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# GC-MS ANALYSIS OF BIOACTIVE COMPONENTS OF AERIAL PARTS OF *KIRGANELIA RETICULATA* POIR (EUPHORBIACEAE)

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## ABSTRACT

The present investigation was carried out to analyze the active constituents present in aerial parts of *Kirganelia reticulata* (Euphorbiaceae). Twenty one compounds were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The prevailing compounds were 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (20.45%), 2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-, [4ar-(4aa, 6a, 8aa)]- (14.87%), a-Sitosterol (8.11%), Hexadecanoic acid, ethyl ester (7.53%), Vitamin E (6.69%), Phenol,2,3,5-trimethyl- (6.64%), Lupeol (4.79%), Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl- (4.72%),Phytol (4.58%),Stigmasterol (4.51%).

**Key words**: *Kirganelia reticulata*, Scandent shrub, GC-MS, Bioactive components.

## INTRODUCTION

*Kirganelia reticulata* Poir is one of the medicinally important plants belonging to Euphorbiaceae is a large, often scandent shrub. The biological work performed so far on this plant showed hypotensive effects and its folkloric use in gastric complaints including colic, constipation etc. and chemical studies demonstrated the presence of octacosanol, teraxerol acetate, friedeline, teraxerone, betulin, sitosterol etc.[1,2] The leaves are employed as a diuretic and cooling medicine. The juice of the leaves is used to cure diarrhoea in infants. The stems are used to treat sore in eyes and the powdered leaf is used in sores, burns, suppurations and chafing of the skin[3]. The bark is used to treat rheumatism, dysentery and venereal diseases[4]. The plant is used for a variety of ailments including small pox, syphilis, asthma, diarrhoea, bleeding gums[5,6]. It is also claimed to have antidiabetic activity in tribal areas, which has been validated[7]. The antibacterial potential of the leaf extracts of this plant has been evaluated recently[8]. By virtue of their photosynthetic machinery, leaves serve as sink for several metabolites and as an important source of several bioactive compounds[9,10]. The main objective of the present study is to analyze the various phytochemical constituents found in aerial parts of *Kirganelia reticulata* using GC-MS study.

## EXPERIMENTAL

### Materials and methods

The aerial parts of *Kirganelia reticulata* Poir were collected from Erachakulam, Kanyakumari District, Tamil Nadu. The aerial parts were shade dried and pulverized to powder in a mechanical grinder.

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Required quantity of powder was weighed and transferred to stoppered flask and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

### GC-MS analysis

GC-MS analysis of the extract was 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 (30 mm X 0.25 mm 1 D X 1 μMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 mL/min and an injection volume of 2 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 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass.

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

The components present in the ethanol extract of aerial parts of *Kirganelia reticulata* was identified by GC-MS (Fig. 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extracts of aerial parts of *K. reticulata* are presented in Table 1.

**Table 1: Phytoconstituents in ethanol extract of aerial parts of *Kirganelia reticulata***

| S. No. | RT | Name of the compound | Molecular formula | MW | Peak area % |
|---|---|---|---|---|---|
| 1 | 7.36 | Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- | $C_{15}H_{22}$ | 202 | 4.72 |
| 2 | 7.67 | 12,15-Octadecadiynoic acid, methyl ester | $C_{19}H_{30}O_2$ | 290 | 2.37 |
| 3 | 10.54 | Phenol, 2,3,5-trimethyl- | $C_9H_{12}O$ | 136 | 6.64 |
| 4 | 10.81 | Ethyl iso-allocholate | $C_{26}H_{44}O_5$ | 436 | 1.39 |
| 5 | 11.20 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 20.45 |
| 6 | 11.47 | 9,12-Octadecadienoic acid (Z,Z)- | $C_{18}H_{32}O_2$ | 280 | 3.21 |
| 7 | 12.98 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284 | 7.53 |

