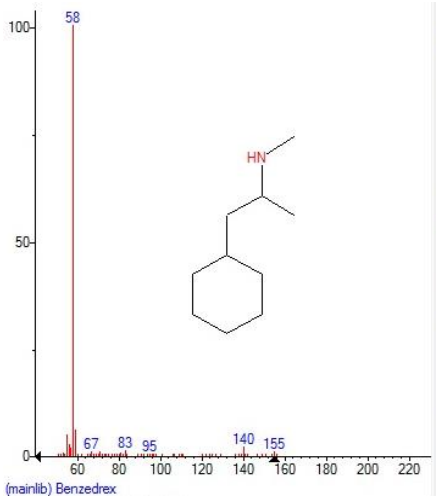


Figure 7: Mass spectrum of Benzedrex with Retention Time (RT)= 5.284

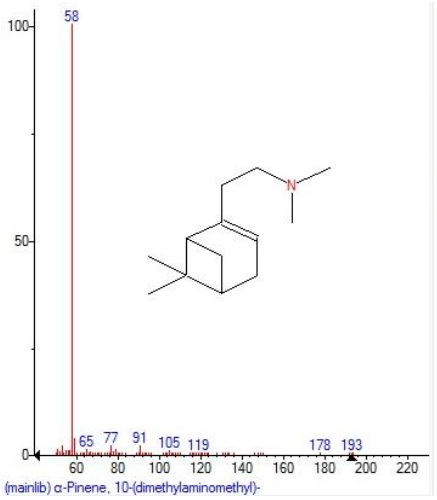


Figure 8: Mass spectrum of α-Pinene , 10-(dimethylaminomethyl)- with Retention Time (RT)= 5.725

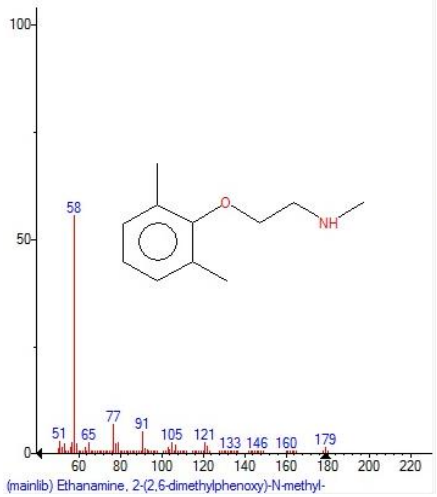


Figure 9: Mass spectrum of Ethanamine ,2-(2,6-dimethylphenoxy)-N-methyl- with Retention Time (RT)= 5.805

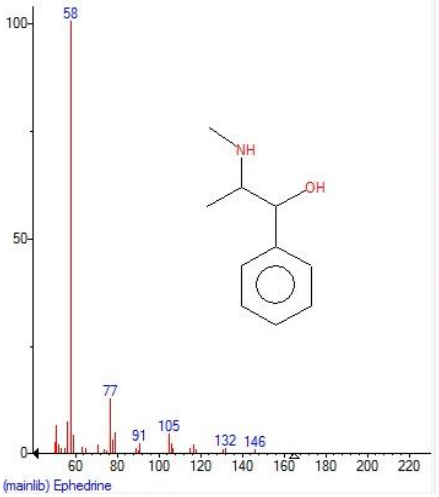


Figure 10: Mass spectrum of Ephedrine with Retention Time (RT)= 5.891

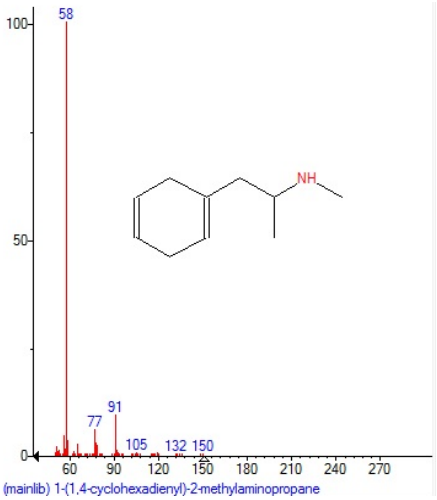


Figure 11: Mass spectrum of 1-(1,4-cyclohexadienyl)-2-methylaminopropane with Retention Time (RT)= 6.600

