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Research Article

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The chemical composition and antimicrobial activity of the essential oil of *Lavandula coronopifolia* growing in Saudi Arabia

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ABSTRACT

The main goal of the present study was to investigate the chemical composition and the antimicrobial activity of Lavandula coronopifolia essential oil, collected in the southern region of Saudi Arabia. The essential oil of the air-dried aerial parts of L. coronopifolia (Lamiaceae) was prepared by hydrodistillation method using a Clevenger-type apparatus and analyzed by gas chromatography/mass spectrometry (GC/MS). The result of GC/MS analysis revealed the presence of forty six volatile components, 7 of which are aromatic and 39 are non-aromatic, representing 88.63% of the total oil. The main components were identified as phenol-2-amino-4,6-bis (1,1-dimethylethyl) (51.18%), carvacrol (4.35%), n-hexadecanoic acid (3.60%), trans-2-caren-4-ol (3.57%), 17-pentartiacontene (2.59%), caryophyllene oxide (2.16%), 1-hexacosanol (2.03%), 2,5-cyclohexadiene-1,4-dione, 2, 6-bis (1,1-dimethylethyl) (1.49%) and 5-amino-1-pentanol (1.23%) representing 76.45% from the total oil. The antimicrobial activity of the essential oil was assessed against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Mycobacterium smegmatis and Candida albicans at a dose of 180µg/ml and showed no activity against all tested organisms.

Keywords: *Lavandula coronopifolia*, Lamiaceae, essential oil, antimicrobial activity

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INTRODUCTION

Lavandula (common name Lavender) is a genus of 39 species of flowering plants in the mint family, *Lamiaceae* [1]. In Saudi Arabia it is represented by three species including *L. coronopifolia Poir*, *L. dentate L.* and *L. pubescens Decne* [2]. Genus *Lavandula* is native to the Mediterranean region and includes annuals herbaceous plants and small shrubs having aromatic foliage and flowers [3]. *L. coronopifolia* is a densely puberulent to glabrescent, woody-based perennial herb with a pleasant or acrid aroma. Stems are many, oppositely branched, erect and stiff. Leaves are 2-3 pinnatisect with oblong-linear and acute lobes. Spikes are often interrupted below, dense and solitary or in panicles, corolla is pale blue. Fruit is an ovoid, reticulate nutlet [4]. By reviewing the current literature, it was found that the essential oils obtained from different species of *Lavandula* (*L. latifolia*, *L. angestifolia* , *L. stoechas*) were commonly used in aromatherapy and massage to achieve many clinical manifestation traditionally ascribed to their antibacterial, antifungal, carminative, sedative and antidepressant actions [5, 6]. Although the essential oils of other *Lavandula* species represent great importance in the fragrance industry and have been intensively studied [7-19], volatile oil components of *L. coronipifolia* have received little attention. Regarding the folk uses of some plants belonging to genus *Lavandula* and growing in Saudi Arabia, *L. dentata* flower infusion is said to be useful in urine retention and removal of kidney and ureter, stone, while the decoction of the leaf of *L. pubscens* is given in some ill cases such as headache and cold [2]. Previous studies on the essential oil of *L. coronopifolia* collected in Tunisia showed that it was characterised by high percentage of *trans-β-* ocimene (26.9%), carvacrol (18.5%), bisabolene

(13.1%), and myrcene (7.5%) [20]. While the Jordanian plant essential oil showed the presence of linalool (41.2%) and 1,8-cineole (25.4%) [21]. The methanolic extract of *L. coronopifolia* growing in Egypt showed strong antioxidant activity [22] also Moroccan *L. coronopifolia* stem, leaves and flower extracts were active against *Clavibacter michiganensis* subsp. *Michiganensis* which is the causal agent of tomatoes` bacterial canker [23]. To the best of our knowledge no phytochmical or biological studies on the essential oil of *L. coronopifolia* growing in Saudi Arabia have been reported so far. In the present study we discuss the result obtained from GC/MS analysis for the titled plant essential oil, its antimicrobial activity and comparing these results with the previously published data.

EXPERIMENTAL SECTION

Plant material:

L. coronopifolia Figure 1 was collected in March 2009 from Shaza Mountains, south Saudi Arabia. Plant identity was proved by Professor Jakob Thomas, College of Science, King Saud University. A voucher specimen (#15799) was deposited at the herbarium of department of pharmacognosy, College of Pharmacy, King Saud University. The aerial parts of *L. coronopifolia* were air-dried and ground into coarse particles till use.

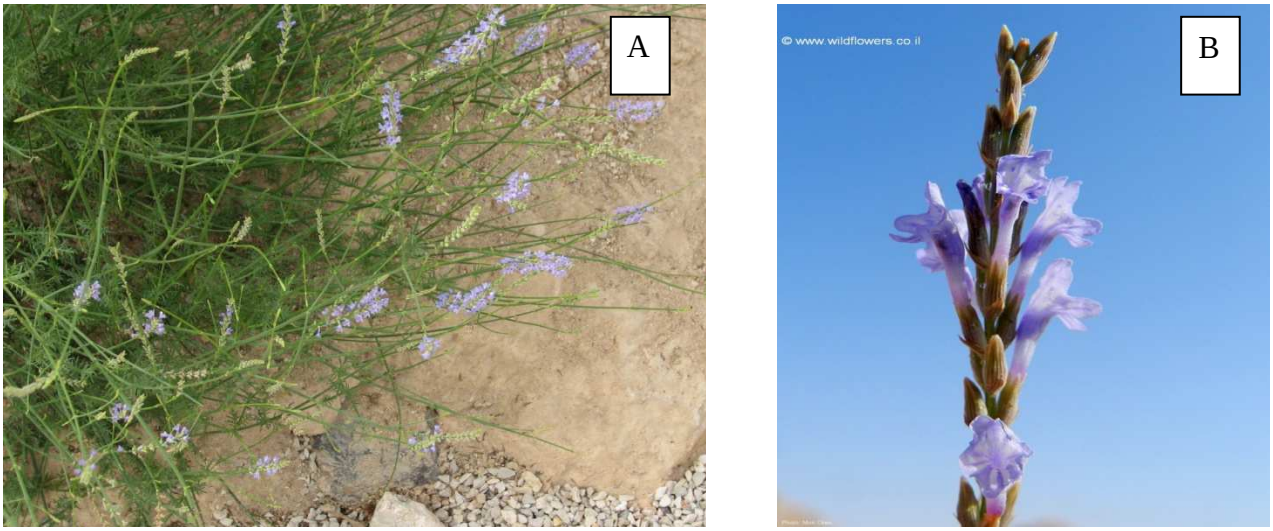


Figure 1: *Lavandula coronopifolia* A Aerial parts B Flowers

Isolation of the essential oil:

The air - dried aerial parts (350 g) were subjected to Hydro distillation for 6 h using Clevenger type apparatus and adopting method prescribed in the British Pharmacopoeia [24]. Distillation was continued until the volume of the essential oil in the graduated stem of the Clevenger apparatus remained constant for at least one hour. The obtained oil was dried over anhydrous sodium sulphate and kept in air- tight, amber coloured glass vial in the refrigerator at 4^{o}C until analysis by GC/MS. The results of GC/MS analysis are shown in Table 1and Fig. 2.