Cont…

| S. No. | RT | Name of the compound | Molecular formula | MW | Peak area % |
|---|---|---|---|---|---|
| 8 | 14.43 | Phytol | $C_{20}H_{40}O$ | 296 | 4.58 |
| 9 | 15.07 | Linoleic acid ethyl ester | $C_{20}H_{36}O_2$ | 308 | 0.63 |
| 10 | 15.16 | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- | $C_{20}H_{34}O_2$ | 306 | 0.49 |
| 11 | 18.25 | Nonadecane, 2-methyl- | $C_{20}H_{42}$ | 282 | 0.67 |
| 12 | 19.65 | Nonadecane | $C_{19}H_{40}$ | 268 | 1.42 |
| 13 | 21.04 | Octadecane, 2-methyl- | $C_{19}H_{40}$ | 268 | 1.72 |
| 14 | 22.42 | Octacosane | $C_{28}H_{58}$ | 394 | 2.00 |
| 15 | 23.79 | Eicosane, 2-methyl- | $C_{21}H_{44}$ | 296 | 1.51 |
| 16 | 23.96 | Squalene | $C_{30}H_{50}$ | 410 | 1.72 |
| 17 | 25.15 | 2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-, [4ar-(4aà,6à,8aá)]- | $C_{15}H_{22}O_2$ | 234 | 14.87 |
| 18 | 28.20 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 6.69 |
| 19 | 30.09 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 4.51 |
| 20 | 31.23 | á-Sitosterol | $C_{29}H_{50}O$ | 414 | 8.11 |
| 21 | 32.92 | Lupeol | $C_{30}H_{50}O$ | 426 | 4.79 |

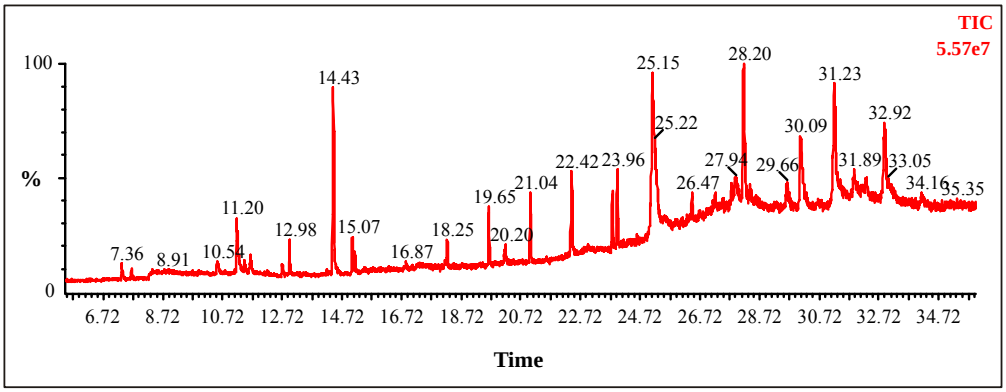


**Fig. 1: GC-MS Chromatogram of ethanol extract of aerial parts of *Kirganelia reticulata***

Twenty one compounds were identified in the ethanol extract of aerial parts of *K. reticulata*. The prevailing compounds were 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (20.45%), 2(1H)-Naphthalenone, 4a,5, 6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-, [4ar-(4aa,6a,8aa)]-(14.87%), a-Sitosterol (8.11%), Hexadecanoic acid, ethyl ester (7.53%), Vitamin E (6.69%), Phenol,2,3,5-trimethyl- (6.64%), Lupeol (4.79%), Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl-(4.72%), Phytol (4.58%), Stigmasterol (4.51%), 9,12-Octadecadienoic acid (Z,Z)- (3.21%), 12, 15-Octadecadiynoic acid, methyl ester (2.37%), Octacosane (2.00%), Octadecane 2-methyl-(1.72%), Squalene (1.72%),Eicosane, 2-methyl-(1.51%), Nonadecane (1.42%), Ethyl iso-allocholate (1.39%). Figs. 2,3,4,5,6,7,8,9,10,11 and 12 show the mass spectrum and structure of phenol, 2,3,5-trimethyl-, ethyl iso-allocholate,3,7,11,15-tetramethyl-2-hexadecen-

1-ol, 9,12-octadecadienoic acid (Z,Z)-, hexadecanoic acid ethyl ester, phytol, linoleic acid ethyl ester, 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)-, squalene, stigmasterol and lupeol. Table 2 listed the major phytocompounds and its biological activities obtained through the GC-MS study of the aerial parts of *K. reticulata*.

