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Original Article

GC-MS analysis of some volatile constituents extracted from stem of *Euphorbia tirucalli* Linn.

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Abstract *Euphorbia tirucalli* Linn. is traditionally used as medicine especially in the treatment of diseases caused by bacterial pathogens. The objectives of the present study were to identify the bioactive compounds in the stem of *Euphorbia tirucalli* Linn. using the gas chromatography-mass spectrometry (GC-MS) analysis, and to investigate their potentials as an alternative for antimicrobial activity. Two-microliters of dried powdered of *Euphorbia tirucalli* Linn. stem were mixed with methanol followed by injection into splitless mode of GC-MS. Separation was achieved by Elite-5MS fused capillary column. The mass spectra were compared with the spectra of known components stored in the NIST and WILEY databases for compound identification. Forty-six chemical constituents were identified. The major constituents were lanosta-8,24-dien-3-ol, (3β)- (13.60%), (23S)-ethylcholest-5-en-(3β)-ol (7.02%), linoleic acid (2.96%) and viminalol (2.57%). Most of the active compounds present in the stem of *Euphorbia tirucalli* Linn. have previously been shown to exhibit antimicrobial properties.

Keywords: Antimicrobial, bioactive compounds, *Euphorbia tirucalli* Linn., GC-MS analysis, phytochemical.

Introduction

Euphorbia tirucalli Linn. or locally known as Tetulang in Malaysia, is one of the most popular herbs that is known to have medicinal properties (Priya and Rao, 2011). In Rajasthan, India, different parts of the plant such as the latex, leaves, stems and roots may have different medicinal purposes (Upadhyay *et al*., 2010). In some parts of the Africa, the people used the plant to cure snakebites, warts, sexual impotence and syphilis while those in Asia use it in the treatment of broken bones, haemorrhoids, pains, warts, swellings and ulcerations (Gupta *et al*., 2013). In Brazil, it is also used for the treatment of scorpion bites, asthma, cancer, spasms and others (Betancur-Galvis *et al.,* 2002). In Peninsular Malaysia, a poultice of the roots or stems is applied to heal nose ulceration, haemorrhoids and swellings (Mwine and Van Damme, 2011). In short, many studies have been conducted to investigate *Euphorbia tirucalli* Linn. phytochemical constituents and their medicinal properties such as antimicrobial (Jyothi *et al.,* 2008; Avelar *et al.,* 2011; Prasad *et al.,* 2011). The presence of various bioactive compounds in this plant is significant evidence in relation to the effectiveness of the plant when employed as traditional medicine (Gopalakrishnan and Vadivel, 2011). Most of the active compounds in *Euphorbia tirucalli* Linn. were identified as having medicinal values (Inbathamizh and Padmini, 2012). Therefore, screening of phytochemical constituents of the herb is important to correlate its therapeutic activity with the specific active constituents (Sugumar *et al.,* 2010). The purpose of the present study is to investigate the phytochemical constituents that are present in the stem of *Euphorbia tirucalli* Linn. by gas chromatography-mass spectrometry (GC-MS) analysis.

Materials and methods

Plant collection and identification

Euphorbia tirucalli Linn. was collected at Baung Bayam, Kota Bharu, Kelantan, Malaysia (Lat. 6^{o}115973' N; Long. 102^{o}27013' E) and a voucher specimen was deposited under the number PID 440915-23 as identified by the Forest Research Institute Malaysia (FRIM).

Preparation of powder

The processing of the stems into powdered form for phytochemical analysis was done according to a previous study with slight modifications (Sugumar *et al.*, 2010). The fresh stems of *Euphorbia tirucalli* Linn. were thoroughly washed 2-3 times with running tap water, and once with distilled water. The stems were then placed in a tray, blotted using a clean tissue paper, followed by drying in the oven at 60^{o}C for 48 hours. The dried stems were then blended using a dry blender until they turned powder. The total amount of the powdered plant was used for GC-MS analysis.