Gas chromatography-mass spectrometry (GC-MS):

The essential oil of *L. coronopifolia* was subjected to GC-MS analysis using Thermo Fisher Scientific U.S.A, TRACE DSQ Mass spectrometer, column type:- THERMO TR-5ms SQC, the injector temperature was 220, temp.. program:- 60 ^{0}C(1min.) then 240 ^{0}C(5 min.) at 6 ^{0}C/min. Instrument Method: C:\Xcalibur\methods\GC.meth, START TIME:- 2.50 min. end time:- 36.00 min., sample type :- liquid, original data path: C:\XCALIBUR\DATA, current data path: C:\Documents and Settings\DATA, Run Time(min): 36 min. ,sample volume (µl): 1µl, ion source temp :- 200 ^{0}C.

Identification of the compounds:

The individual compounds were identified by comparing their retention indices relative to C8-C26 *n* alkane series and by comparing their mass spectra fragmentation pattern and retention time with those reported before in the NIST library and literature [25, 26].

Antimicrobial activity:
Microorganisms:
American Type of Culture Collection (ATCC) standard against various microorganisms namely: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis* and *Candida albicans* were used.

Antimicrobial assay:
The antimicrobial activity of the essential oil was tested according to the National Committee of Clinical Laboratory Standards (National committee for clinical laboratory standards, 2002) against various microorganisms, Bacillus subtilis (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 15036), *Pseudomonas aeruginosa*, *Candida albicans* and *Mycobacterium smegmatis* (ATCC 10231). The positive antibacterial and antifungal activities were established by the presence of inhibition zones after 24 h for bacteria and 48 h for fungi [27].

RESULTS AND DISCUSSION

The chemical composition of the investigated oil is presented in Table 1, where the identified components are listed in order of their elution on the Trace TR-5 column with their retention time and percentages. Fragmentation pattern for some identified compounds are presented in Fig.3. A total of forty six volatile components were identified in the essential oil, phenol-2-amino-4,6-bis (1,1-dimethylethyl) (51.18%) was present as the major compound. Other significant constituents were carvacrol (4.35%), *n*-hexadecanoic acid (3.60%), *trans*-2-caren-4-ol (3.57%), 17-pentartiacontene (2.59%), caryophyllene oxide (2.16%) and 1-hexacosanol (2.03%). The essential oil contains other minor constituents as *cis*-*z*-å -bisabolene oxide (0.99%), cedrol (0.68%), geranyl isovalerate (0.55%), tridecan-1-ol (0.48%), *trans*-linalol oxide (0.34%), *p*-anisic acid ethyl ester (0.28%) and others. Our findings are in contrast with some previous observations of *L. coronopifolia* essential oils [20, 21]. As the essential oil of Tunisian *L. coronopifolia* was characterized by high percentage of *Trans*-*β*- ocimene (26.9%), carvacrol (18.5%), *β*-bisabolene (13.1%) and myrecene (7.5%) [20], while the essential oil of *L. coronopifolia* collected from Jordon is characterized by the presence of linalool in percentage of 41.2% and 40.7% for flower and of leaves respectively, while 1,8-cineole (25.4% , 7.7% and 7.3%) were identified in the aerial parts, of leaves and flower respectively [21]. By careful inspection of literature data and our results it was obvious that the chemical composition of the essential oils isolated from different species of genus *Lavandula* and collected from different localities is quite different in both quality and quantity of their constituents. Perhaps this difference in essential oils composition and ratios of the individual components may be partially attributed, at least, by differences in the ecological conditions, harvesting time of the investigated plant materials, the environmental conditions such as water, nutrient stress or time of year, temperature, and geographical source [28, 29]. Although the essential oil of many plants showed strong antimicrobial activity [30-33], unfortunately the antibacterial activity of the essential oil of *L. coronopifolia* against Gram positive bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, Gram negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against Mycobacterium *smegmatis* and *Candida albicans* was assessed at a dose of 180µg/ml and showed no activity against all tested microorganisms.

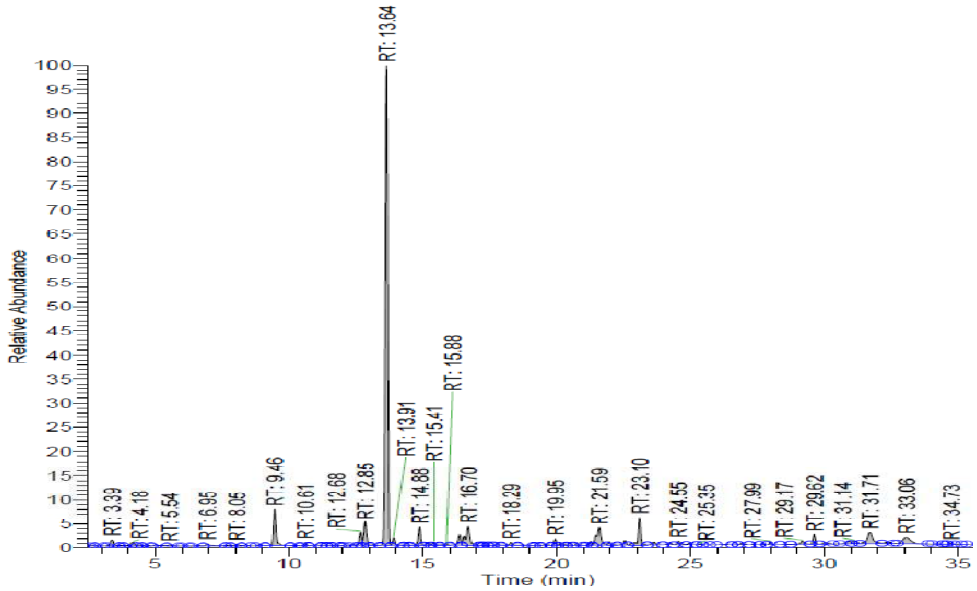
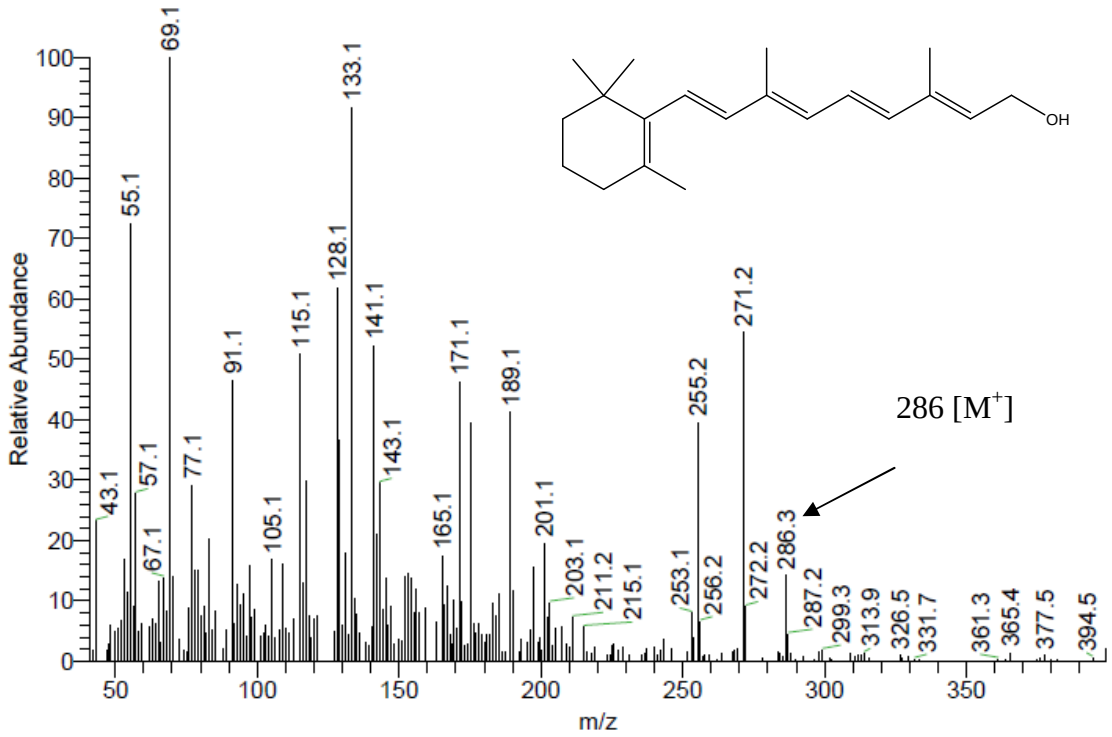