**Table 2: Activity of components identified in the aerial parts of *Kirganelia reticulata***

| S. No. | RT | Name of the compound | Molecular formula | MW | Peak area% | Compd. nature | **Activity |
|---|---|---|---|---|---|---|---|
| 1 | 10.54 | Phenol, 2,3,5-trimethyl- | $C_9H_{12}O$ | 136 | 6.64 | Phenolic compound | Antimicrobial, antioxidant, anti-inflammatory |
| 2 | 10.81 | Ethyl iso-allocholate | $C_{26}H_{44}O_5$ | 436 | 1.39 | Steroid | Antimicrobial, anticancer antiarthritic, antiasthma, diuretic, anti-inflammatory |
| 3 | 11.20 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 20.45 | Terpene alcohol | Antimicrobial, anti-inflammatory |
| 4 | 11.47 | 9,12-Octadecadienoic acid (Z,Z)- | $C_{18}H_{32}O_2$ | 280 | 3.21 | Linoleic acid | Hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, anti androgenic, hypocholesterolemic 5-alpha reductase inhibitor, antihistaminic anticoronary, insectifuge, antieczemic, antiacne |
| 5 | 12.98 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284 | 7.53 | Palmitic acid ester | Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic, flavor, hemolytic, 5-alpha reductase inhibitor |
| 6 | 14.43 | Phytol | $C_{20}H_{40}O$ | 296 | 4.58 | Diterpene | Antimicrobial, anticancer, diuretic, anti-inflammatory |
| 7 | 15.07 | Linoleic acid ethyl ester | $C_{20}H_{36}O_2$ | 308 | 0.63 | Linoleic acid ethyl ester | Hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, anti androgenic, hypocholesterolemic 5-alpha reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne |
| 8 | 15.16 | 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- | $C_{20}H_{34}O_2$ | 306 | 0.49 | Linolenic acid ethyl ester | Hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, anti-androgenic, hypocholesterolemic 5-alpha reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne |
| 9 | 23.96 | Squalene | $C_{30}H_{50}$ | 410 | 1.72 | Triterpene | Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide |

Cont…

| S. No. | RT | Name of the compound | Molecular formula | MW | Peak area% | Compd. nature | **Activity |
|---|---|---|---|---|---|---|---|
| 10 | 28.20 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 6.69 | Vitamin E compound | Antiageing, analgesic, antidiabetic, anti inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary |
| 11 | 30.09 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 4.51 | Steroid | Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory |
| 12 | 31.23 | á-Sitosterol | $C_{29}H_{50}O$ | 414 | 8.11 | Steroid | Antimicrobial, anticancer, antiarthritic, antiasthma, diuretic, anti-inflammatory |
| 13 | 32.92 | Lupeol | $C_{30}H_{50}O$ | 426 | 4.79 | Triterpene compound | Antimalarial, antioxidant, antiflue, antihyperglycemic, antitumor, antiviral, pesticide, cytotoxic, anti-inflammatory |

