ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 4, 2013

ISSN - 0974-2441

Research Article

CHARACTERIZATION AND MEDICINAL IMPORTANCE OF PHYTOCONSTITUENTS OF C. PAPAYA FROM DOWN SOUTH INDIAN REGION USING GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY

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Received:8 June 2013, Revised and Accepted: 8 July 2013

ABSTRACT

Objective: To investigate the phytocompounds of the methanolic extract of *C. papaya* using GC-MS. Method: Fresh leaves of *Carica papaya* were collected, washed thoroughly and shade dried for 8- 10 days. Two hundred grams of crushed fresh young leaves were sequentially extracted in a Soxhlet apparatus using methanol. The chemical compositions of the extract of was investigated using Perkin – Elmer Gas Chromatography – Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standard and Technology (NIST) library.
Result: Analysis shows different peaks out of which some important eleven peaks of phytochemical compounds chosen namely Benzyl nitrile, 2-Methoxy-4-vinylphenol, Citronellyl butyrate, N-Aminomorpholine, Methyl-2[methoxy(methyl)amino]-2-methylpropanoate , n Hexadecanoic acid , 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Crotonoyl bromide, 4-Cyclopropylcarbonyloxytetradecane, 2-Chloro-11H-pyrido[3',2'-4,5]pyrrolo[3,2-c]quinoline and beta sitosterol.

Conclusions: From the results it can be concluded that the plant extract shows the presence of 25 important compounds.

Keywords: *Carica papaya* L., *Colletotrichum gloeosporiodes,* , carpaine and pseudocarpaine.

INTRODUCTION

Many components of human diet such as vegetables and fruits are excellent medicinal values. The medicinal value of plants lies in some chemical substances (plant secondary metabolites) that produce a specific biological action on the human body [1](Hassan A,2009). It has been estimated roughly, that presently more than half of the total population of the world use herbal drugs[2] (Change et al 1987). Knowledge of the chemical constituent of plants is desirable for the discovery of therapeutic agents and in discovering the actual value of folklore remedies[3] (G.Selvamangai et al.2012)

Papaya (*Carica papaya* L.) is grown in tropical and sub-tropical regions around the world[4] (Corral-Aguayo RD et. al.2008). Extracts from different papaya tissues have been shown to contain bioactive compounds. It is a common man's fruit. It is low in calories and rich in natural vitamins and minerals. *Carica papaya* has been used to treat diabetes mellitus and hypertension[5](Singh VP, et. al.1980). Papaya leaves contain the bitter alkaloids, carpaine and pseudocarpaine, which act on the heart and respiration papaya leaves shows antiasthamatic property [6](Watt JMet. al. 1984). Aqueous extracts of leaves and seeds are known to have antifungal activity against *Colletotrichum gloeosporiodes* [7] (Bautista-Ban˜os et. al. 2002). The leaves are used for treatment of intestinal worms and venereal diseases [8] (Dalziel JM 1985), recently the juice of leaves of *C. papaya* have been used as treatment for dengue fever, however there is no scientific evidence. Hence we have undertaken to study the phytocompounds in leaf extract and further evaluation of the same for its antiviral nature. [12]

MATERIALS AND METHODS

Collection and Identification of plantMaterials

The leaves of *Carica papaya* were handpicked at local area in Thanjavur city, identified and authenticated by Department of Botany, PRIST University; they were washed, air-dried and ground into fine powder using homogenizer in the laboratory.

Extraction

Plant material extraction carried out by sequential Soxhlet extraction with solvent of increasing polarity. Two hundred grams of crushed fresh young leaves were sequentially extracted in a Soxhlet extractor using 1500 ml of methanol. The extraction time was about 30 cycles in the same solvent. The resulting extract was evaporated using rotary evaporator stored at 4°C.

GC-MS analysis

GC-MS analysis of the extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1µ Mdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1 injector temperature 250 °C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min). With an increase of 10 °C /min, to 200 °C then 5 °C /min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550 Da.

Identification of components

Data obtain from the National institute of standard and Technology has put as standard to interpret the results of GC-MS with more than 62,000 patterns. Resulting data in spectrum was compared with known spectrum available in NIST. The credentials of plant material tested include molecular weight and structures of the constituents were ascertained.