Figure 2: **GC/MS chromatogram of the essential oil of *Lavandula coronopifolia* growing in Saudi Arabia**

Table 1. Chemical composition of the essential oil of the air-dried aerial parts of *L. coronopifolia*

No.	Name of compound	Retention time	Area (%)	M^+
1	Pentanol	2.65	0.09	88
2	5-Amino-1-pentanol	3.39	1.23	103
3	Benzene acetaldehyde	4.18	0.74	120
4	*Trans* linalool oxide	5.02	0.34	170
5	Phenyl ethyl alcohol	5.54	0.22	122
6	1-Cyclohexene-1-carboxaldehyde, 5,5-dimethyl-3-oxo	6.10	0.04	152
7	*n*-Decanoic acid	8.93	0.30	172
8	Carvacrol	9.46	4.35	150
9	Ttidecandial	10.18	0.05	212
10	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	10.61	0.35	234
11	a-Diamascenone	11.05	0.27	190
12	2,5-Octadecadiynoic acid, methyl ester	11.33	0.09	290
13	*P*-Anisic acid methyl ester	12.41	0.28	180
14	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	12.68	1.49	220
15	5-Methoxy-2,2,6-trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]heptane	12.85	2.78	236
16	Geranyl isovalerate	13.20	0.55	238
17	Phenol, 2-amino-4,6-bis(1,1-dimethylethyl)-	13.64	51.18	221
18	cis-Z-à-Bisabolene epoxide	13.91	0.99	220
19	Caryophyllene oxide	14.88	2.16	220
20	Cedran-diol, 8S,13	15.20	0.46	238
21	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	15.41	0.58	342
22	Alloaromadendrene oxide-(1)	15.88	0.44	220
23	Verrucarol	16.38	1.45	250
24	Benzoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	17.83	0.13	264
25	Tetradecanoic acid	18.29	0.62	228
26	Hexadecanoic acid, ethyl ester	18.72	0.25	284
27	2-Dodecanone	19.55	0.34	184
28	*n*-Tridecan-1-ol	20.15	0.48	200
29	1-Heptatriacotanol	20.49	0.13	536
30	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	20.79	0.36	276
31	*n*-Hexadecanoic acid	21.59	3.60	256
32	3,5-Heptadienal, 2-ethylidene-6-methyl	22.56	0.67	150
33	Hexadecanoic acid, ethyl ester	21.96	0.60	284
34	*trans*-2-Caren-4-ol	23.10	3.57	152
35	Cedrol	23.65	0.68	222
36	Octadecanoic acid	24.55	0.70	284
37	Ethyl iso-allocholate	25.35	0.10	436
38	1-Hexadecanol, 2-methyl	26.34	0.27	256
39	Retinol	26.90	0.27	286
40	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	27.34	0.11	320
41	Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	28.64	0.13	446
42	1,2-Benzenedicarboxylic acid, diisooctyl ester	29.62	1.04	390
43	7, 8-Epoxylanostan-11-ol, 3-acetoxy-	30.03	0.38	502
44	17-Pentatriacontene	31.71	2.59	490
45	1-Hexacosanol	33.06	2.03	382
46	Hexadecan-1,1-bis (dodecyloxy)	34.73	0.12	594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55.1
128.1
141.1
115.1
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189.1
271.2
255.2
77.1
57.1
43.1
67.1
105.1
165.1
201.1
143.1
203.1
211.2
215.1
253.1
256.2
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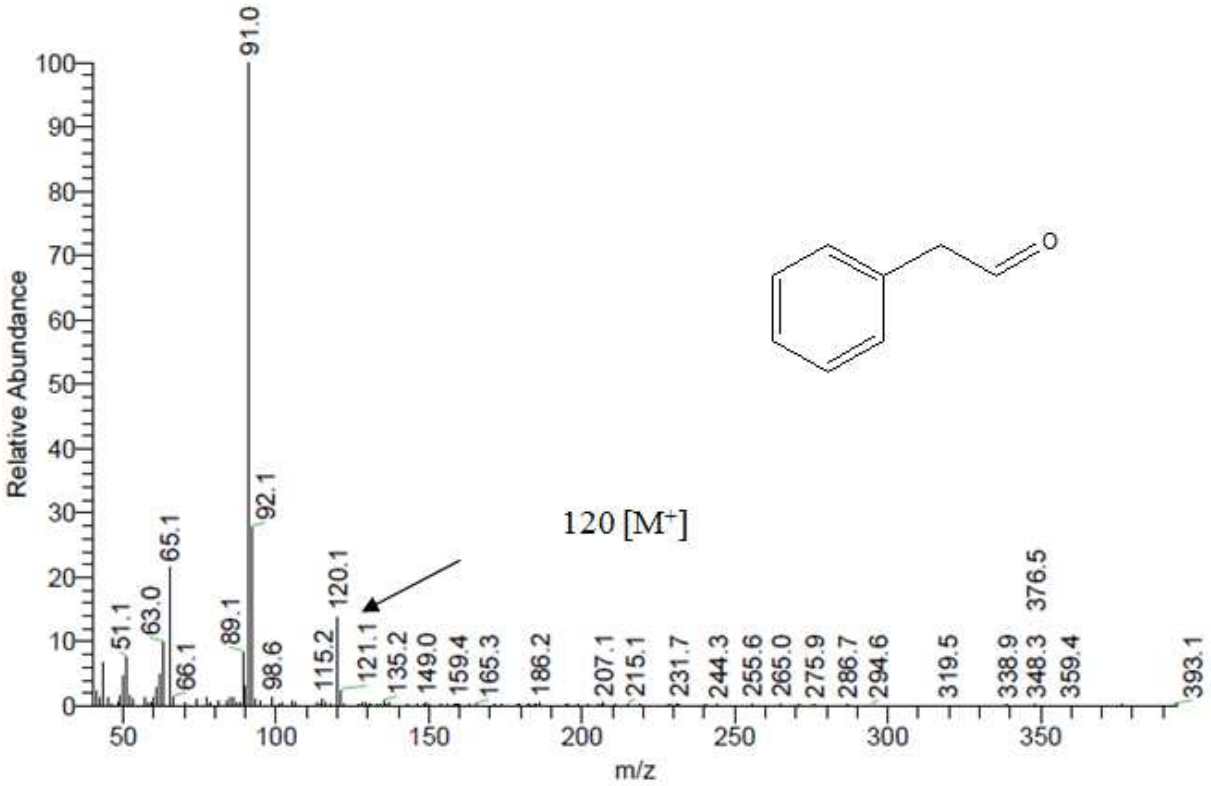
365.4
377.5
394.5


