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Research Article

Analysis of Bioactive Compounds of Methanolic Leaves extract of *Mentha pulegium* Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique

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ABSTRACT

The objective of this study was analysis of the secondary metabolite products. Bioactives are chemical compounds often referred to as secondary metabolites. Sixteenth bioactive compounds were identified in the methanolic extract of *Mentha pulegium*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Mentha pulegium* revealed the existence of the Erythritol , Cyclohexanone , 3-methyl-,(R)- , 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one , 1-Oxaspiro[2.5]octan-4-one ,2,2,6-trimethyl-, cis- , Terpinyl formate , Acetamide , N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]- , Pulegone , 2-Oxabicyclo[3.3.0]oct-7-en-3-one , 7-(1-hydroxypentyl)- , 2(3H)-Naphthalenone ,4,4a,5,6,7,8-hexahydro-1-methoxy- , 2-Cyclopenten-1-one , 2-(2-butenyl)-4-hydroxy-3-methyl-,(Z)- , (5β)Pregnane-3,20β-diol 14α,18α-[4-methyl-3-oxo-(1-oxa-4- , 2-(4-(But-2-yl)phenyl) propnoic acid , Nootkaton-11,12-epoxide , 2-Heptanone , 6-methyl-6-[3-methyl-3-(1-methylethenyl)-1-cyclo , Cholestan-3-ol , 2-methylene-, (3β,5α)- , 1-Heptatriacotanol and Digitoxin.

Keywords: Bioactive Compounds, Leaves, GC-MS, *Mentha pulegium.*

INTRODUCTION

The leaves of the plant were used to flavor pudding. Even though pennyroyal oil is extremely poisonous, people have relied on the fresh and dried herb for centuries. Early settlers in colonial Virginia used dried pennyroyal to eradicate pests[1-3]. Pennyroyal was such a popular herb that the Royal Society published an article on its use against rattlesnakes in the first volume of its Philosophical Transactions in 1665. It has been traditionally employed as an emmenagogue (menstrual flow stimulant) or as an abortifacient[4]. Pennyroyal is also used to settle an upset stomach and to relieve flatulence. The fresh or dried leaves of pennyroyal have also been used when treating colds, influenza, abdominal cramps, and to induce sweating, as well as in the treatment of diseases such as smallpox and tuberculosis, and in promoting latent menstruation[5]. However, when treating infestations such as fleas, using the plant's essential oil should be avoided due to its toxicity to both humans and animals, even at extremely low levels. The metabolite menthofuran is thought to be the major toxic agent. The distribution of phenolic compounds in the methanolic extract showed a variation among studied plants. *Mentha pulegium* can be considered as a source of gallocatechin. In an in vitro study, the most suitable solvent for extraction of antioxidants was investigated and correlation existed between plant growth stage and its antioxidant capacity was examined. Water extract was more potent than the methanol extract. Essential oil did not show considerable antioxidative effect. It seems that water extract of *M. pulegium* is a potent antioxidant which makes it as a potential antioxidant for oil and oily products during storage[6]. The compounds were also tested for kinase inhibitory activity in an assay involving 24 different kinases. Compounds 1, 2, 3, and the mixture of 4 and 5 were the most potent inhibitors, displaying EC (50) values between 0.64 and 1.4 microg/mL toward individual kinases.

MATERIALS AND METHODS

Gas chromatography – Mass Spectrum analysis

Interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA). The database consists of more than 62,000 patterns of known compounds. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library. *Mentha pulegium* GC–MS analysis were carried out in a GC system (Agilent 7890A series, USA). The flow rate of the carrier gas, helium (He) was set to beat 1 mL min−1, split ratio was 1:50. The injector temperature was adjusted at 250◦C, while the detector temperature was fixed to 280