GC-MS Analysis

GC-MS analysis was performed using a Hewlett Packard 5890 series Gas Chromatograph with 5973N Mass Selective Detector. The gas chromatography (GC) was interfaced to a mass spectrometer (MS) equipped with an Elite-5MS (5% diphenyl / 95% dimethyl poly saline) fused capillary column (30 x 0.25μm ID x 0.25μm Df). Electron impact mode at 70 Ev were used for ionization in the GC-MS analysis. The initial oven temperature was 50^{o}C for 2 min, increased at 20^{o}C/min to 280^{o}C, and held for 10 min. The injection was performed in the split-less mode with injection port temperature maintained at 250^{o}C. Data acquisition was conducted in the MS scan mode (range 40-650 m/z). The relative percentage area of each component was calculated by comparing it to the total area.

Identification of bioactive components

The test components were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Wiley) to ascertain its name, molecular weight, and structure.

Results

The present study has identified forty-six compounds in the stem of *Euphorbia tirucalli* Linn. by GC-MS analysis. However, only eighteen compounds were listed based on inclusion criteria of more than 80% library matching, and more than 0.5% of peak area. The peak report of the total ion chromatogram has the details of peak number, retention time (RT), the name of the compound, molecular formula, molecular weight (MW) and area percentage as presented in Table 1. The fragmentation patterns of the peak were characterized, and compared based on mass spectra of the constituents with NIST and WILEY libraries. Chromatogram with the peaks of the test compounds with respect to retention time is shown in Fig. 1 and the mass spectrum of major chemical constituents are shown in Fig. 2-4. The major chemical constituents were identified as lanosta-8,24-dien-3-ol, (3β)- (13.60%), (23S)-ethylcholest-5-en-(3β)-ol (7.02%), linoleic acid (2.96%), and viminalol (2.57%). The potential of phytochemical constituents which contributed to the antibacterial value is presented in Table 2. The major constituents in the *Euphorbia tirucalli* powder are lanosta-8,24-dien-3-ol, (3β), (23S)-ethylcholest-5-en-(3 β)-ol, and viminalol, and these three components have been shown to inhibit gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Bacillus cereus* (*B. cereus*), and *Escherichia coli* (*E. coli*) (Kuete *et al.,* 2011; Negi *et al.,* 2012; Mujeeb *et al.,* 2014).

Discussion

In the present study, GC-MS analysis was used to identify the bioactive compounds. On the other hand, phytochemical analysis of active compound by thin layer chromatography of *Euphorbia tirucalli* Linn. has also shown high concentration of alkaloids, cardiac glycoside, polyphenols, flavonoids, and saponin, which are important to fight microorganism (Upadhyay *et al*., 2010). The present study has revealed that most of the compounds present in the stem of

Euphorbia tirucalli Linn. have antibacterial properties, and some of the compounds are commonly present in the medicinal plants. Analysis of plant constitutes is important in the determination of their potential value due to a synergistic effect (Sulaiman *et al.,* 2008). The active compounds of *Euphorbia tirucalli* Linn. plant have the potential for anti-tumor, antibacterial, antifungal, antioxidant, and hepatoprotective (Sugumar *et al*., 2010; Uchida *et al*., 2010; Upadhyay *et al*., 2010; Priya and Rao, 2011). Each compound has different function and capacity to fight bacteria either by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters (Upadhyay *et al*., 2010). The major chemical constituents identified by GC-MS analysis in *Euphorbia tirucalli* powder have antibacterial properties, and commonly present in many types of research of medicinal plants. Most of the active compounds have identical name or synonym name which is important for searching their function and application. Based on the results from GC-MS analysis in Table 1, it is shown that lanosta-8,24-dien-3-ol, (3β)- (13.60 %), (23S)-ethylcholest-5-en-(3β)-ol (7.02 %), linoleic acid (2.96 %), and viminalol (2.57 %) were among the highest compounds in the stem of *Euphorbia tirucalli* Linn*.* Previous study has shown that lanosta-8,24-dien-3-ol, (3β)- is able to inhibit *S. aureus* and *B. subtilis* at 150 mg/ml of minimal inhibitory concentration (MIC) of methanol extract of *B. purpure* (Negi *et al.,* 2012). Extraction of propolis from the beehive *Trigona* showed that lanosta-8,24-dien-3-ol, (3β) is one of the compounds which negatively works against of *E. coli* growth (Hasan *et al.,* 2014). The second major compound in *Euphorbia tirucalli* is (23S)-ethylcholest-5-en-(3β)-ol, and this compound has been proven to inhibit *K. pneumoniae*, *B. cereus* and *S. aureus* (Mujeeb *et al.,* 2014). Perianayagam *et al.* (2012) also proved that isolated (23S)-ethylcholest-5-en-(3β)-ol inhibits Gram-positive and Gram-negative bacteria. In addition, the linoleic acid is also able to inhibit Gram-positive bacteria like *S. aureus, Listeria monocytogenes* and *B. subtilis* (Dilika *et al.,* 2000; Shin *et al.,* 2005). Viminalol or better known as α-amyrinl, is able to inhibit *K. pneumoniae, S. aureus* and *E. coli* (Kuete *et al*., 2011).