**Source: Dr. Duke's : Phytochemical and Ethnobotanical databases

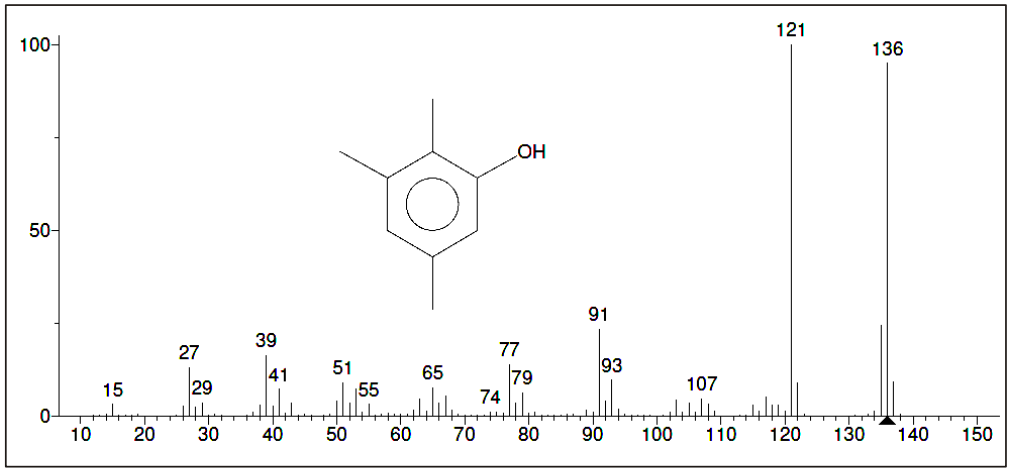


**Fig. 2: Mass spectrum and structure of Phenol, 2,3,5- trimethyl-**

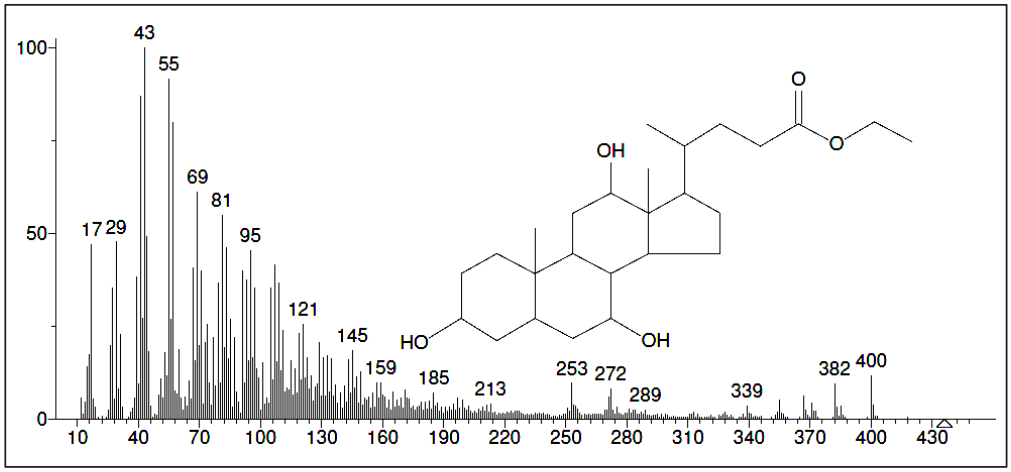


**Fig. 3: Mass spectrum and structure of Ethyl iso-allocholate**

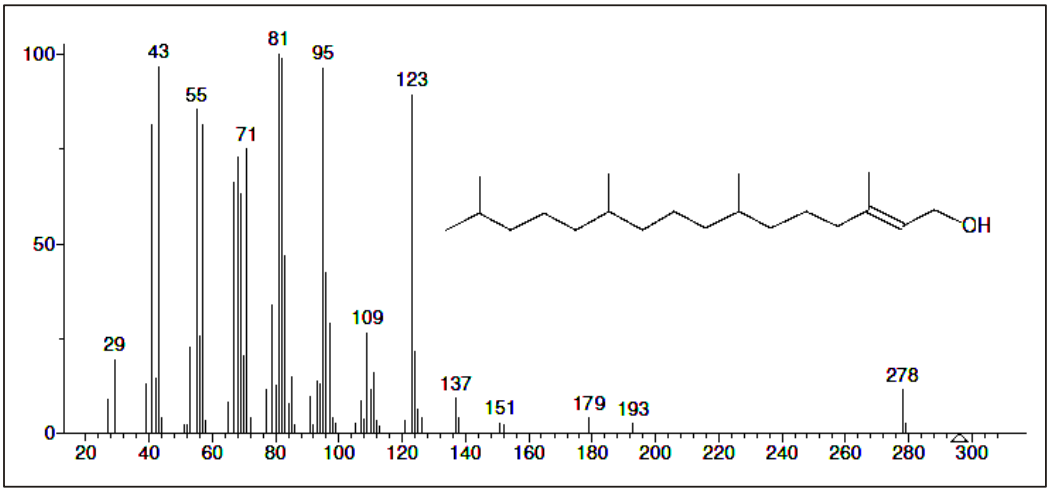


**Fig. 4: Mass spectrum and structure of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol**

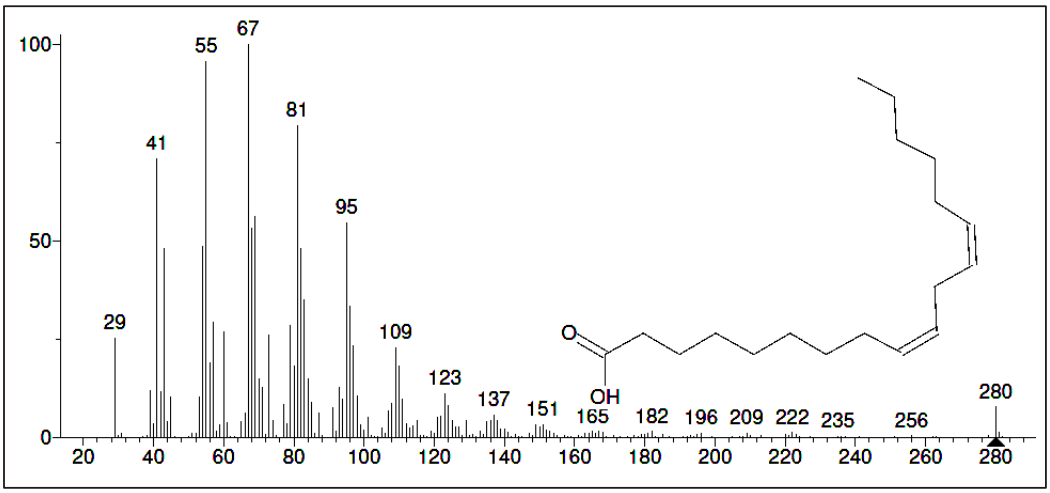


**Fig. 5: Mass spectrum and structure of 9,12-Octadecadienoic acid (Z,Z)-**

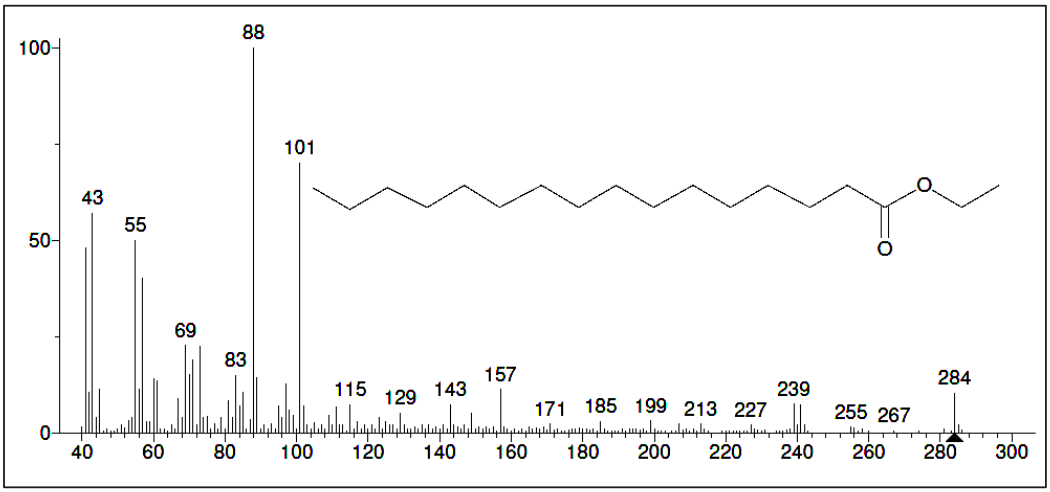