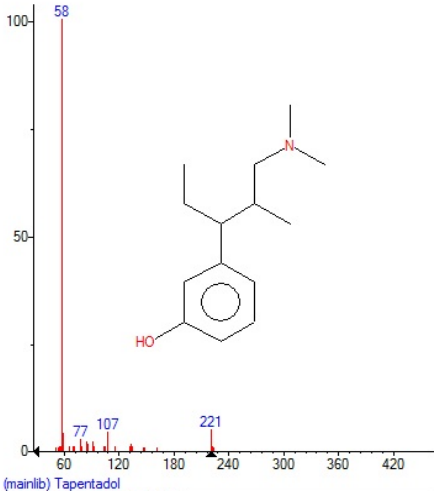


Figure 12: Mass spectrum of Tapentadol with Retention Time (RT)= 7.888

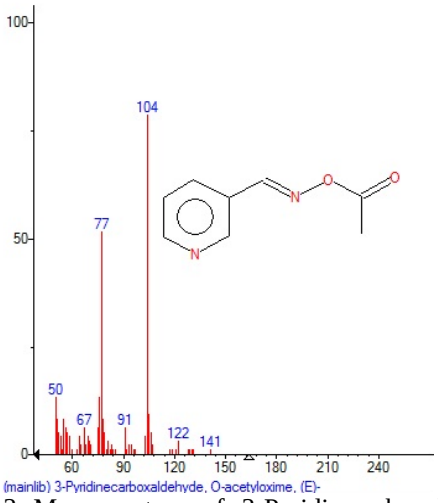


Figure 13: Mass spectrum of 3-Pyridinecarboxaldehyde , O-acetyloxime , (E)- with Retention Time (RT)= 8.769

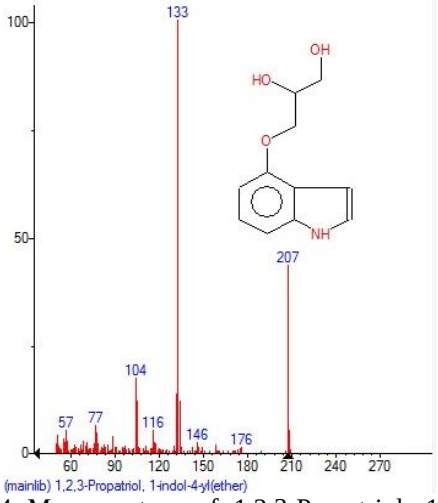


Figure 14: Mass spectrum of 1,2,3-Propatriol , 1-indol-4-yl(ether) with Retention Time (RT)= 9.043

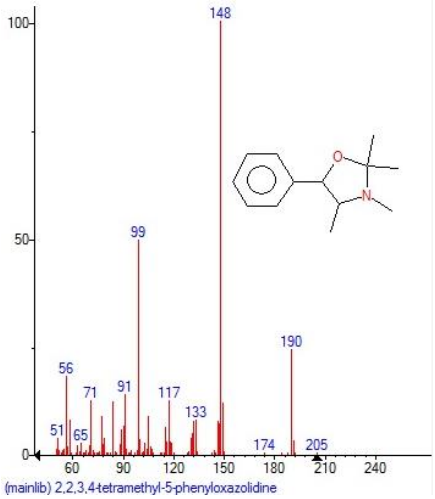


Figure 15: Mass spectrum of 2,2,3,4-tetramethyl-5-phenyloxazolidine with Retention Time (RT)= 9.478

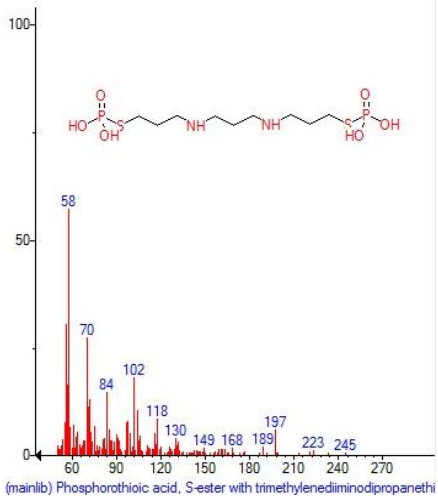


Figure 16: Mass spectrum of Phosphorothioic acid, S-ester with trimethylenediiminodipropanthi with Retention Time (RT)= 10.005

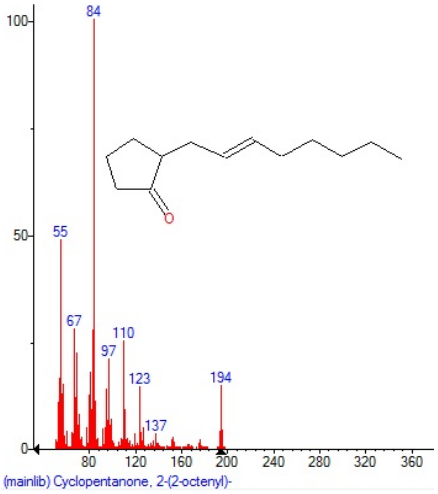


Figure 17: Mass spectrum of Cyclopentanone ,2-(2-octenyl)- with Retention Time (RT)= 14.502