Based on the references presented in Table 2, it could be concluded that all analysed compounds, which peak area is 0.5% and above, extracted from the stem of *Euphorbia tirucalli* Linn. do have antimicrobial properties. This conclusion is derived based on the fact that those extracted compounds should have similar antimicrobial effects, even though the previous studies were conducted using other different types of plant extracts.

Conclusion

In the present study, eighteen chemical compounds have been identified from the powdered form of the *Euphorbia tirucalli* Linn. stem using the GC-MS. Previous studies on all the 18 compounds have proven that they have antimicrobial activities. Thus, further investigations of the active compounds present in the plant are important to establish *Euphorbia tirucalli* Linn. as an antimicrobial herb. One important study to be undertaken is the type of solvents used in extraction process to maximize the quantity of the active compounds.

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Table 1 Phytoconstituents identified in the stem of *Euphorbia tirucalli* Linn. by GC-MS

No	RT (Min)	Constituents	Molecular Formula	Molecular weight	Peak area (%)
1	7.94	4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	$C_6H_{10}O_3$	130.14	0.54
2	8.70	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4(4*H*)-one	$C_6H_8O_4$	144.13	1.02
3	9.25	5-hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126.11	0.99
4	10.43	(E)-2-methoxy-4-(prop-1-enyl) phenol	$C_{10}H_{12}O_2$	164.20	0.54
5	11.34	Megastigmatrienone	$C_{13}H_{18}O$	190.28	0.53
6	11.65	2-hydroxy-1-(4-isopropylphenyl)-2-methyl-1-propanone	$C_{13}H_{18}O_2$	206.28	1.03
7	11.84	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	1.36
8	12.70	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	1.81
9	13.40	Linoleic acid	$C_{18}H_{32}O_2$	280.45	2.96
10	13.46	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	0.82
11	15.21	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	280.45	0.56
12	15.24	Ethyl linoleate	$C_{20}H_{36}O_2$	308.50	0.64
13	15.66	Spinacene	$C_{30}H_{50}$	410.72	0.53
14	17.14	Vitamin E	$C_{31}H_{54}O_2$	453.76	1.60
15	17.88	Ergost-5-en-3-ol,(3β)-	$C_{28}H_{48}O$	400.68	0.89
16	14.37	Lanosta-8,24-dien-3-ol,(3β)	$C_{30}H_{50}O$	426.72	13.60
17	18.70	(23S)-ethylcholest-5-en-(3β)-ol	$C_{29}H_{50}O$	414.71	7.02
18	19.03	Viminalol	$C_{30}H_{50}O$	426.72	2.57

Table 2 List of compounds found in the stem of *Euphorbia tirucalli* Linn. and its antibacterial activity reported previously