**Fig. 6: Mass Spectrum and structure of Hexadecanoic acid, ethyl ester**

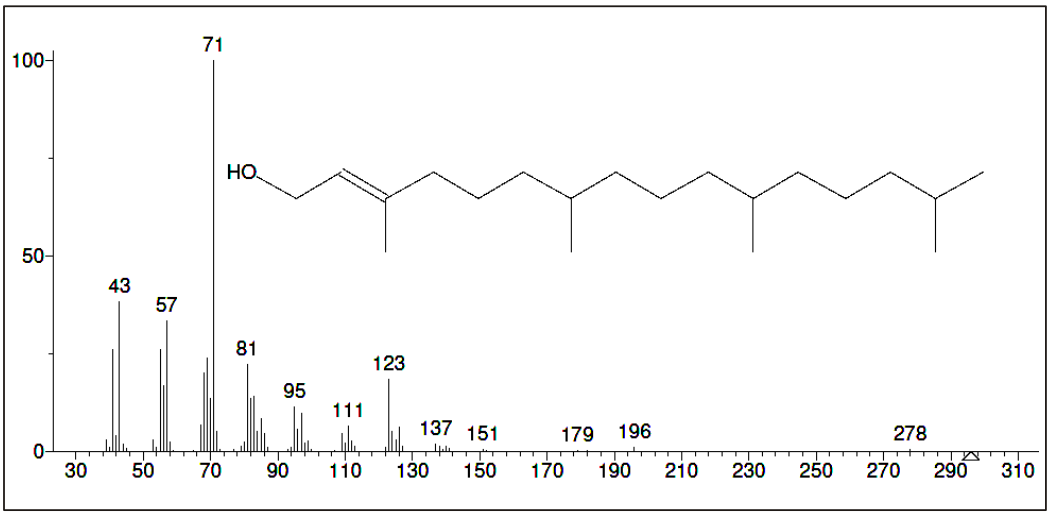


**Fig. 7: Mass Spectrum and structure of Phytol**

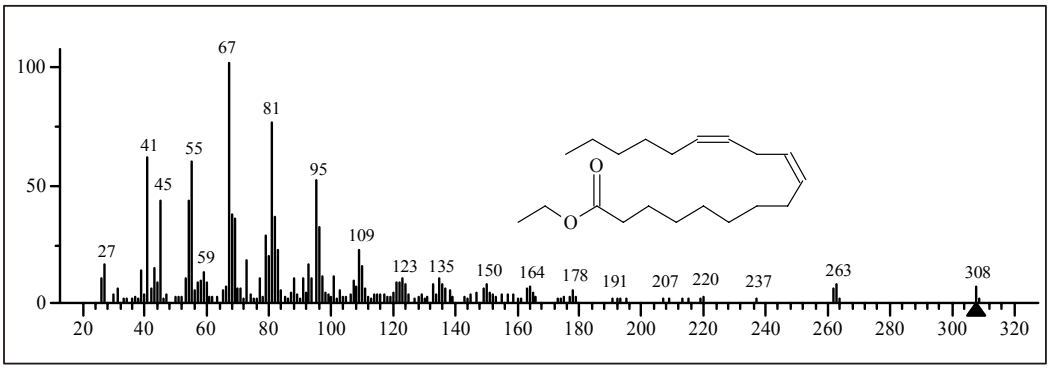


**Fig. 8: Mass Spectrum and structure of Linoelic acid ethyl ester**

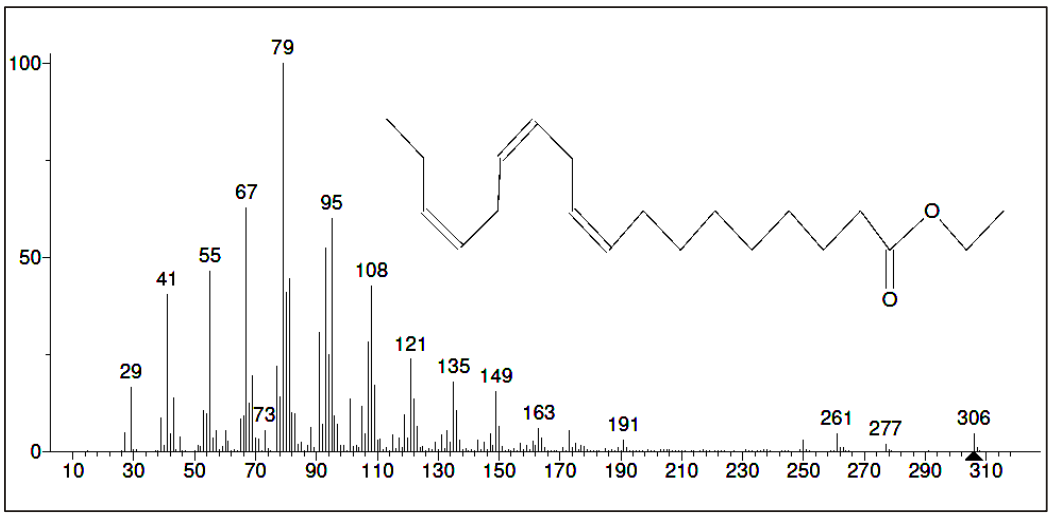


**Fig. 9: Mass Spectrum and structure of 9, 12, 15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)**

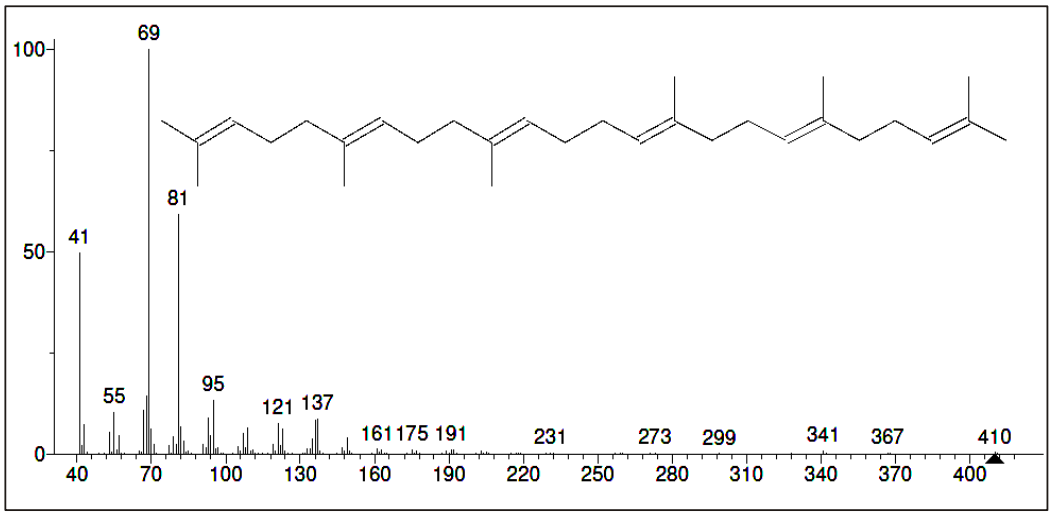


**Fig. 10: Mass spectrum and structure of Squalene**

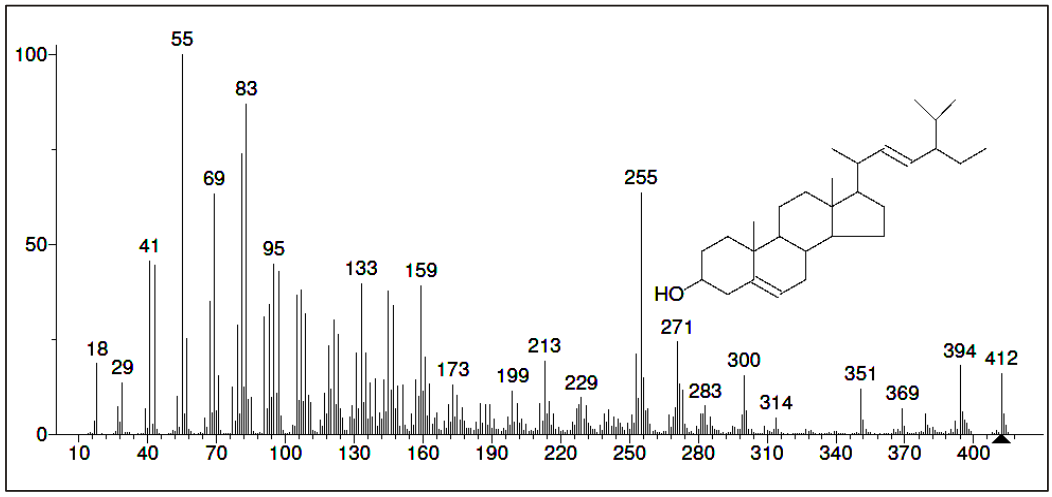