RESULTS

The identified compounds of the leaves of *C.papaya*, their percentage of composition, chemical structures and biological activities have been described in table 1. The compounds prediction is on the basis

of literature as well as Dr. Duke's Phytochemical and Ethno botanical Database.

The result showed the presence of glycerine (4.15%), benzyl nitrile (1.04%), 2-Phenyl-1,3-oxazol-2-ine (2.95%), 2-Methoxy-4-vinylphenol (1.04%), choloroacetic acid, 2,2-dimethyl (1.22%), 9-octadecyne (4.14%), 3,4-altrosan (2.64%), L- arabinitol (3.48%), citronellyl butyrate (0.83%), 3,7,11,15Tetramethyl-2-hexadecanoic acid (1.21%), N-aminomorpholine (1.30%), Methyl-2[methoxy(methyl)amino]-2-methylpropanoate (1.30%), n-hexadecanoic acid (14.73%), 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (1.7%), phytol (3.64%), 9,12,15-Octadecatrienoic acid (2.11%), Crotonoyl bromide (1.56%), 4-Cyclopropylcarbonyloxytetradecane (1.56%), 2-Chloro-11H-pyrido[3',2'-4,5]pyrrolo[3,2-c]quinoline (0.71), (3-Bromo_1-methylpropoxymethyl) (3.47%), vitamin E (2.70%), 5-Methyl-2-(N-ethyl-p-chlorophen (3.20%), 1,2-Dihydropyridol(3,2,1-kl)phenol (3.27%) and beta sitosterol(11.40%) are the major compounds available in the plant. Spectrum and individual structural fragmentation of some important compound are shown in fig.2A-2K.

DISCUSSION

In the present study GCMS analysis of methanolic extract of *C.papaya* showed presence of twenty four compounds. Gas Chromatography – Mass Spectrometry (GC-MS) is a valuable for tool for reliable identification of phytocompounds [9] [10](Sampath Kumar et. al 2011 and Johnson M et al.2011)from the compounds identified in the extract n- hexadecanoic acid was found to be in higher percentage (14.73%) and this compound acts as an antioxidant,

hypocholesterolemic and anti-hemolytic. Beta sitosterol is another important compound present in higher amounts (11.03%) plays a very important role in prostate cancer. 2-Methoxy-4-vinylphenol, 9-Octadecyne, n Hexadecanoic acid, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol and Phytol are compounds present in the extract show antioxidant activity. Compounds such as 3-Bromo-1-methylpropoxymethyl-benzene and Vitamin E also show antiviral activity.

In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs [11] (Chew YL, et. al.2009) GC-MS analyzes is the first step in understanding the nature of bioactive compounds in the plant extract. Isolation of these compounds and subjecting it to further studies will help to elucidate their biological activities.