No.	Name of compound	Compound nature	Antibacterial activity (References)
1	4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	Cyclic Ester	Inhibit formation biofilm of *Pseudomonas aeruginosa* PAO1 (Choi *et al.,* 2014)
2	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-pyran-4(4*H*)-one	Ketone	Greatly inhibit *S. sonnei, S. aureus* and *C. albicans* (Ara *et al.,* 2013)
3	5-hydroxymethyl-2-furaldehyde	Aryl aldehydes	Inhibit *X. axonopodis, P. carotovorum , P. crhysanthemi* and E. *amylovora* (Espinoza *et al.,* 2008)
4	(E)-2-methoxy-4-(prop-1-enyl) phenol	Alcohol	Against *P. aeruginosa*, *A. baumanii*, *S.aureus*, *E. coli*, *B. cloacae*, *K. pneumoniae*, *S. epidermidis* and *E. faecalis* (Cai *et al.,* 2012)
5	Megastigmatrienone	Cyclic Ketone	Inhibit *E. tarda*, *E. coli*, *Flavobacterium sp*, *P. aeruginosa* and *V. cholerae* (Lee *et al*., 2011)
6	2-hydroxy-1-(4-isopropylphenyl)-2-methyl-1-propanone	Ketone	Coating medical device for antibacterial activity (Van Dongen and Kluijtmans, 2011)
7	Tetradecanoic acid	Fatty Acid	Inhibit *S. epidermidis* (Liu and Huang, 2012)
8	Hexadecanoic acid	Fatty Acid	Inhibit *S. aureus* and *E. coli* (Alamin *et al.,* 2016)
9	Linoleic acid	Fatty Acid	Inhibit Gram-positive bacteria (*B. cereus, B. pumilus*, *B. subtilis* and *S. aureus* (Dilika *et al.,* 2000)
10	Octadecanoic acid	Fatty Acid	Against *S. aureus* and *S. pyogenes* (Zheng *et al.,* 2005)
11	9,12-Octadecadienoic acid	Fatty Acid	Inhibit *S. aureus* and *E. coli* (Alamin *et al.,* 2016)
12	Ethyl Linoleate	Fatty Acid Ethyl Ester	Inhibit *S. aureus* and *E. coli* (Keawsa-Ard *et al.,* 2012)
13	Spinacene	Isoprenoid Polyenes	Against *X. oryzae* and *P. syringae* (Alsultan *et al.,* 2016)
14	Vitamin E	Tocopherols	Inhibit *S. aureus* and *S. epidermidis* (Al-Salih *et al,.* 2013)
15	Ergost-5-en-3-ol,(3β)-	Steroid Alcohol	Effective inhibit *P. mirabilis* (Singariya *et al,.* 2012)
16	Lanosta-8,24-dien-3-ol,(3β)	Tetracyclic Triterpenoid	Inhibit gram positive bacteria (*S aureus* and *B subtilis*) (Negi *et al.,* 2012)
17	(23S)-ethylcholest-5-en-(3 β)-ol	Steroid Alcohol	Against *K. pneumoniae, B. cereus* and *S. aureus* (Mujeeb *et al.,* 2014)
18	Viminalol	Pentacyclic Triterpenoid	Inhibit *K. pneumoniae, S. au*reus and *E. coli* (Kuete *et al.,* 2011)

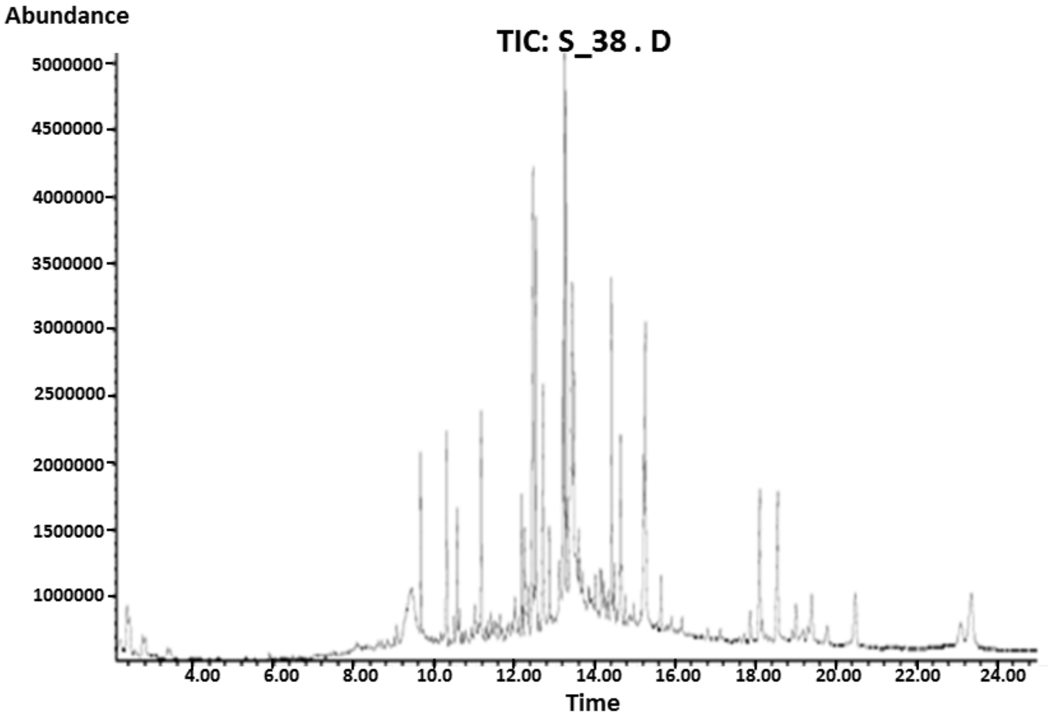

Fig. 1 GC-MS chromatograms of the stem of *Euphorbia tirucalli* Linn.