Relative Abundance
m/z
O
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65.1
120.1
51.1
63.0
89.1
66.1
98.6
115.2
121.1
135.2
149.0
159.4
165.3
186.2
207.1
215.1
231.7
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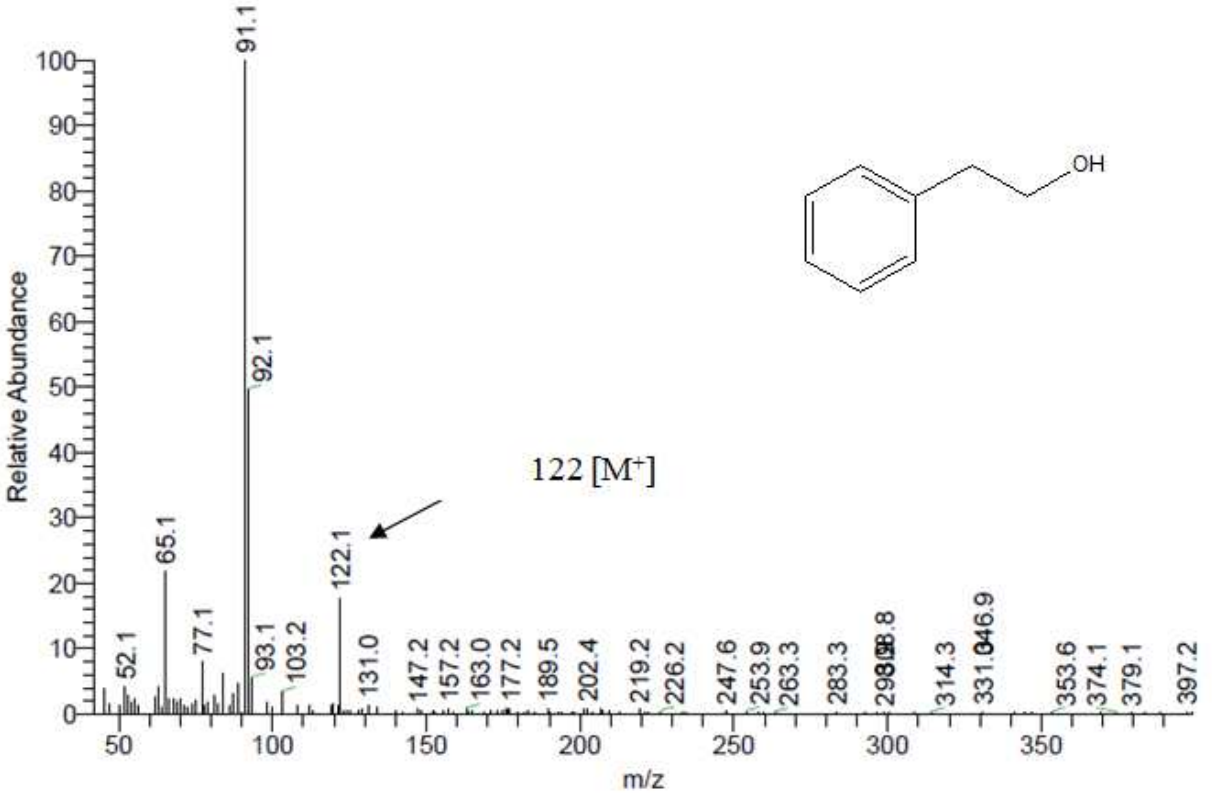
338.9
348.3
376.5
359.4
393.1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157.2
163.0
177.2
189.5
202.4
219.2
226.2
247.6
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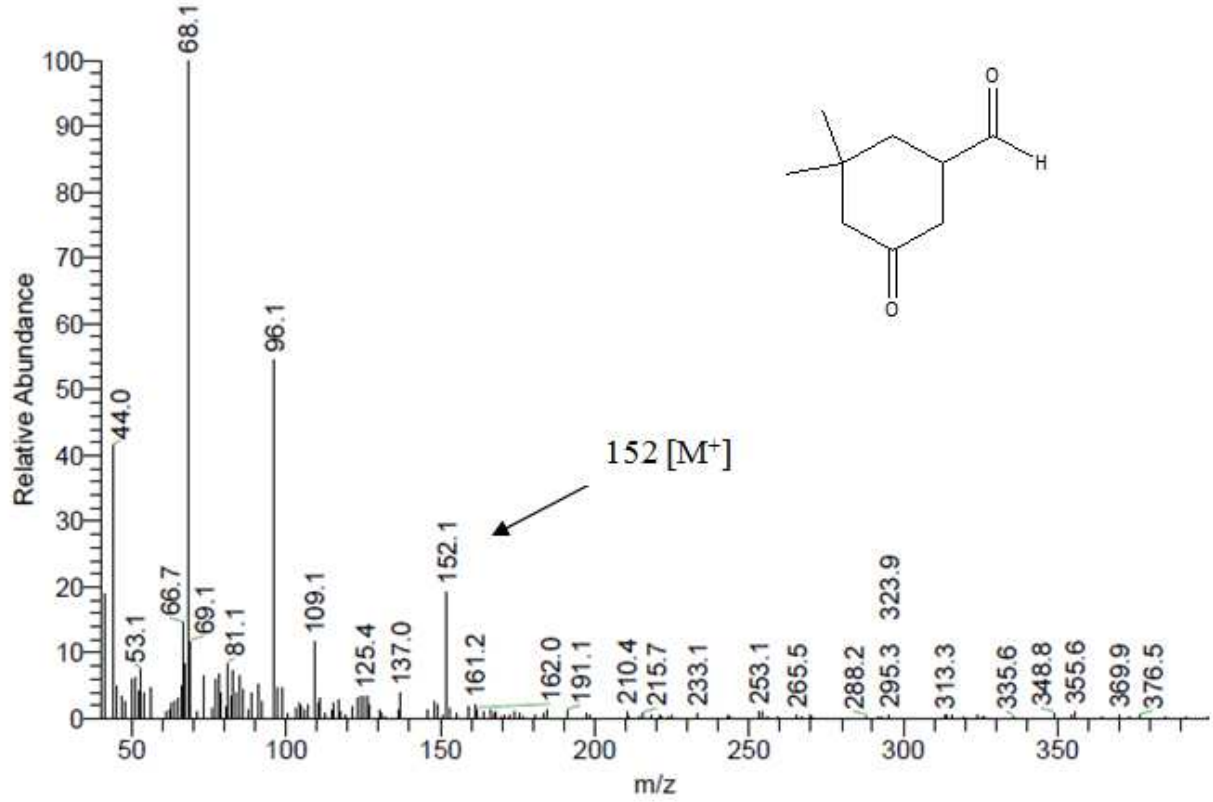
374.1
379.1
397.2
122 [M+]
OH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233.1