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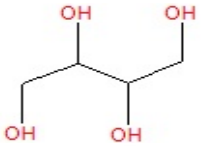
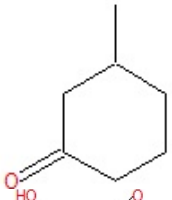
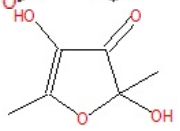
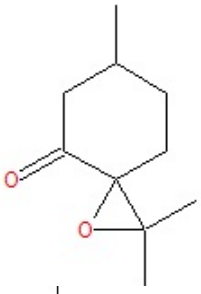
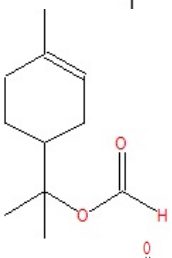
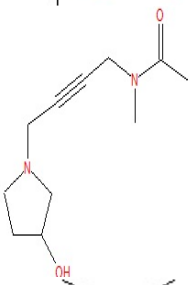
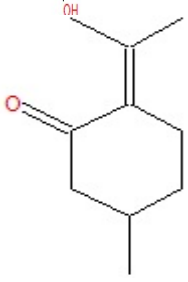
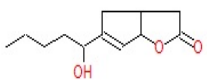
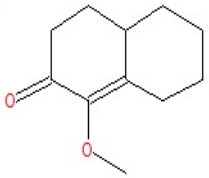
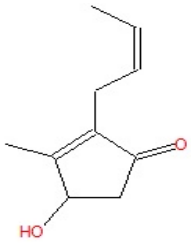
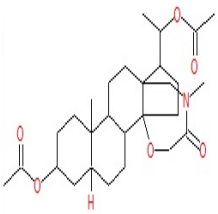
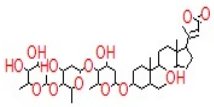
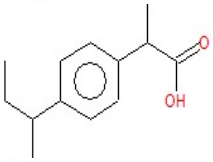
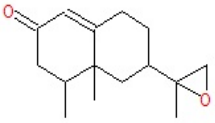
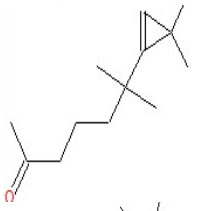
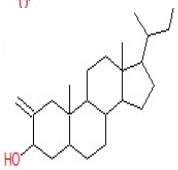
C°[7-14]. The column temperature was kept at 40 C° for 1 min followed by linear programming to raise the temperature from 40◦to 120 C° (at 4 C° min−1with 2 min hold time), 120 C° to 170 C° (at 6 C° min−1with 1 min hold time) and 170 C° to 200 C° (at10◦C min−1with 1 min hold time). The transfer line was heated at 280 C°. Two microliter of FAME sample was injected for

Table 1: Major phytochemical compounds identified in methanolic extract of *Mentha pulegium*.

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1.	Erythritol	3.539	122	122.057909		61,74,91,101	Anti-cariogenic effects
2.	Cyclohexanone, 3-methyl-,(R)-	4.311	112	112.088815		56,69,84,97,112	anti-oxidant
3.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	4.437	144	144.042258		55,73,84,101,144	antioxidant activity
4.	1-Oxaspiro[2.5]octan-4-one ,2,2,6-trimethyl-, cis-	5.885	168	168.115029		55,81,108,125,150	antibacterial activity
5.	Terpinyl formate	5.942	182	182.13068		59,93,121,136,152,182	anticancer
6.	Acetamide , N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	6.200	210	210.136827		56,68,124,192	anti-inflammatory activities
7.	Pulegone	6.383	152	152.120115		55,67,81,95,109,137,152	anti-inflammatory and antioxidant activities