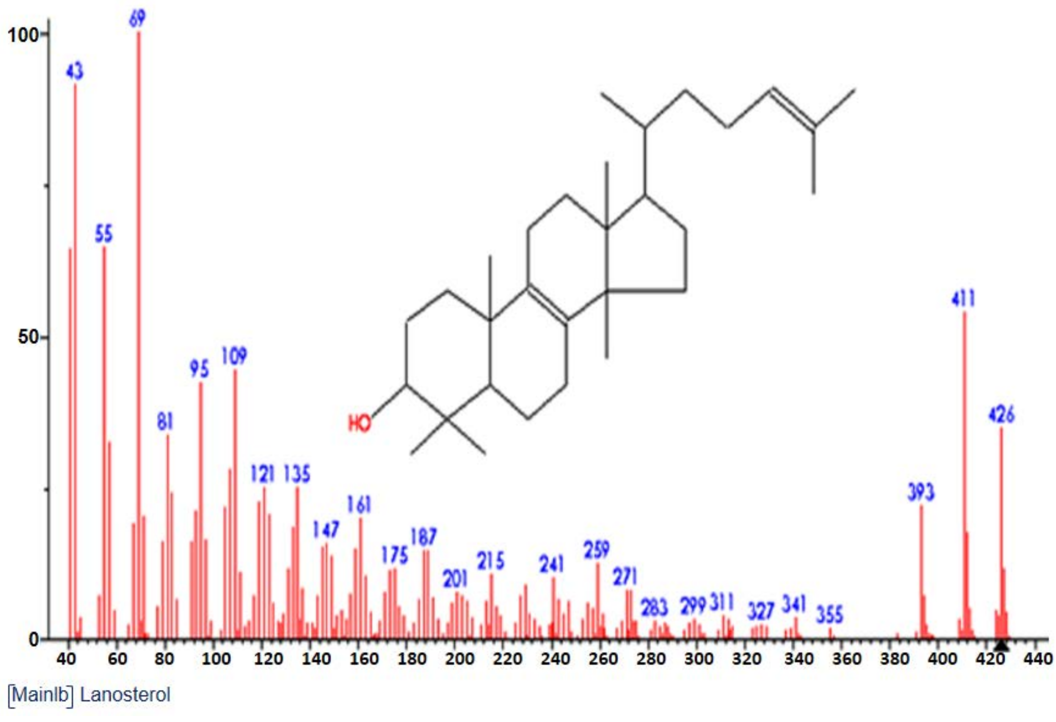

Fig. 2 Mass spectrum of lanosta-8, 24-dien-3-ol, (3β) (RT: 14.37).