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376.5
152 [M+]
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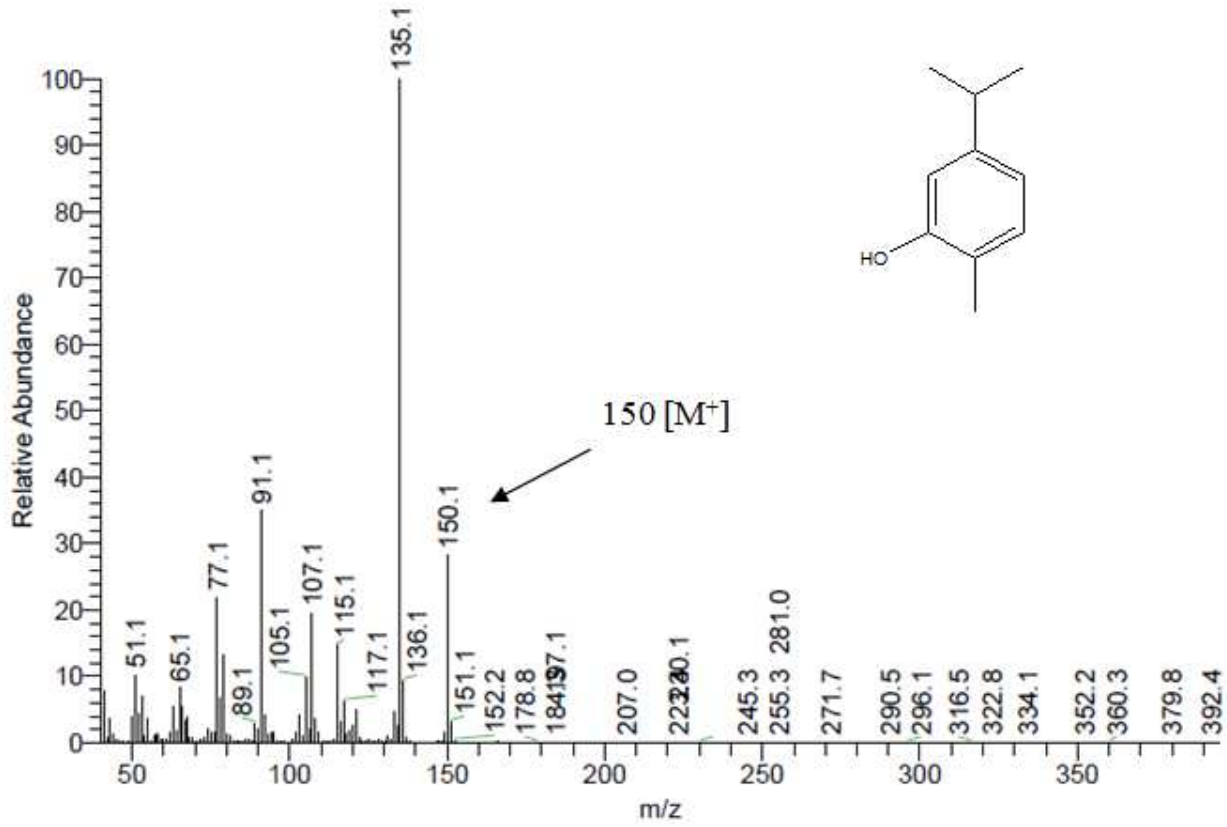
H
O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152.2
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207.0
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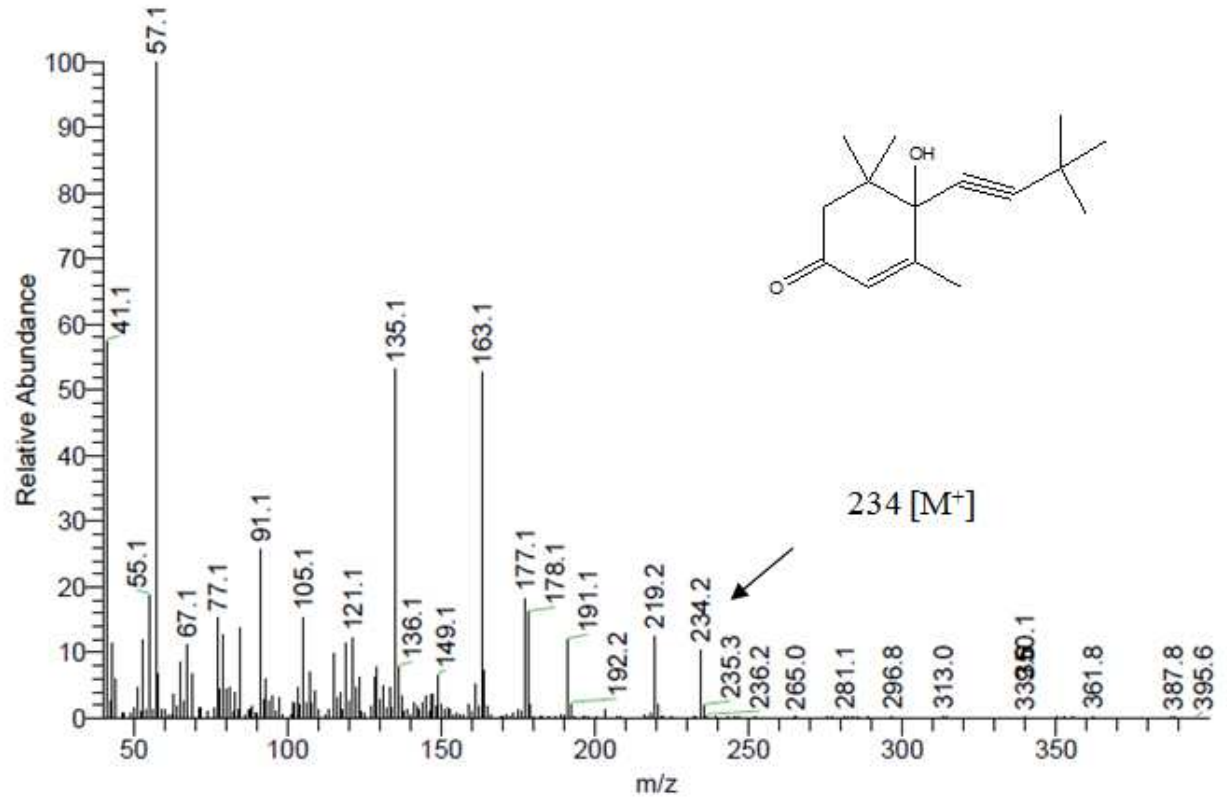
392.4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OH
O
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136.1
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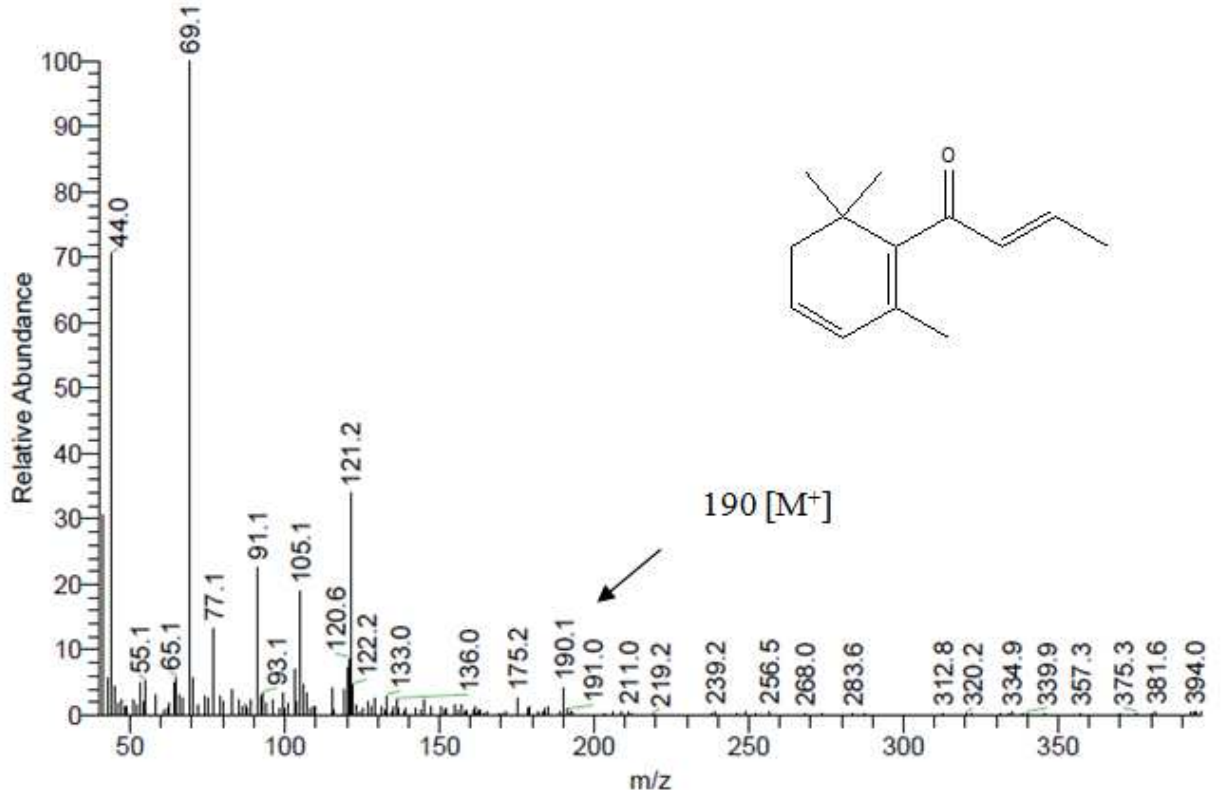
