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Full Length Research Paper

Screening of antioxidant activity, total phenolics and gas chromatograph and mass spectrometer (GC-MS) study of delonix regia

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The present study was carried out for identification of the phytochemicals present in the *Delonix regia* leaves and also evaluates the total phenols, total flavonoids and antioxidant activity of the leaf extract. Total phenols were carried out by Folin Ciocalteu method and the phenolic content was 16.00 mg/100 g of gallic acid equivalent (GE). Antioxidant activity was evaluated by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method and the leaves of *D. regia* showed 10.73 mg/100 g of ascorbic acid equivalent antioxidant capacity (AEAC). The gas chromatograph and mass spectrometer (GC-MS) study was also carried out and it showed the presence of phytochemicals like phytol (RT: 15.49), Cumarin 7, 8-dihyddro-7-hydroxy-6-methoxy-8-oxo (RT: 15.92), Squalene (RT: 25.41) and Vitamine (RT: 29.97).

Key words: Total phenols, total flavonoids, antioxidant activity, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), gas chromatograph and mass spectrometer (GC-MS), delonix regia.

INTRODUCTION

The *Delonix regia* belongs to the family of Caesalpinioideae. The members of the genus are flowering trees, native to the East Africa, has been used in traditional Indian medicine for the treatment of rheumatism, stomach disorders. (Thirugnanam et al., 2003) and its leaves are used in the treatment of bronchitis and pheumonia in infants. Leaf extracts of *D. regia* are reported for strong anti-inflammatory activity. (Sethuraman et al., 1986). The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potential antioxidant activities, no side effects and economic viability (Auudy et al., 2003). Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. *D. regia* contains many polyphenolic compounds, terpenoids, tannins, cardiac glycosides, anthroquinones. Since these compounds have an antioxidant potential. The antioxidant potency of *D. regia* was investigated by employing various established *in vitro* systems. (Om Prakash and Yamini, 2007). The present study was carried out to study the flavonoids, antioxidant activity of *D. regia* and the chemical constituents were studied by GC-MS.

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Abbreviations: GE, Gallic acid equivalent; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **AEAC,** acid equivalent antioxidant capacity; **GC-MS,** gas chromatograph and mass spectrometer; **UV,** ultraviolet; **NIST,** National Institute Standard and Technology; **O.D,** optical density.

MATERIALS AND METHODS

Collection and processing of plant material

The leaves of the plant *D. regia* collected from Thanjavur District in the month of July, 2010 and authenticated by Dr. John Britto, Rapinet Herbarium, ST. Joseph's College, Tiruchirappalli. The leaves were cleansed and shade dried for a week and grounded

into uniform powder. 1 g of plant material was added to 20 ml of aqueous ethanol (20%, v/v) for 18 h at room temperature. The extracts were filtered and used for the estimation of total phenols and antioxidant activity.

Total phenols

0.5 ml of freshly prepared sample was taken and diluted with 8 ml of distilled water. 0.5 ml of Folin Ciocalteu Reagent (1 N) was added and kept at 40°C for 10 min. 1 ml of Sodium Carbonate (20%) was added and kept in dark for one hour. The color was read at 650 nm using Shimadzu ultraviolet (UV)-1650 Spectrophotmeter (Malick and Singh, 1980). The same procedure was repeated for all standard gallic acid solutions and standard curve obtained. The sample concentration was calculated as GE.

Total flavonoids

0.5 ml of aqueous extract of sample is diluted with 3.5 ml of distilled water at zero time and 0.3 ml of 5% sodium nitrate was added to the tubes. After 5 min, 0.3 ml of aluminium chloride (10%) was added to all the tubes. At the 6 min, 2 ml of sodium hydroxide (1 M) was added to the mixture. Immediately, the contents of the reaction mixture were diluted with 2.4 ml of distilled water and mixed thoroughly. Absorbance of the mixture was determined at 510 nm versus a prepared blank immediately. Gallic acid was used as the standard compound for quantification of total flavonoids as mg/100g (Zhisen et al., 1999).