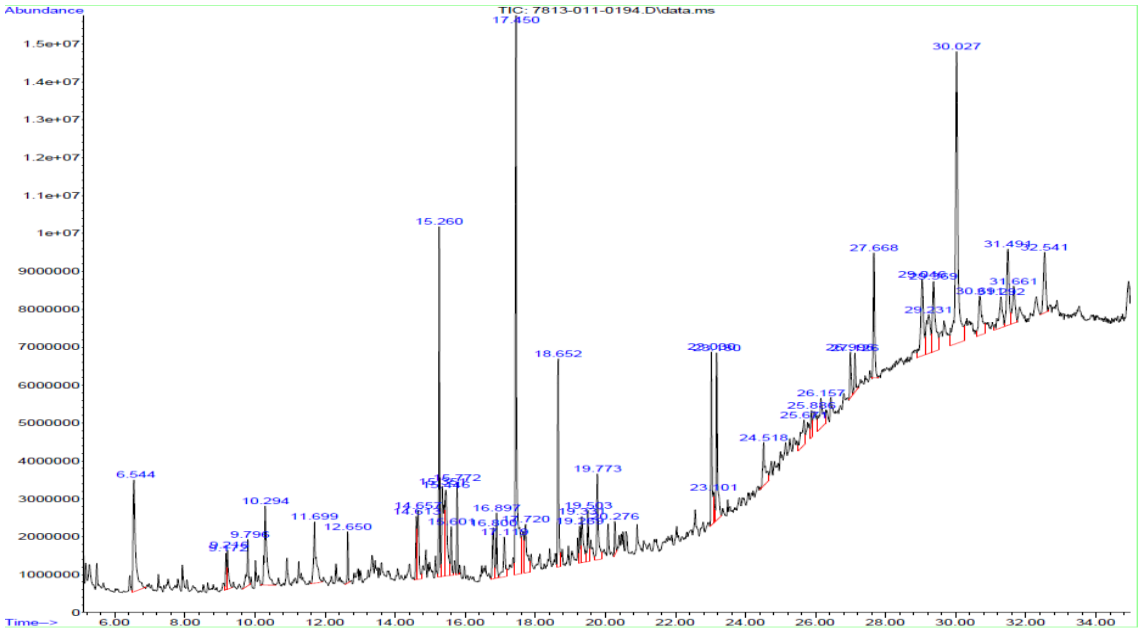


Fig.1: Graph Showing the GC-MS analysis of C. papaya

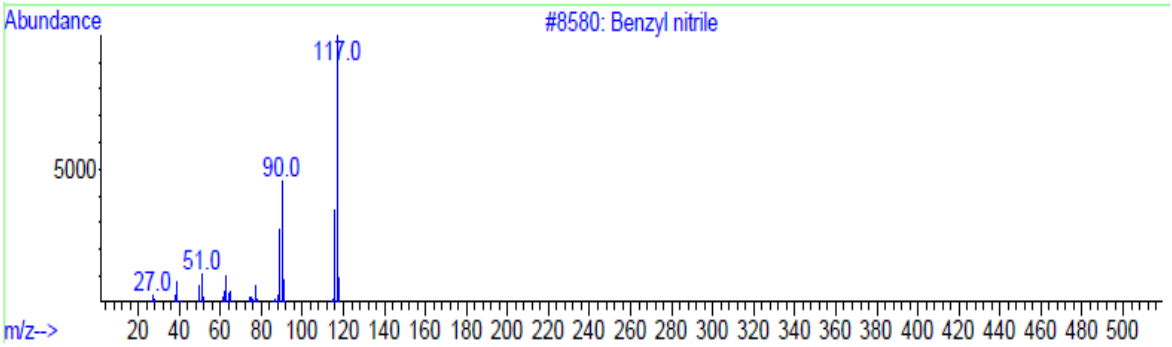
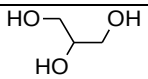
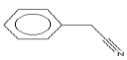
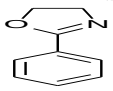
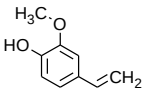
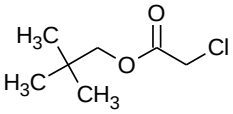
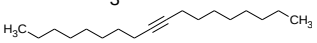
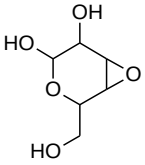
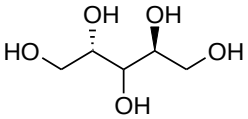
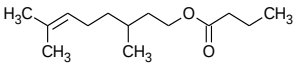
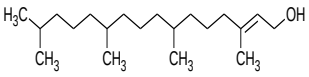
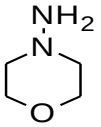
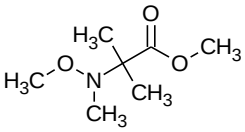
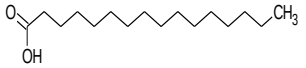
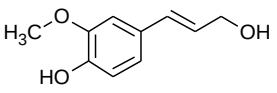
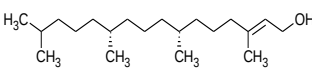
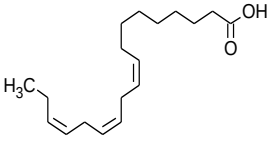
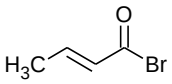
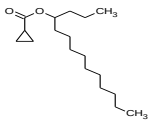
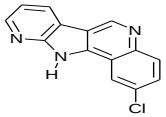
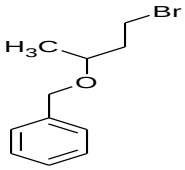
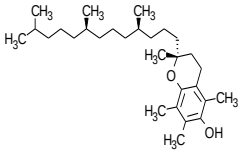
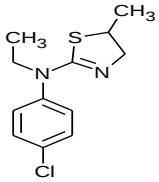
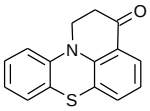
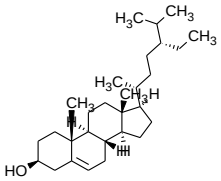
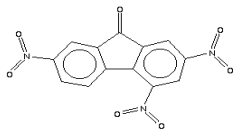