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136.0
175.2
190.1
191.0
211.0
219.2
239.2
256.5
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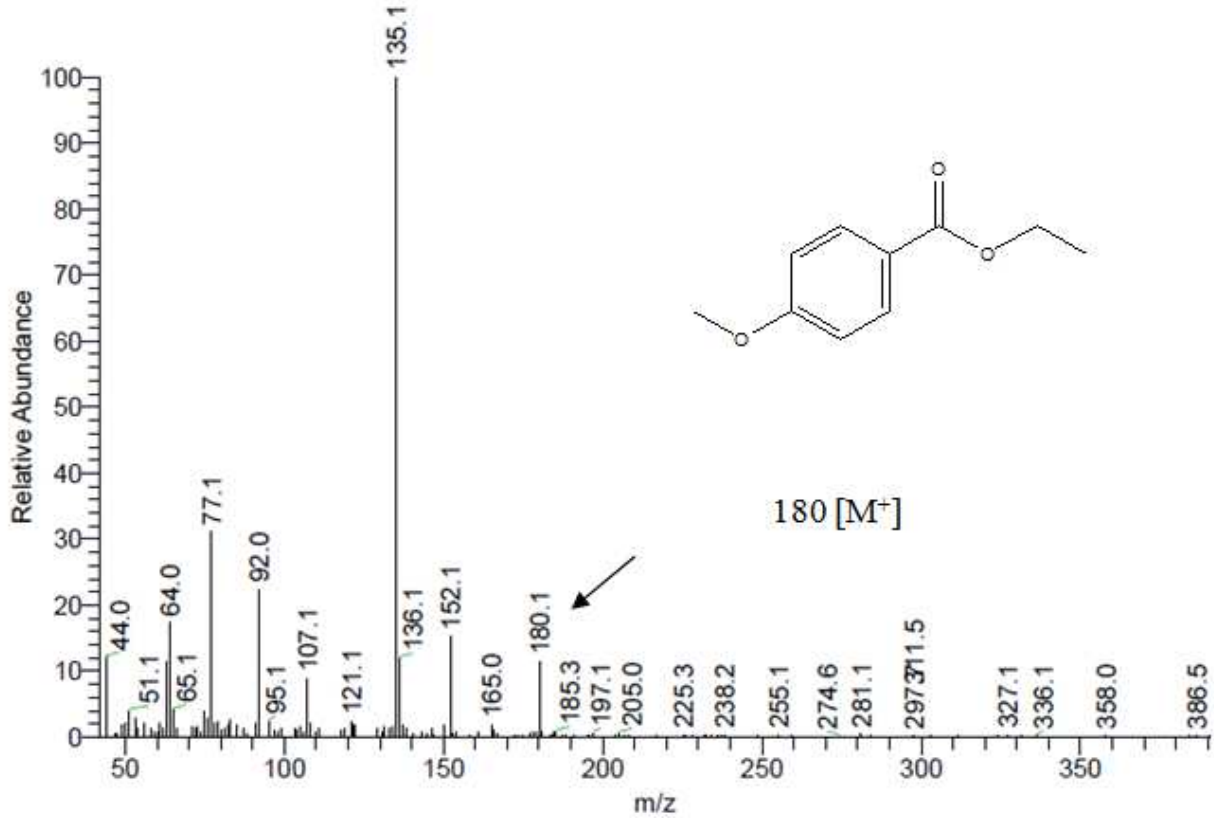
375.3
381.6
394.0
190 [M+]
O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180.1
185.3
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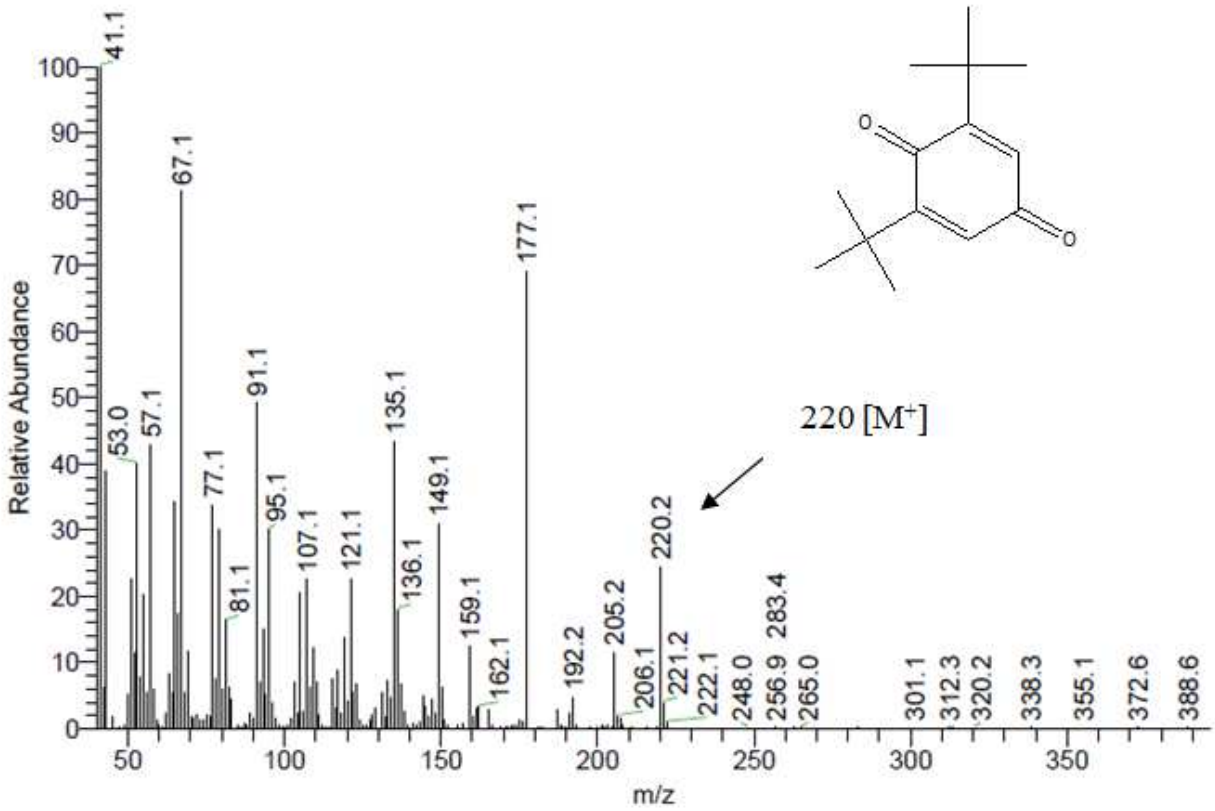
O
O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162.1
177.1
192.2
205.2
206.1
220.2
221.2
222.1
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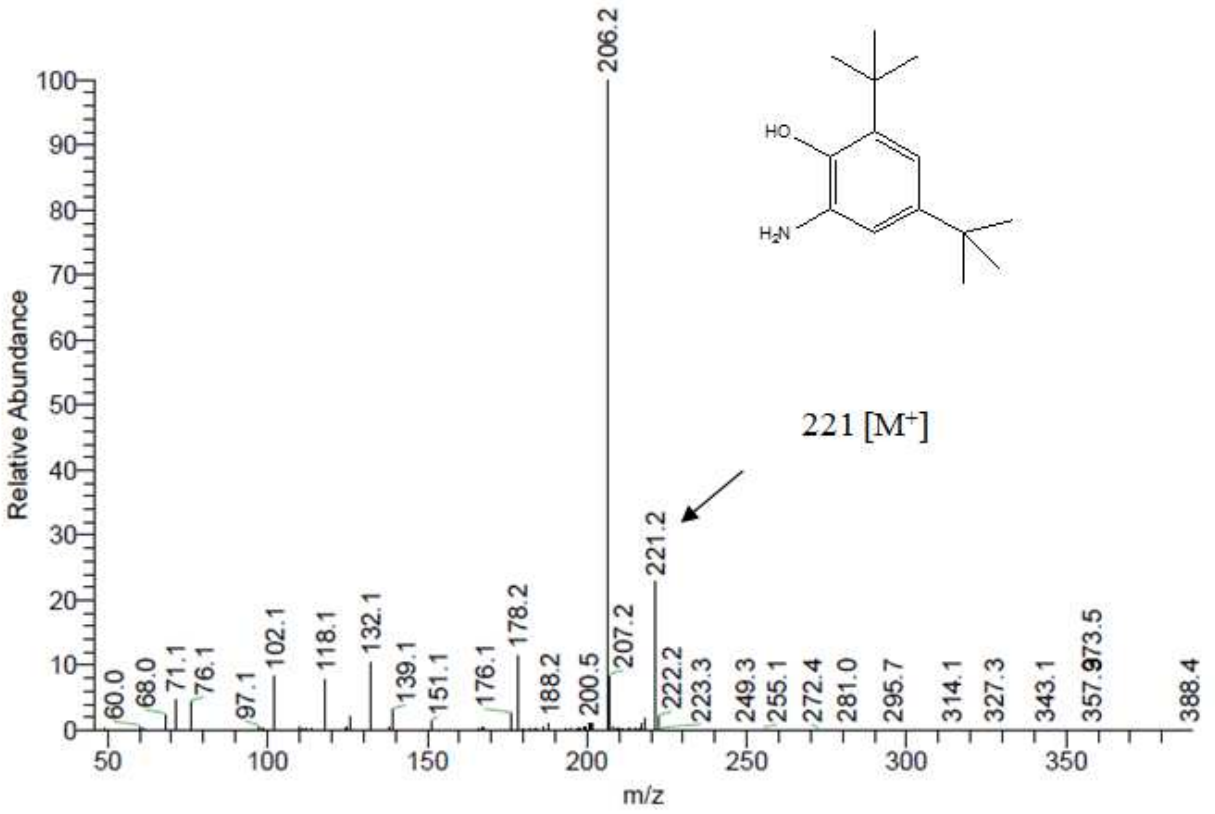
372.6
388.6
O
O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132.1