8.	2-Oxabicyclo[3.3.0] oct-7-en-3-one , 7-(1-hydroxypentyl)-	6.503	210	210.125594		57,85,97,126, 153,210	Unknown
9.	2(3H)-Naphthalenone ,4,4a,5,6,7,8-hexahydro-1-methoxy-	7.579	180	180.115029		53,67,79,123, 180	anti-inflammatory
10.	2-Cyclopenten-1-one , 2-(2-butenyl)-4-hydroxy-3-methyl-,(Z)-	7.956	166	166.09938		55,79,109,137 ,151,166	Unknown
11.	(5β)Pregnane-3,20β-diol 14α,18α-[4-methyl-3-oxo-(1-oxa-4-	8.374	489	489.309038		57,73,133,161 ,267,328,360, 399,459,489	anti-bacterial *activity*
12.	Digitoxin	15.046	764	764.434692		55,69,113,131 ,203,221,246, 339,401	anti-inflammatory effects
13.	2-(4-(But-2-yl)phenyl)propnoic acid	9.719	206	206.13068		65,77,91,107, 161,191,206	anti-inflammatory activity
14.	Nootkaton-11,12-epoxide	10.720	234	234.16198		55,67,79,91,105,119,134,161,176,216,234	anti-inflammatory activity
15.	2-Heptanone , 6-methyl-6-[3-methyl-3-(1-methylethenyl)-1-cyclo	11.138	220	220.182715		55,69,91,119, 135,205,220	Unknown
16.	Cholestan-3-ol , 2-methylene-, (3β,5α)-	12.459	400	400.370516		69,81,95,149, 227,315,400	antioxidant activity

analysis. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z[15-21].

Statistical analysis

Results of the study were based on analysis of variance (ANOVA) using Statistica Software. A significance level of 0.05 was used for all statistical tests[22-28].

RESULTS AND DISCUSSION

Identification of biochemical compounds

Analysis of compounds was carried out in methanolic extract of *Mentha pulegium*, shown in Table 1. The GC-MS chromatogram of the peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Mentha pulegium* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined

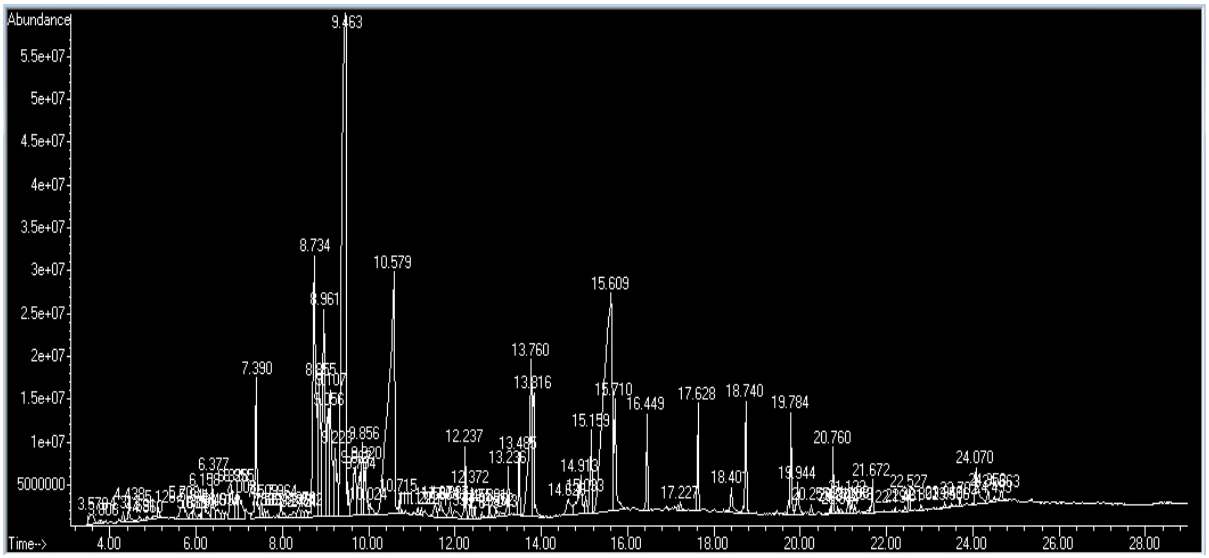


Figure 1: GC-MS chromatogram of methanolic extract of *Mentha pulegium*.