**Fig. 11: Mass spectrum and structure of Stigmasterol**

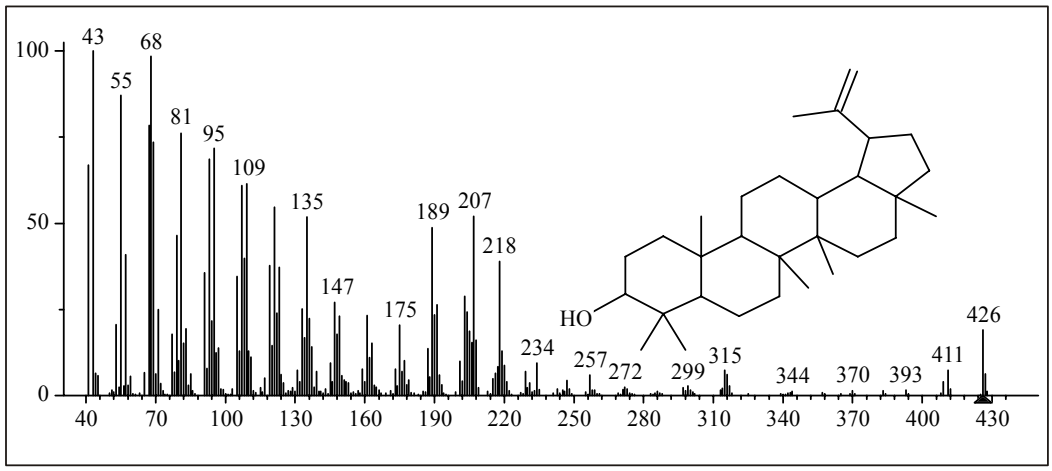


**Fig. 12: Mass spectrum and structure of Lupeol**

Among the identified phytochemicals, hexadecanoic acid ethyl ester, squalene have the property of antioxidant activity[11,12]. Recently squalene possesses chemopreventive activity against colon carcinogenesis[13,14]. Phenolic compounds are a class of antioxidant agents which act as free radical terminators[15]. Phytol is detected in *K. reticulata* aerial parts, which was also found to be effective in different stages of arthritis. It was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases[16]. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K[17]. 9,12, octadecadienoic acid (Z,Z)- has the property of anti-inflammatory and antiarthritic as reported by earlier worker[18]. Stigmasterol is used as a precursor in the manufacture of semi synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanism related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens and corticoids. Lupeol exhibits a broad spectrum of biological activities and can be used as antiprotozoal, anti-inflammatory, antitumour and chemopreventive agents[19]. The above said compounds found in the ethanol extract of *K. reticulata* aerial parts which are being used for the pharmacological work. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. However, isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *K. reticulata* contains various bioactive compounds. So it is recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

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