Figure 2A: Benzyl nitrile

Table 1: Phytocomponents identified in Methanolic leaf extract of *C. papaya* by GC-MS

RT	Name	Mol Formula	Mol. Wt.	Peak area %	Structure		Activity
6.539	Glycerin	$C_3H_8O_3$	920.9	4.15		Alcoholic compound	Lubricant, Moisturizer
9.792	Benzyl nitrile	C_8H_7N	117.1479	1.04		Benzyl group	Biofumigation, antifungal
10.30	2-Phenyl-1,3-oxazol-2-ine	C_9H_9NO	147.17	2.95		Phenolic	Strong antimycobacterial
11.69	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.174	1.04		Phenolic	Antimicrobial, Antioxidant, Anti-inflammatory, Analgesic
14.614	Choloroacetic acid, 2,2-dimethyl	$C_7H_{13}ClO_2$	164.62	1.22		Acidic	Antibacterial, expectorant, mucolytic
15.267	9-Octadecyne	$C_{18}H_{34}$	250.46	4.14		Alkene	Antioxidant antimicrobial
15.355	3,4-Altrosan	$C_6H_{10}O_5$	162.140	2.64		Poly Hydroxy compound	Bacteriostat Fungicide
15.442	L/(D) Arabinitol	$C_5H_{12}O_5$	152.14	3.48		Alcohol	Antidiabetic, anti-HIV, activity
15.601	Citronellyl butyrate	$C_{14}H_{26}O_2$	226.355	0.83		Citronellyl	inhibitor of the mitogenic activity of epidermal growth factor (EGF), antifungal
15.776	3,7,11,15Tetramethyl-2-hexadecanoic acid	$C_{20}H_{40}O$	296.53	1.21		Terpene alcohol	Flavor, Lubricanting agent
16.801	N-Aminomorpholine	$C_4H_{10}N_2O$	102.135	1.30		enol-imines	The antimicrobial activities
16.807	Methyl-2[methoxy(methyl)amino]-2-methylpropanoate	$C_7H_{15}NO_3$	161.198	1.30		Esteric acids	Glucose Transport-Enhancing and Hypoglycemic Activity
17.446	n Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14.73		Palmitic acid	Antioxidant, hypocholesterolemic, nematicide, hemolytic, 5-alpha reductase inhibitor
17.722	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.200	1.70		Phenolic compound	Antioxidant Antimicrobial
18.651	Phytol	$C_{20}H_{40}O$	296.53	3.64		Diterpene	Anticancer, antioxidant, anti-inflammatory, diuretic
19.77	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.42	2.11		Linolenic acid	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase

							inhibitor Antiandrogenic,
24.519	Crotonoyl bromide	C_4H_5BrO	148.98	1.56		Crotonyll	Antimicrobial,anticarcinogenic
24.501	4-Cyclopropylcarbonyloxytetradecane	$C_{18}H_{34}O_2$	282.461	1.56		Cyclodecane	Unknown
27.133	2-Chloro-11H-pyrido[3',2'-4,5]pyrrolo[3,2-c]quinoline	$C_{14}H_8ClN_3$	253.68	0.71		Quinoline	Antimalarial, antiparasitic
23.182	(3-Bromo-1-methylpropoxymethyl)benzene	$C_{11}H_{15}BrO$	243.140	3.47		Aromatic	Anesthetic activity, antiviral agent
27.670	Vitamin E	$C_{29}H_{50}O_2$	430.70	2.70		Methylated Phenols	Antioxidant activity, anti-atherogenic, antithrombotic, anticoagulant, neuroprotective, antiviral, immune-modulatory, cell membrane-stabilizing and antiproliferative actions
29.05	5-Methyl-2-(N-ethyl-p-chlorophenylamino)-2-thiazoline	$C_{12}H_{15}ClN_2S$	254.77	3.20		Amines	Analgesic
29.396	1,2-Dihydropyrido(3,2,1-kl)phenothiazin-3-one	$C_{15}H_{11}NOS$	253.31	3.27		Pyridocarbazoles	Pulmonary hypertension, ischemic heart diseases, erectile insufficiency, female sexual dysfunction or diseases
30.023	β-Sitosterol	$C_{29}H_{50}O$	414.711	11.04		Sterol	Antilipidic, anticancer prostate
32.535	2,4,7-Trinitrofluorenone	$C_{13}H_5N_3O_7$	315.1947	2.18		Aromatic compounds	Antimalarial activity, antioxidants,antiparasitic