139.1
151.1
176.1
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188.2
200.5
207.2
222.2
223.3
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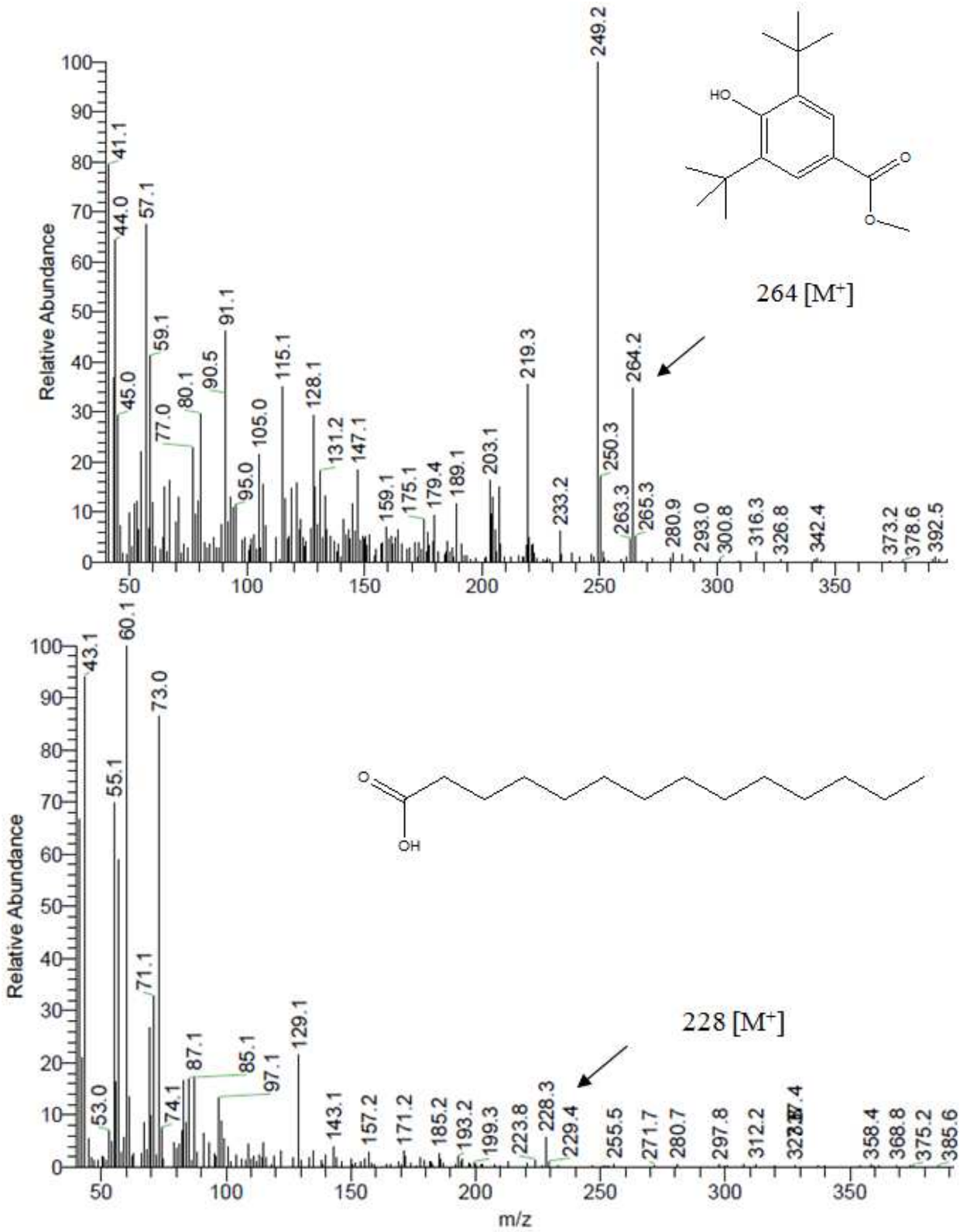
388.4
HO
H2N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233.2
250.3
263.3
264.2
265.3
280.9
293.0
300.8
316.3
326.8
342.4
373.2
378.6
392.5
HO
O
O
264 [M+]
Relative Abundance
60.1
43.1
73.0
55.1
71.1
87.1
85.1
129.1
97.1
74.1
53.0
143.1
157.2
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193.2
199.3
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m/z