as follows. All peaks were determined to be Erythritol , Cyclohexanone , 3-methyl-,(R)- , 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one , 1-Oxaspiro[2.5]octan-4-one ,2,2,6-trimethyl-, cis- , Terpinyl formate , Acetamide , N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]- , Pulegone , 2-Oxabicyclo[3.3.0]oct-7-en-3-one , 7-(1-hydroxypentyl)- , 2(3H)-Naphthalenone ,4,4a,5,6,7,8-hexahydro-1-methoxy- , 2-Cyclopenten-1-one , 2-(2-butenyl)-4-hydroxy-3-methyl-,(Z)- , (5β)Pregnane-3,20β-diol 14α,18α-[4-methyl-3-oxo-(1-oxa-4- , 2-(4-(But-2-yl)phenyl) propnoic acid , Nootkaton-11,12-epoxide , 2-Heptanone , 6-methyl-6-[3-methyl-3-(1-methylethenyl)-1-cyclo , Cholestan-3-ol , 2-methylene-, (3β,5α)- , 1-Heptatriacotanol and Digitoxin Figure 2-19. Hepatic and neurologic injury developed in two infants after ingestion of mint tea. Examination of the mint plants, from which the teas were brewed, indicated that they contained the toxic agent pennyroyal oil. It is a possible cause of hepatic and neurologic injury in infants, particularly if the infants may have been given home-brewed mint teas. Benefits and phytochemicals of this plant was evaluated. Results showed consistent evidence that *Pterospartum tridentatum* and *Mentha pulegium* are an important reservoir of phytochemicals with antiradical activity and antibacterial capacity and thus they might be used in a preventive way or in a combined pharmaceutical and antibiotic therapy against pathogenic bacteria. Anti-oxidant capacity, anti-oxidant activity and anti-genotoxic effects of methanolic extract of *Mentha pulegium* were investigated. A significant decrease in the level of MDA was observed when compared with CCl_4 alone treated group. In addition, anti-genotoxic effect of ME was studied by using sister chromatid exchange (SCE) method. As a result, ME has shown anti-genotoxic effect depend on anti-oxidative effect on human lymphocyte culture. The inhibitory effect of *Mentha pulegium* extract on steel corrosion in 1 M HCl solution was investigated. The remarkable inhibition efficiency of MPE was discussed in terms of blocking of electrode surface by adsorption of inhibitor molecules through active centers. The adsorption of MPE was found to accord with the Temkinisotherm. The relaxant activity of the essential oil of *Mentha pulegium* L. (EOMP) and pulegone in rat isolated tracheal and bladder smooth muscles was evaluated. The findings suggests that EOMP induced relaxant responses in precontracted smooth muscles of rat trachea and bladder, which are likely to be mediated via inhibition of calcium entry, mainly by its major compound, pulegone [29-32]. These effects are coherent with the popular use of EOMP as an antispasmodic agent. Organic extracts from aerial parts were evaluated to determine their spasmolytic action on rat isolated ileum test. Findings indicate that dichloromethanic extract of *M. pulegium* induced its spasmolytic effect through Ca2+-influx blockade, which may explain its traditional use against diarrhea. Anti-oxidant capacity, anti-oxidant activity and anti-genotoxic effects of methanolic extract of *Mentha pulegium* were investigated. A significant decrease in the level of MDA was observed when compared with CCl_4 alone treated group. In addition, anti-genotoxic effect of ME was studied by using sister chromatid exchange (SCE) method. As a result, ME has shown anti-genotoxic effect depend on anti-oxidative effect on human lymphocyte culture. Two new terpenoidal compounds 1α, 6βdimethyl-5β-hydroxy-4β-(prop-1-en-2-yl)-decahydronaphthalen-2-one (1) and 1-(O-β-D-glucopyranosyl)-2,7-dimethyloct-5-en-3-one (2) were isolated from the chloroformic extract of *Mentha pulegium* L. Compound 1 displayed moderate anti-microbial effect. The antibacterial activity of *Mentha pulegium* essential oil on isolates of *Klebsiella* was investigated. Thirty nine isolates were collected from urine specimens submitted to two educational hospitals in Urmia, Iran. The results suggest the potential use of the *Mentha pulegium* essential oil for the control of multi-drug resistant *Klebsiella* sp. infections. However, more adequate toxicological study must be carried out to verify the possibility of using it for fighting microorganisms in human. The effects of the essential oil of *Mentha pulegium* L. were assessed on the isolated rat

myometrium. Studies show that the essential oil of the abortifaceant plant *Mentha pulegium* exerts an inhibitory effect on the contractile activity of the isolated rat