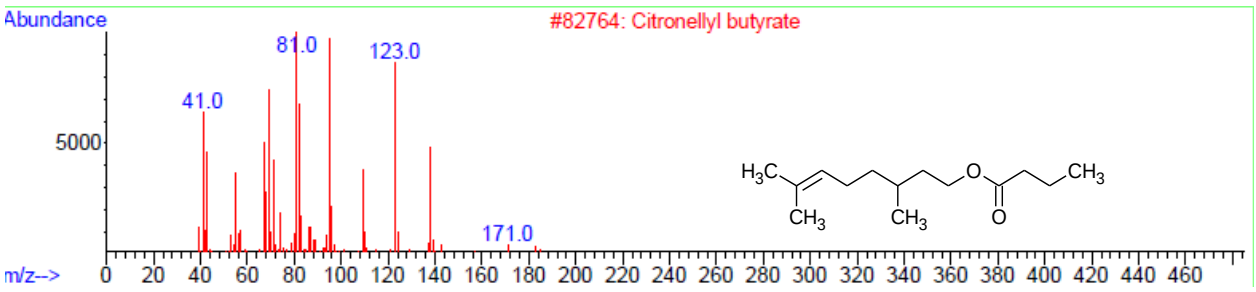


Figure 2C: Citronellyl butyrate

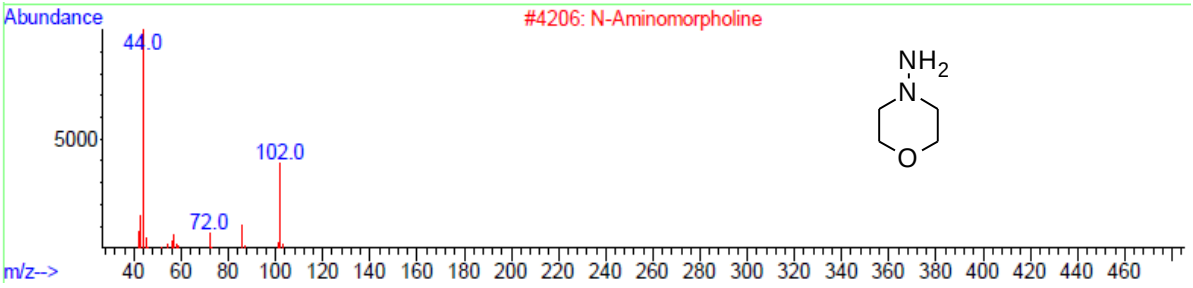


Figure 2D: N-Aminomorpholine

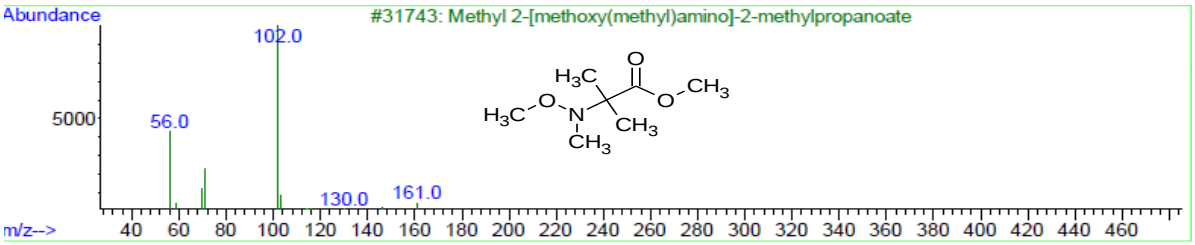


Figure 2E: Methyl-2[methoxy(methyl)amino]-2-methylpropanoate

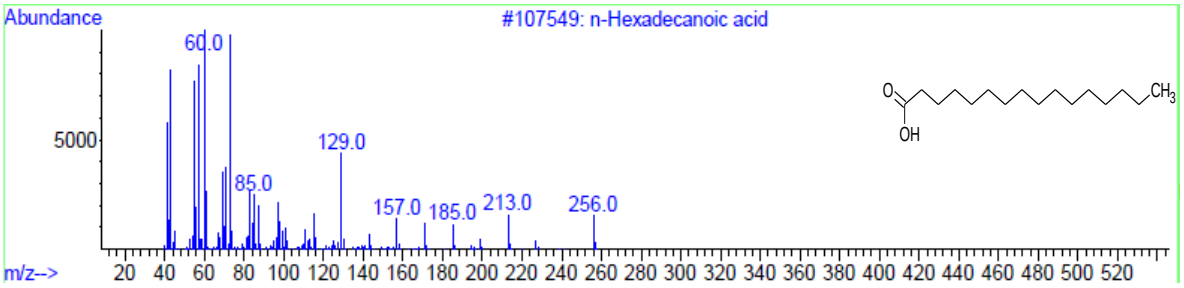


Figure 2F: n-Hexadecanoic acid

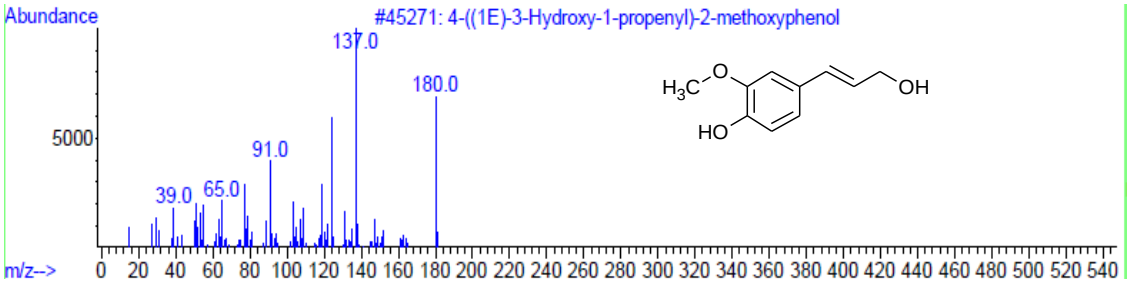


Figure 2G: 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol

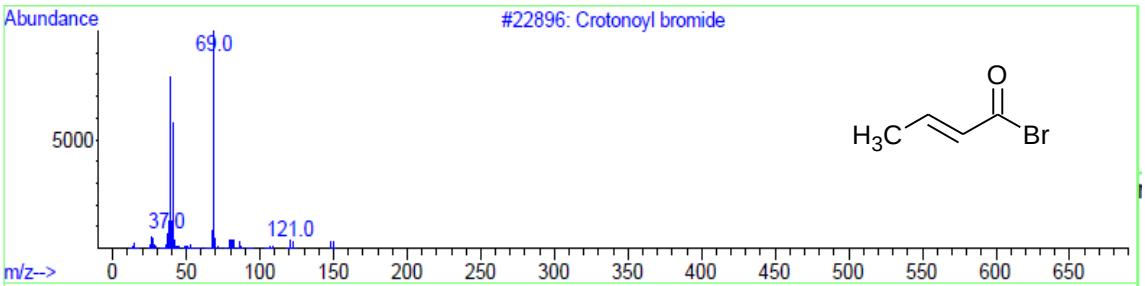


Figure 2H: Crotonoyl bromide

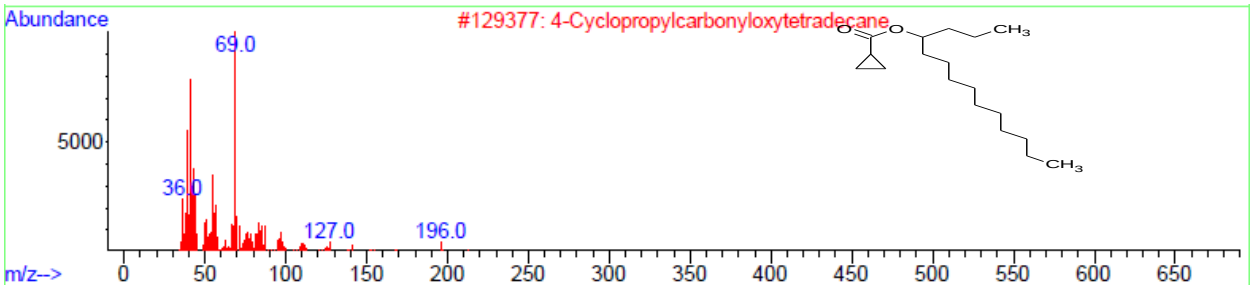


Figure 2I: 4-Cyclopropylcarbonyloxytetradecane

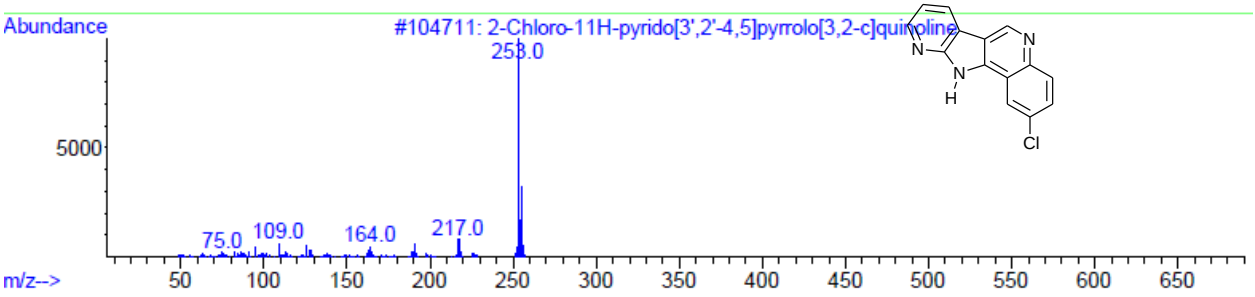


Figure 2J: 2-Chloro-11H-pyrido[3',2'-4,5]pyrrolo[3,2-c]quinolone

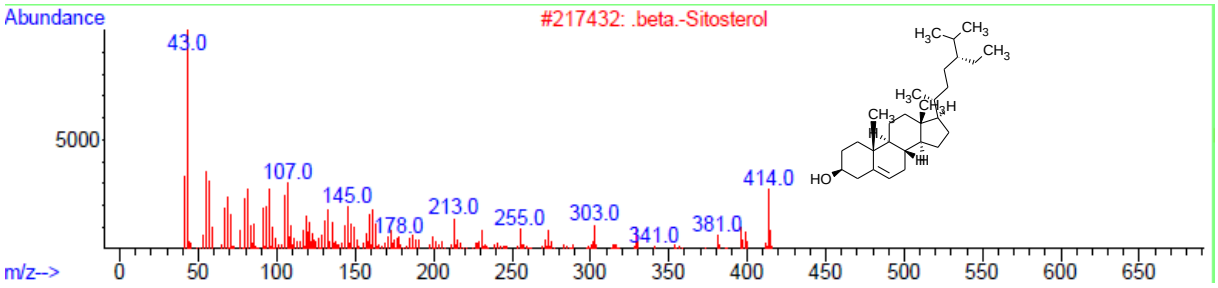


Figure 2K: beta-Sitosterol

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