Antioxidant activity

DPPH method

0.1 ml of the freshly prepared sample was taken in test tubes. 6 ml of DPPH solution (0.1 mM) was added and the tubes kept in dark for one hour. The color was read at 517 nm. The difference in the optical density (O.D) of DPPH solution and DPPH solution+ sample was calculated. The decrease in OD with sample addition is used for calculation of the antioxidant activity. Ascorbic acid standards were prepared in different concentrations and antioxidant was determined as ascorbic AEAC mg/100 g of sample (Koleva et al., 2002).

GC-MS analysis

Preparation of extract

Leaves of *D. regia* were shade dried. 20 g of the powdered leaves were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatmann filter paper No.41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. 2 µl of these solutions was employed for GC-MS analysis (Merlin et al., 2009).

GC analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and GC gas interfaced to a MS instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID × 1 µM df, composed of 100% Dimethyl poly diloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl 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 0.5 s and fragments from 40 to 450 Da. Total GC running time is 36 min.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Total phenolics and flavonoid content in the leaves of Delonix regia

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Om Prakash and Yamini, 2007). The flavonoid contents of the extracts in terms of GE (Table 1). Total phenolic content of the ethanolic extract of *D. regia* leaves is 16.00 mg/ 100 g of GE. The value of phenolic content indicates that the plant has antioxidant activity.

GC-MS study

The GC-MS study of *D. regia* leaves has shown many phytochemicals which contributes to the medicinal activity of the plant (Tables 2 and 3). The major components which present Benzenetriol (RT: 6.74), Butyl 8-methylnonyl ester (RT: 13.51), Lupeol (RT: 35.90) and Vitamin E (RT: 29.97). The other compounds like Hexadecanoic acid, 2-hydroxy-1- (RT: 21.20), 1, 6-Anhydro-a-D-glucopyranose (RT: 8.21) and 1,3,5-Benzenetriol also present in the leaves of *D. regia* (Figure 1). Figures 2, 3 and 4 shows mass spectrum and structure of phytol, lupeol and coumarin, 7,8-dihydro-7-hydroxy-6-methoxy-8-oxo- compound which is suggested to be a diterpenoid, triterpenoid and coumarin compound and is used as an anticancer, anti-inflammatory, antioxidant, antimicrobial and diuretic.

Conclusion

The study clearly indicates that the leaf extract was

Table 1. Total phenolics, flavonoids and antioxidant activity in the leaves of *Delonix regia*.

S/N	Parameter analyzed	Values obtained
1	Total phenols (mg/100 g) GE*	16.00
2	Total flavonoids (mg/100 g) GE*	0.20
3	Antioxidant activity (mg/100 g) AEAC**	10.73

The values are mean value of three replicates. Gallic acid equivalent, ascorbic acid equivalent antioxidant capacity.

Table 2. Phytocomponents identified in the ethanolic extract of the leaves of *Delonix regia* by GC-MS.

RT	Name of the compound	Peak area (%)
2.17	Butane, 1,1-diethoxy-2-methyl-	0.49
3.03	Propane, 1,1,3-triethoxy-	0.47
6.74	1,2,3-Benzenetriol[Synonyms: Pyrogallol]	50.51
8.21	1,6-Anhydro-á-D-glucopyranose(levoglucosan)	2.05
9.43	1,3,5-Benzenetriol	1.73
11.16	3-O-Methyl-d-glucose	40.17
12.03	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.81
13.51	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	0.14
15.49	Phytol	1.59
15.92	Coumarin, 7,8-dihydro-7-hydroxy-6-methoxy-8-oxo-	0.18
21.20	Hexadecanoic acid, 2-hydroxy-1 (hydroxymethyl)ethyl ester	0.10
25.41	Squalene	0.10
29.97	Vitamin E	1.19
35.90	Lupeol	0.47

Table 3. Activity of phytocomponents identified in *Delonix regia* extract- by GC-MS.

RT	Name of the compound	Compound nature	**Activity
2.17	Butane, 1,1-diethoxy-2-methyl-	Ether compound	No activity reported
3.03	Propane, 1,1,3-triethoxy-	Ether compound	No activity reported
6.74	1,2,3-Benzenetriol [Synonyms: Pyrogallol]	Pyrogallol	Antioxidant, antiseptic antibacterial, antidermatitic fungicide, pesticide, antimutaginic dye candidicide
8.21	1,6-Anhydro-á-D-glucopyranose (levoglucosan)	Sugar moiety	Preservative
9.43	1,3,5-Benzenetriol	Poly Hydroxy compound	Antioxidant, antiseptic antibacterial, antidermatitic fungicide, pesticide, antimutaginic dye candidicide

Table 3. Contd.