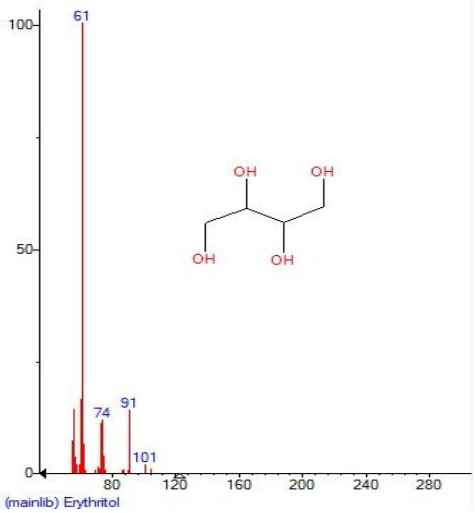


Figure 2: Mass spectrum of Erythritol with Retention Time (RT)= 3.539

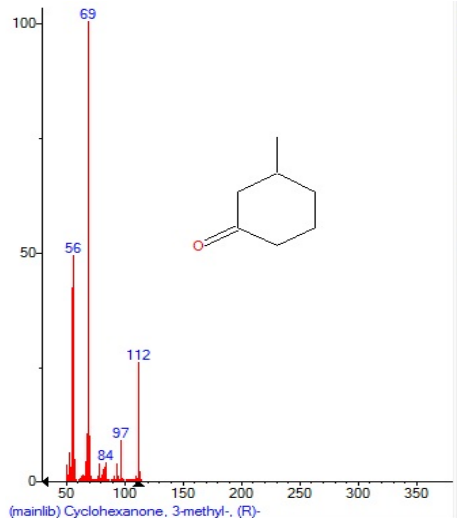


Figure 3: Mass spectrum of Cyclohexanone , 3-methyl-,(R)- with Retention Time (RT)= 4.311

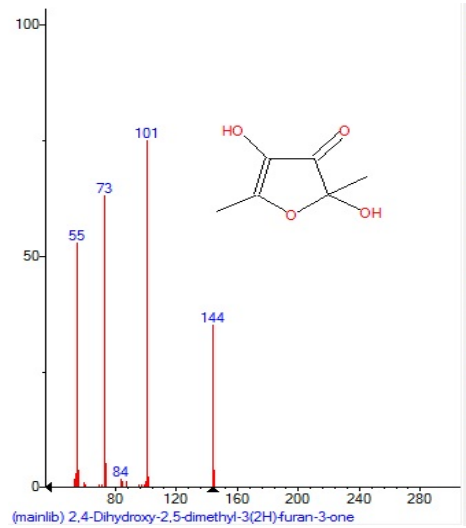


Figure 4: Mass spectrum of 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one with Retention Time (RT)= 4.437

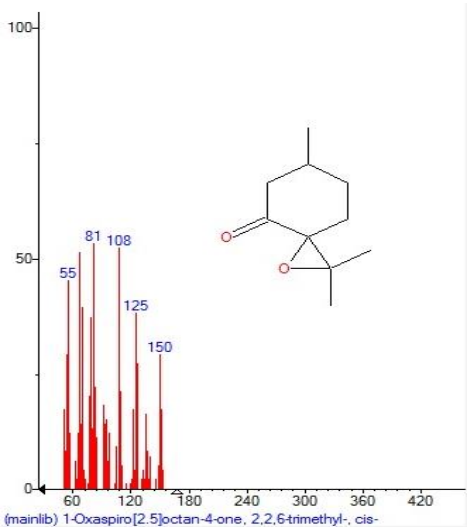


Figure 5: Mass spectrum of 1-Oxaspiro[2.5]octan-4-one ,2,2,6-trimethyl-, cis- with Retention Time (RT)= 5.885