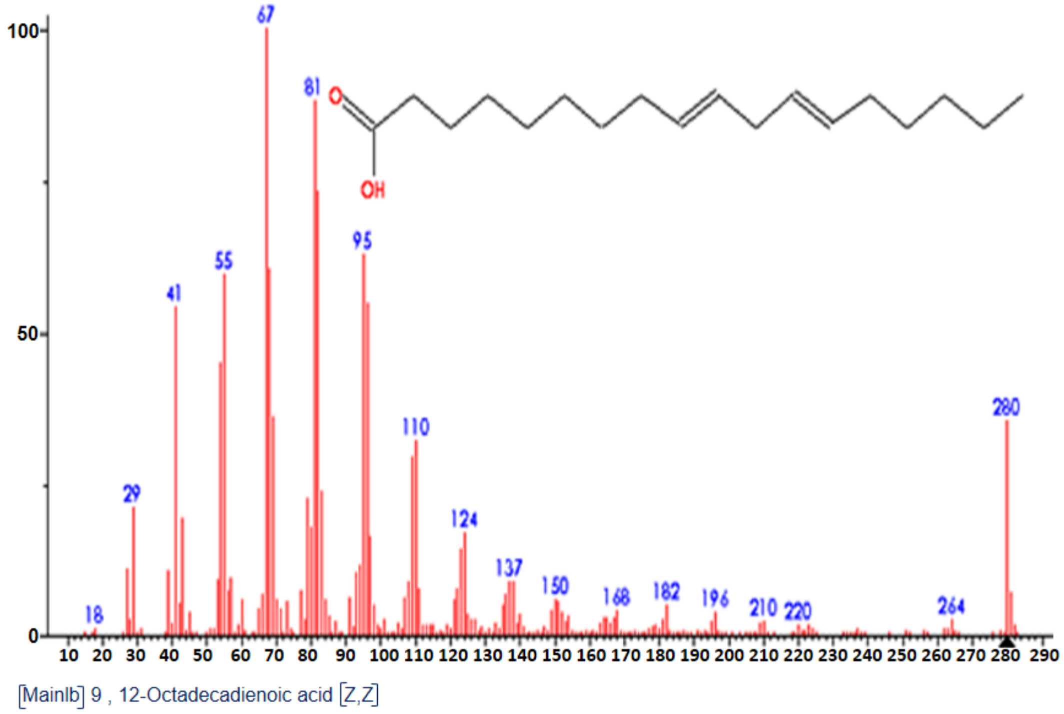

Fig. 3 Mass spectrum of linoleic acid (RT: 13.40).

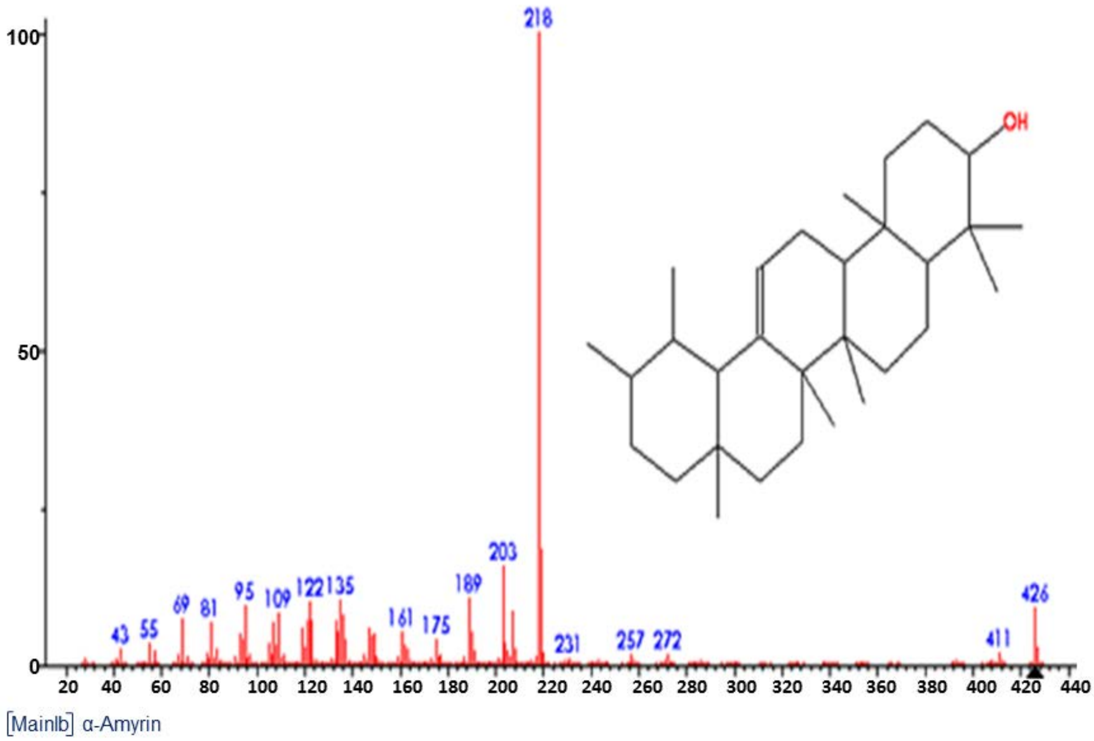

Fig. 4 Mass spectrum of viminalol (RT: 19.03).

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