11.16	3-O-Methyl-d-glucose	Sugar moiety	Preservative
12.03	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial, antiinflammatory
13.51	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	Plasticizer compound	Antimicrobial, antifouling
15.49	Phytol	Diterpene	Antimicrobial, antiinflammatory, anticancer diuretic
15.92	Coumarin, 7,8-dihydro-7-hydroxy-6-methoxy-8-oxo-	Coumarin compound	Antimicrobial
21.20	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Fatty acid ester	No activity reported
25.41	Squalene	Triterpene	Antibacterial, antioxidant, antitumour, cancer preventive, immunostimulant, chemo preventive and lipoxygenase-inhibitor pesticide
29.97	Vitamin E	Vitamin compound	Antiageing, analgesic, antidiabatic antiinflammatory, antioxidant, antidermatitic, antileukemic, antitumour, anticancer, hepatoprotective, hypocholesterolemic antiulcerogenic, vasodilator, antispasmodic, antibronchiti and anticoronary
35.90	Lupeol	Triterpenoid compound	Antimicrobial, antiinflammatory and anticancer

**Source: Dr. Duke's phytochemical and ethnobotanical databases (Online database).

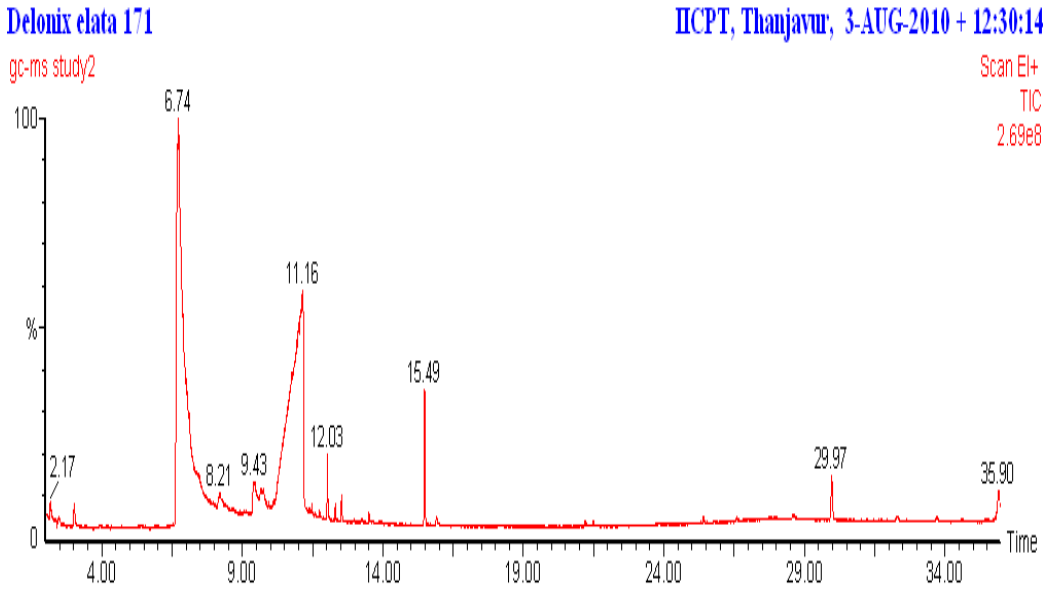
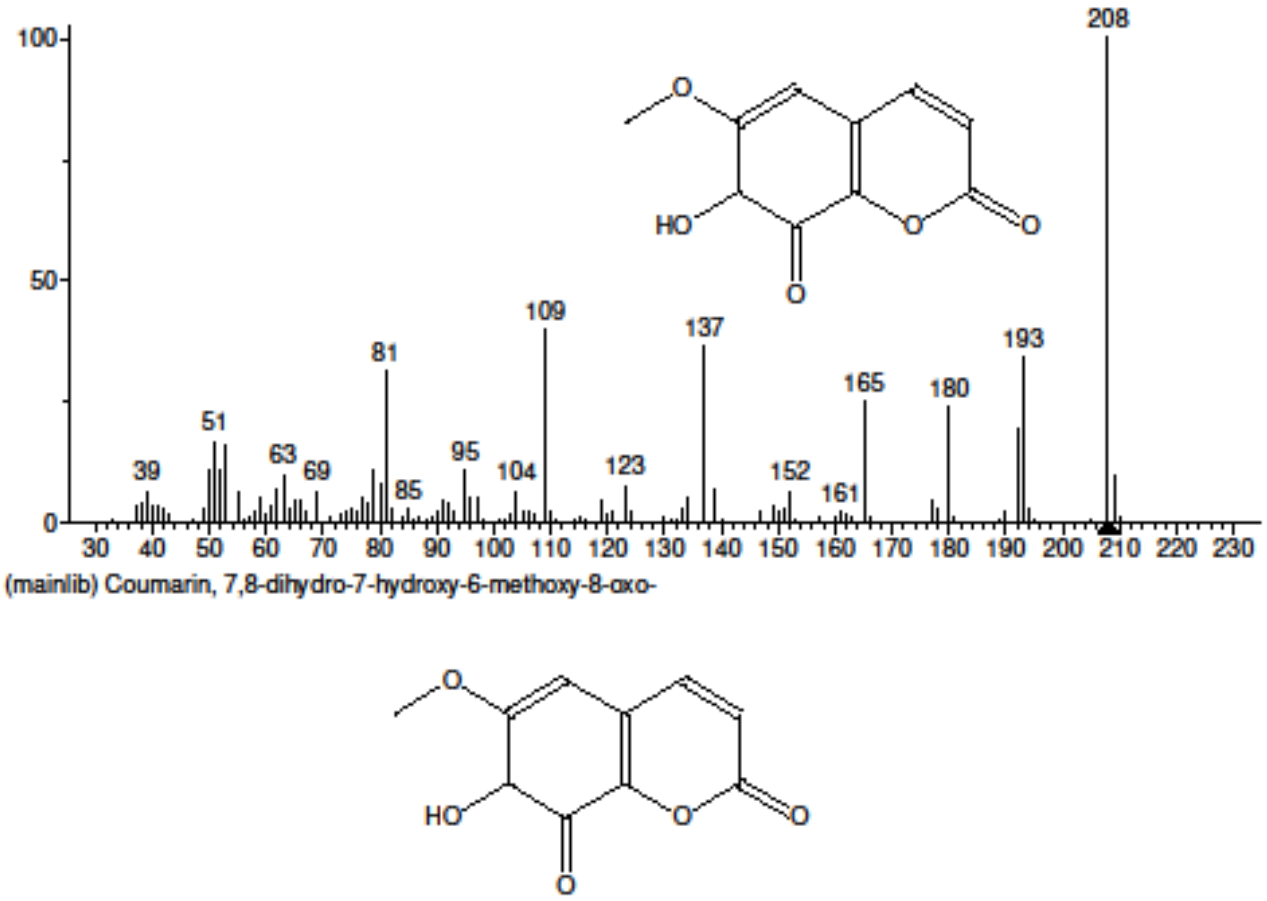


Figure 1. Chromatogram of *Delonix regia* leaves by GC-MS.



Name: Coumarin, 7,8-dihydro-7-hydroxy-6-methoxy-8-oxo-
Formula: $C_{10}H_8O_5$
MW: 208 CAS#: N/A NIST#: 263136 ID#: 114276 DB: mainlib
Other DBs: None
Contributor: A.A.Kutin, Moscow, Russia
10 largest peaks:
208 999 | 109 398 | 137 363 | 193 340 | 81 311 | 165 251 | 180 239 | 192 194 | 51 167 | 53 161 |
Synonyms:
no synonyms.