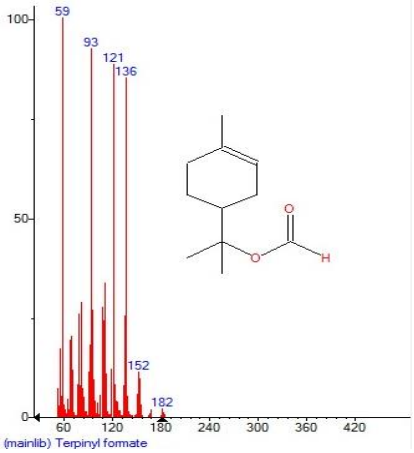


Figure 6: Mass spectrum of Terpinyl formate with Retention Time (RT)= 5.942

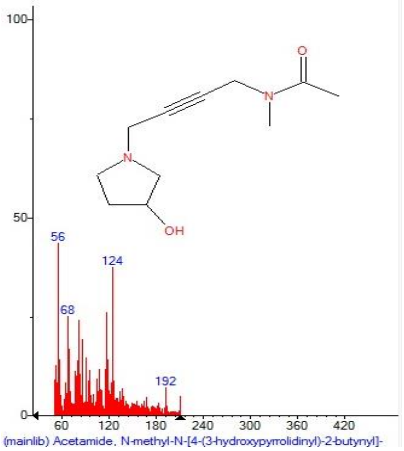


Figure 7: Mass spectrum of Acetamide , N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]- with Retention Time (RT)= 6.200

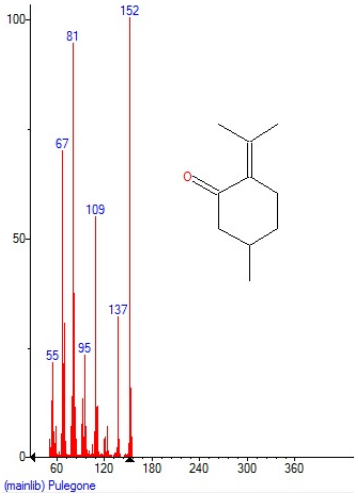


Figure 8: Mass spectrum of Pulegone with Retention Time (RT)= 6.383

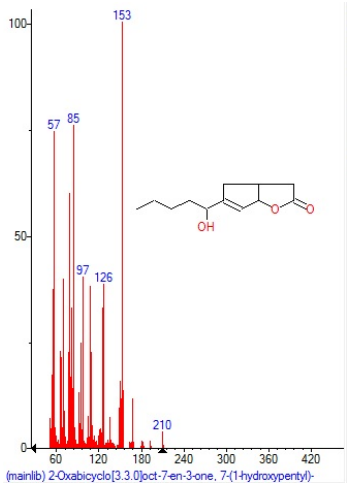


Figure 9: Mass spectrum of 2-Oxabicyclo[3.3.0]oct-7-en-3-one , 7-(1-hydroxypentyl)- with Retention Time (RT)= 6.503

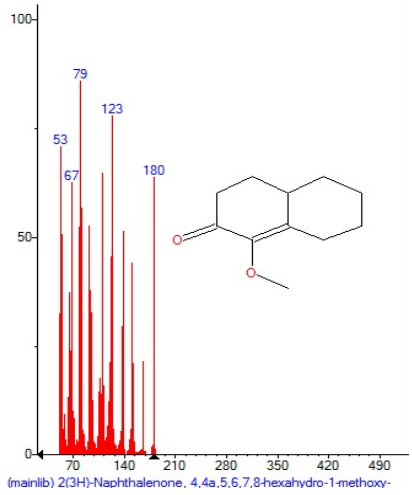


Figure 10: Mass spectrum of 2(3H)-Naphthalenone ,4,4a,5,6,7,8-hexahydro-1-methoxy- with Retention Time (RT)= 7.579