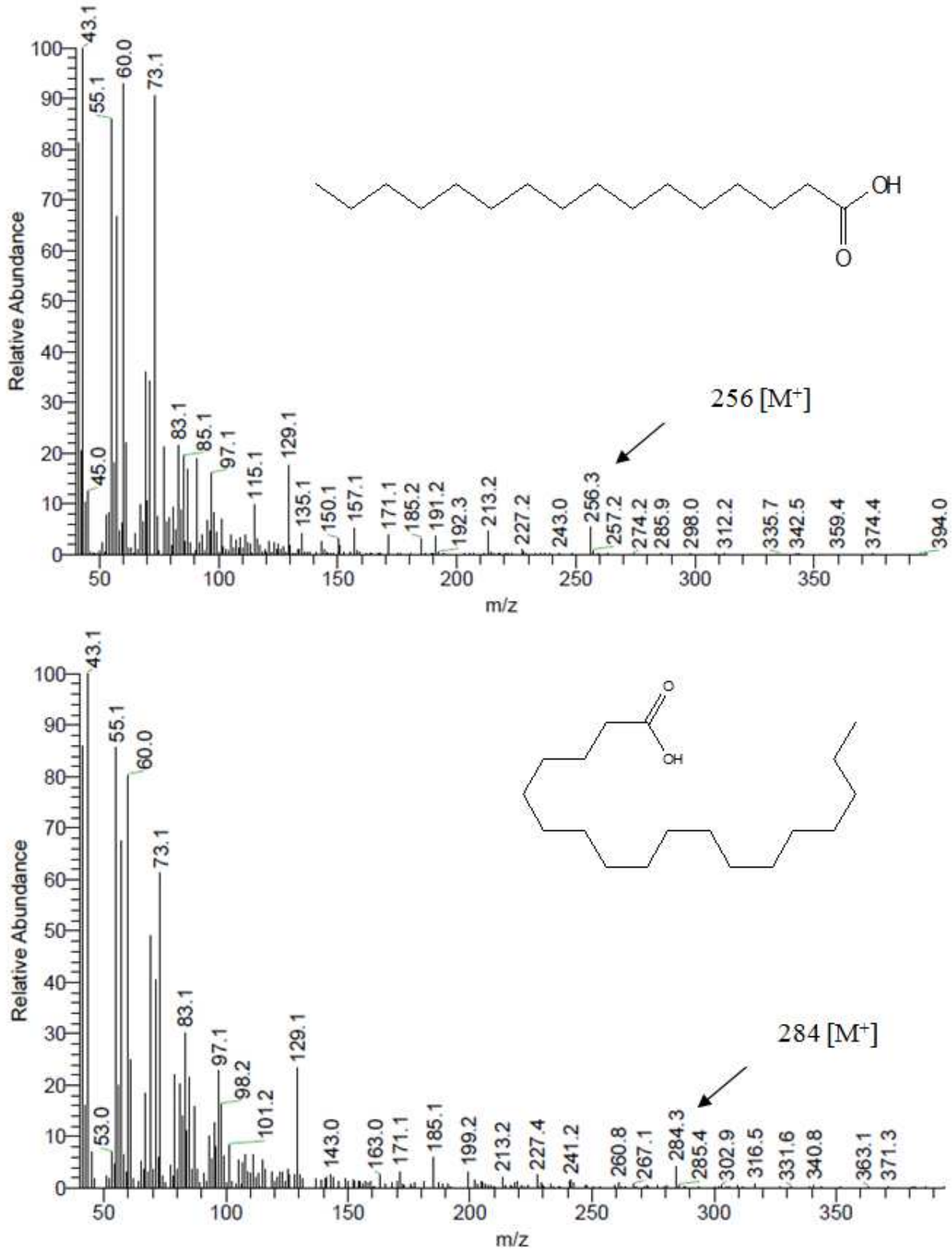

Figure 3. Fragmentation pattern for some identified compounds

Pharmacological results:

Antimicrobial activity:

Antimicrobial screening by determination of zone of inhibition for the essential oil of *L. coronopifolia* was assessed against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia* coli, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis* and *Candida albicans* at a dose of 180µg/ml test and showed no activity against all tested organisms.

Acknowledgments
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