Figure 2. Mass spectrum and structure of coumarin, 7, 8-dihydro-7-hydroxy-6-methoxy-8-oxo compound.

normal in antioxidants, phenolics and flavonoids. The GC-MS study also showed many Phytochemicals Benzenetriol, Butyl 8-methylnonyl ester, Lupeol, VitaminE, Hexadecanoic acid, 2-hydroxy-1- ,1,6-Anhydro-a-D-glucopyranose, 1,3,5-Benzenetriol, Phytol, Coumarin, 7,8-dihydro-7-hydroxy-6-methoxy-8-oxo-, phytol, lupeol and Squalene which contributes the activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, antiulcerogenic and other activities. This investigation has helped to identify the compounds present in the leaves of *D. regia*, a hitherto uninvestigated species. This investigation has helped to identify the compounds present in the leaves of *D. regia,* evaluation of pharmacological activity in the ethanol extract is in progress.

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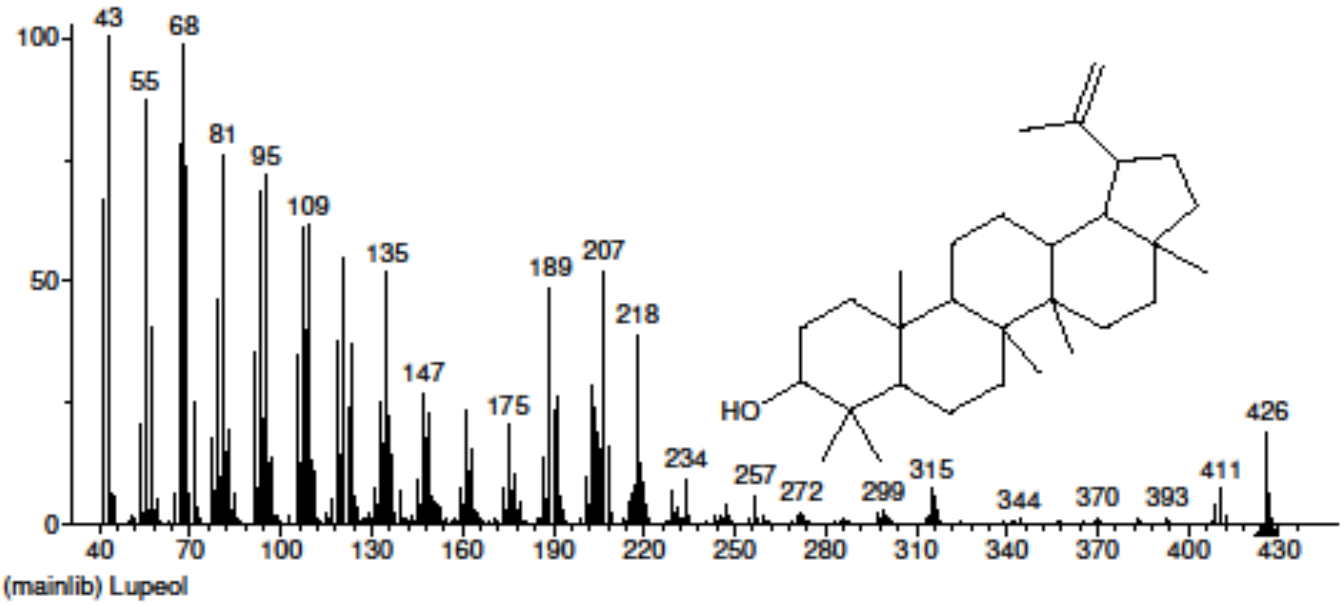
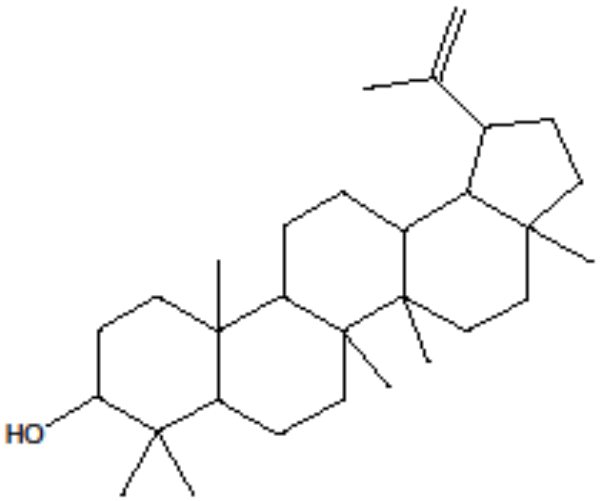
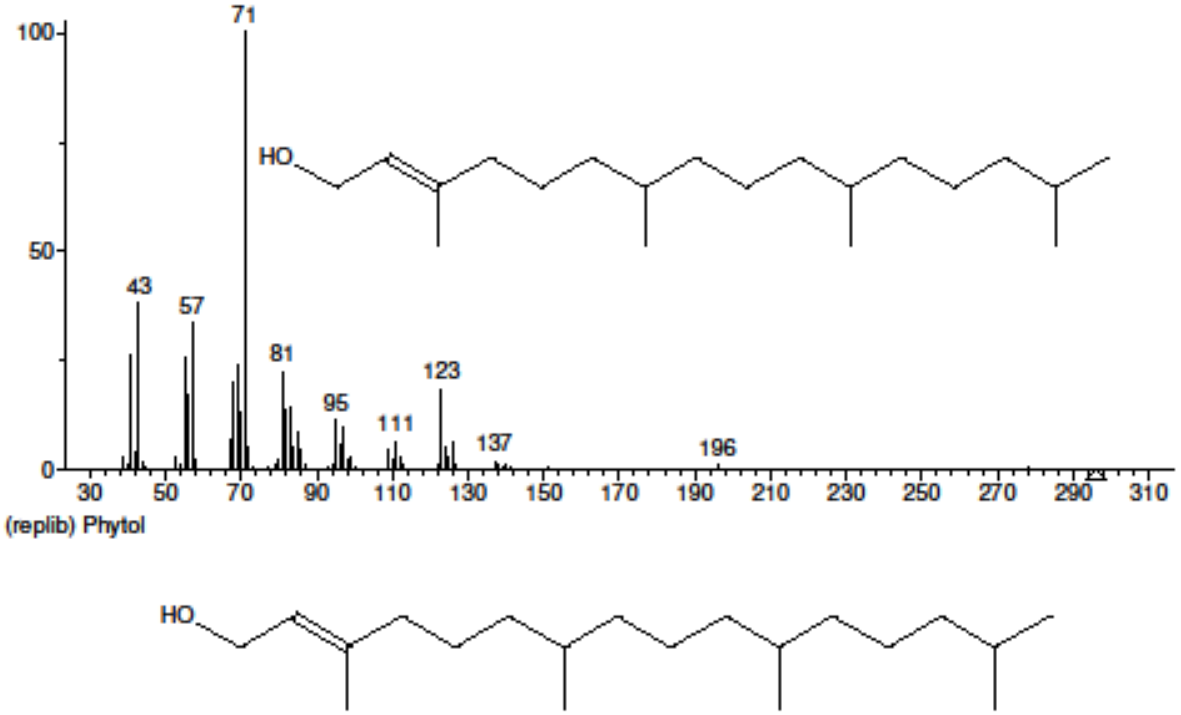