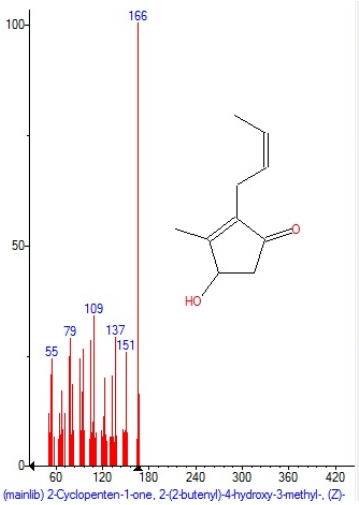


Figure 11: Mass spectrum of 2-Cyclopenten-1-one , 2-(2-butenyl)-4-hydroxy-3-methyl-,(Z)- with Retention Time (RT)= 7.956

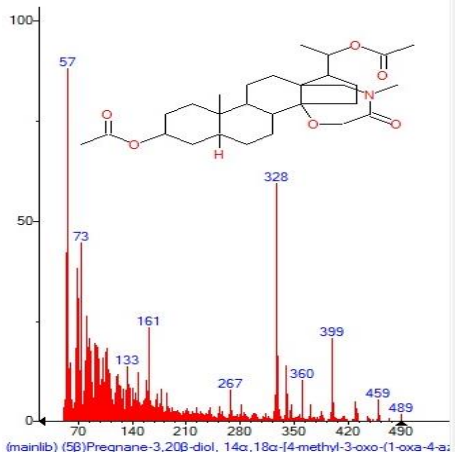


Figure 12: Mass spectrum of (5β)Pregnane-3,20β-diol 14α,18α-[4-methyl-3-oxo-(1-oxa-4- with Retention Time (RT)= 8.374

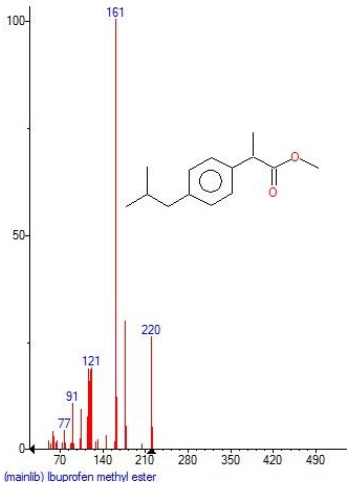


Figure 13: Mass spectrum of Ibuprofen methyl ester with Retention Time (RT)= 8.717

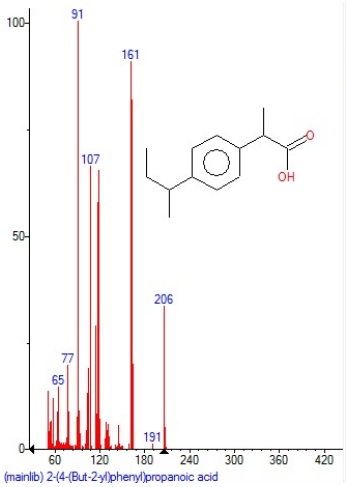


Figure 14: Mass spectrum of 2-(4-(But-2-yl)phenyl)propnoic acid with Retention Time (RT)= 9.719

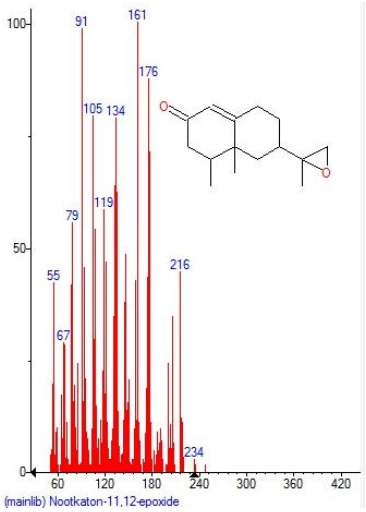


Figure 15: Mass spectrum of Nootkaton-11,12-epoxide with Retention Time (RT)= 10.720