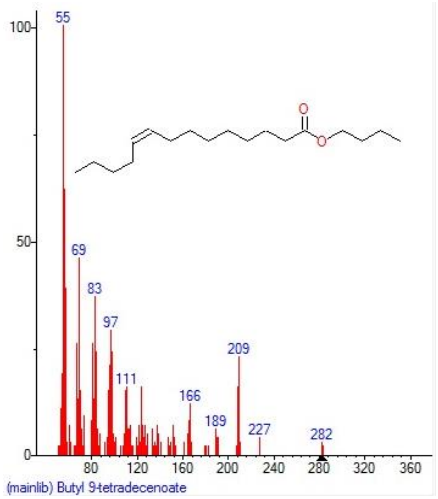


Figure 18: Mass spectrum of Butyl 9-tetradecenoate with Retention Time (RT)= 15.458

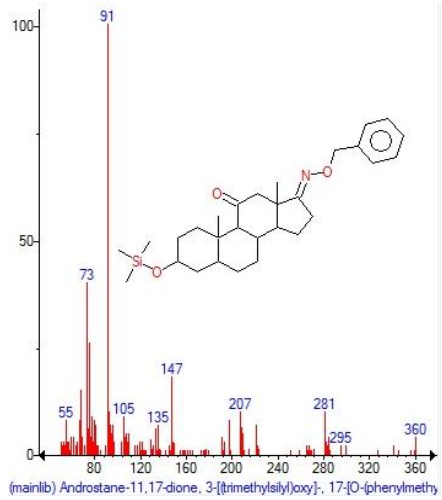


Figure 19: Mass spectrum of Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17-[O-(phenylmethyl) with Retention Time (RT)= 15.652

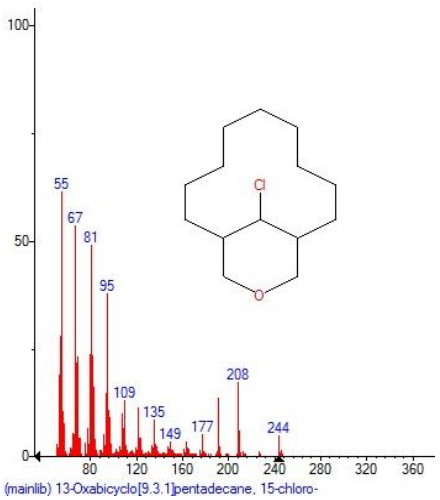


Figure 20: Mass spectrum of 13-Oxabicyclo[9.3.1]pentadecane , 15-chloro-with Retention Time (RT)= 16.144

Benzenemethanol, 4-hydroxy-α-[1-methylamino)ethyl]-,(R*,S*)-, Quinolin-2(1H)-one, 3,4,5,6,7,8-hexahydro-3-dimethylaminomethyl-, 5-Hepten-2-amine, N,6-dimethyl, 2-Pentanone, 4-amino-4-methyl, Benzedrex, α-Pinene, 10-(dimethylaminomethyl)-, Ethanamine, 2-(2,6-dimethylphenoxy)-N-methyl-, Ephedrine, 1-(1,4-cyclohexadienyl)-2-methylaminopropane, Tapentadol, 3-Pyridinecarboxaldehyde, O-acetyloxime, (E)-, 1,2,3-Propatriol, 1-indol-4-yl(ether), 2,2,3,4-tetramethyl-5-phenyloxazolidine, Phosphorothioic acid, S-ester with trimethylenediiminodipropanthi, Cyclopentanone, 2-(2-octenyl)-, Butyl 9-tetradecenoate, Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17-[O-(phenylmethyl) and 13-Oxabicyclo[9.3.1] pentadecane, 15-chloro.

CONCLUSION

Cumin is the second most popular spice in the world, after black pepper, and used as a medicinal plant for aromatherapy and various illnesses. For this reason, the standardization of the plant material from cultivation to storage is too important. To insure the achievement of quality, acceptance criteria for plant material and validating of the technical process in manufacturing are considered. Standardized seeds and essential oils are qualitatively optimized the products or preparations with reproducible content. Determination of the physicochemical characteristics of the oil may establish by measurement of extraction yield, refractive index, density, carbonyl and steric indexes together with aldehyde, alcohol and acid contents. Microscopic analyzing is very important in the products containing grinded seeds. In addition, thin layer chromatography may help to detect the pinenes and phellandrenes in the seeds as the main and characteristic monoterpenes. Cumin aldehyde is not only the active constituent of the Cumin seed and its oil but also sometimes added to the oil as a fraud which can difficulty detected by changing the refractive index.

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