Name: Lupeol
Formula: $C_{30}H_{50}O$
MW: 426 CAS#: 545-47-1 NIST#: 124852 ID#: 7258 DB: mainlib
Other DBs: None
Contributor: H. Fales, LC, NHLBI, NIH, Bethesda, MD 20892
10 largest peaks:
43 999 | 68 983 | 55 869 | 67 782 | 81 759 | 69 733 | 95 716 | 93 684 | 41 667 | 109 613 |
Synonyms:
1.Lup-20(29)-en-3-ol, (3.beta.)-
2.Lup-20(29)-en-3.beta.-ol
3.beta.-Viscol
4.Clerodol
5.Fagarasterol
6.Fagarsterol
7.Lupenol
8.Monogynol B
9.Triterpene lupeol

Figure 3. Mass spectrum and structure of pentacyclic lupeol.



Name: Phytol
Formula: $C_{20}H_{40}O$
MW: 296 CAS#: 150-86-7 NIST#: 108727 ID#: 8157 DB: replib
Other DBs: None
Contributor: Philip Morris R&D
10 largest peaks:
71 999 | 43 381 | 57 334 | 41 260 | 55 259 | 69 239 | 81 223 | 68 199 | 123 184 | 56 169 |
Synonyms:
1.2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
2.trans-Phytol
3.3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4.(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol #

Figure 4. Mass spectrum and structure of phytol.

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