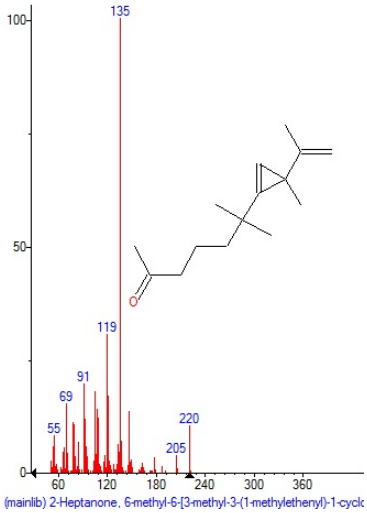


Figure 16: Mass spectrum of 2-Heptanone , 6-methyl-6-[3-methyl-3-(1-methylethenyl)-1-cyclo with Retention Time (RT)= 11.138

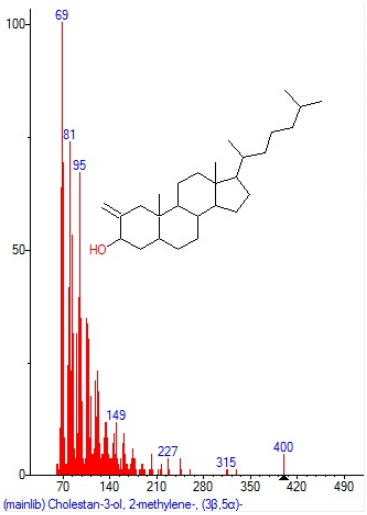


Figure 17: Mass spectrum of Cholestan-3-ol , 2-methylene-, (3β,5α)- with Retention Time (RT)= 12.459

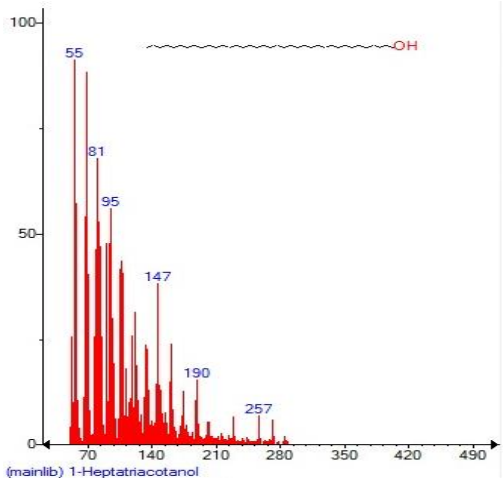


Figure 18: Mass spectrum of 1-Heptatriacotanol with Retention Time (RT)= 12.620

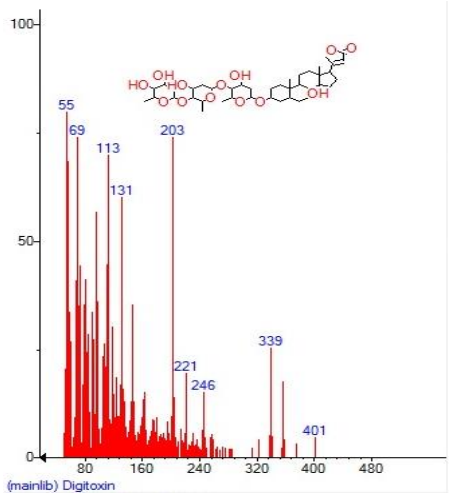


Figure 19: Mass spectrum of Digitoxin with Retention Time (RT)= 15.046

myometrium. This oil shares a common effect with the voltage-dependent calcium channel (VDCC) blocker nifedipine, although ostensibly acting via a different mechanism[33-36].

CONCLUSION

Further research is required to evaluate the practical values of therapeutic applications. *Mentha pulegium* can be considered as a source of gallocatechin.

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