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GC-MS Analysis of Bioactive components of *Cordia retusa* (Boraginaceae)

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ABSTRACT

Plan: *The present study is structured to analyse the chemical constituents of the plant Cordia retusa (vahl.), belongs to the family Boraginaceae using GC-MS.*
Methodology: *The ethanolic crude extract of aerial part of plant C. retusa was analyzed. The GC Clarus 500 (Perkin Elmer) used in the investigation employed a column packed with Elite- 5MS (5%Diphenyl / 95% Dimethyl poly siloxane, 30mm x 0.25mm x0.25µmdf) and the components were separated using Helium (1mL/min) as the carrier gas. The 2 µl sample extract injected into the instrument was detected by the Turbo mass gold detector (Perkin Elmer) with the aid of the Turbo mass 5.2 software.*
Outcome*: Qualitative analysis of ethanolic crude extract of C.retusa by using GC MS showed that the presence of fourteen different phytochemical compounds. The components were recognized by comparing their retention time and fragmentation patterns with those data stored in the National Institute of Standard and Technology (NIST) library. The reported chief constituents are Alpha Amyrin, (1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- and 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-,acetate, (3á,4à,5à).*
Keywords: *Cordia retusa (vahl.), GC-MS, NIST, Secondary metabolites*

1. INTRODUCTION

Herbal Plants have been widely used for medicinal purposes for lots of centuries. At the moment, herbal medicines are being engaged worldwide in a variety of health care settings and as home medication. In some developing countries, communities rely profoundly on traditional health practitioners and medicinal plants to meet their primary health care requirements. From the period of the last decade, employ of traditional medicine has long-drawn-out globally and has gained reputation.

With the remarkable development in the use of traditional medicine worldwide, assurance and potency as well as quality control of herbal medicines and traditional therapies have turn out to be important concerns for both health authorities and the public. Essential products from medicinal plants, both as isolated compounds or as extracts, provide unrestricted opportunities for new drug leads.

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Due to mounting demand for chemical multiplicity in testing programs, searching therapeutic drugs from natural products has grown all over the Universe. Botanicals and herbal formulations for medicinal usage enclose a variety of bioactive compounds[1]. In many urbanized countries well-liked use of Complementary and Alternative Medicine (CAM) is fuelled by concern about the undesirable effects of synthetic drugs. In budding countries, broad use of Traditional Medicine (TM) is often attributable to its ease of access and availability. For the last few years, there has been a global trend for the regeneration of awareness in the traditional system of treatments. Simultaneously investigation of medicinal plants using indigenous medical systems has become ever more important for speed up better and effective treatment[2].

There is still a noteworthy lack of research records in this field. In the absence of pharmacopoeia statistics on a variety of plant extracts, it is not doable to isolate or regiment the active contents having the desired effects from plants[3]. Screening of active components from plants has unswerving to the development of new medicinal drugs which have well-organized fortification and treatment role against various diseases[4]. *C. retusa* (Family: *Boraginaceae*) is an evergreen shrub to undersized tree. Leaves are in clusters of 3-5, blade obovate or oblanceolate. Flowers are 3-12 flowered scorpioid cymes, unbranched or branched once. Fruit are globose, 4-5 mm in diameter, ripening fruits are brownish orange in color[5].

C. retusa leaf decoction is being used to treat cough and stomach ache, root as antidote[6]. The sap of leaves is taken internally for three days prior to and later than the menstrual period for three to four months to intensify fertility[7]. The leaves are used to make tea which is used for abdominal colic and for the treatment of diarrhea and dysentery. Further, the leaves are anti-inflammatory[8]. Lot of medicinal plants and their purified constituents have shown advantageous therapeutic potentials. Today, rummage around for natural compounds rich in antioxidant and antimicrobial properties are getting higher due to their medicinal properties in controlling many interrelated chronic diseases[9]. Ehretianone a quinoid xanthane was isolated from methanol extract of the root bark which possesses the anti-snake venom activity [10]. With these circumstances, the present study meant to spot out the phytoconstituents present in ethanolic extract of *C. retusa* by means of GC-MS analysis.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Plant material

The aerial parts of plant *C. retusa* were collected from the natural habitats of Kalakad, Thirunelveli District of Tamilnadu, India. The plant was authenticated by Botanist Dr. V. Chelladurai, Research officer-Botany (Retd.), Central council for research in Ayurveda and Siddha, Government of India and the herbarium of voucher specimen number T135 has been deposited at the herbarium of Entomology research Institute, Loyola College, Chennai (India). The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The aerial parts of plant were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

2.2. Extraction

Fifty grams of powdered sample was extracted with ethanol overnight and filtered through ash less filter paper with anhydrous sodium sulphate and the extract was concentrated. The % yield of this extraction was 14%. The extract was analyzed using the Clarus 500 GC-MS (Perkin Elmer). 2 µL of the ethanolic extract of *C.retusa* was employed for GC-MS analysis.

2.3. GC-MS analysis

The Clarus 500 GC (Perkin Elmer) used in this analysis. It employed a fused silica column packed with Elite -5MS (5%Diphenyl / 95% Dimethyl poly siloxane, 30mm x 0.25mm x0.25µm df) and the components were separated using helium as carrier gas at a constant flow of 1 mL/ min. The 2 µL sample extract injected into the instrument. It was detected by the turbo gold mass detector (Perkin Elmer) with the aid of Turbo mass 5.2 software. During the GC process the oven was maintained at a temperature of 110^0c with 2 min holding. The injector temperature was set at 250^0c.
The different parameters involved in the operation of the Clarus 500 MS were also standardized. The inlet line temperature was 200^0C and source temperature was 200^0C. Mass spectra were taken at 70 eV; a scan period of 0.5 s and fragments from 45-450 Da. The MS detection was completed in 36 min. The NIST ver. 2.0 year 2005 library was employed for detection.

3. RESULTS

The consequences concerning to GC-MS investigation led to the recognition of lot of compounds from the GC fractions of the ethanolic extract of *C. retusa*. These compounds were acknowledged through mass spectrum attached with GC. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) are accessible in Table 1.

The outcome exposed that the presence of 1,6-Octadiene, 3,7-dimethyl-,(S)-(0.26%), trans-2-Undecen-1-ol(0.57%), 1,14-Tetradecanediol (0.13%), Phytol (1.04%), 1-Iodo-2-methylundecane (0.07%), 3-Hexadecycloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion (0.17%), Octadecane, 1-(ethenyloxy)- (0.17%), 2-[(2-methoxyphenyl)-(5-methyl-2-furyl)-methyl]-5-methyl-furan (0.52%), Squalene (1.24%), Vitamin E (1.82%), 2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- (21.54%), à-Amyrin (59.24%), 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-,acetate, (3á,4à,5à)- (11.03%),d-Norandrostane (5à,14à) (2.19%). The spectrum sketch of GC-MS deep-rooted the presence of fourteen components with the retention time 10.82, 10.97, 11.41, 14.11, 17.76, 19.14, 21.88, 22.85, 23.42, 27.60, 31.14, 32.22, 33.82, 33.92 min correspondingly which is shown in Figure. 1. The individual fragmentation patterns of imperative components were illustrated in Figures A-H.

The present study characterized the chemical profile of *C. fruticosa* using GC-MS. The GC chromatogram shows the relative concentration of various compounds getting eluted as a function of retention time.

The heights of the peak point out the relative concentration of the presented components. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are figure print of that compound which can be identified from the National Institute of Standards and Technology (NIST) data library. 1, 6-Octadiene, 3, 7-dimethyl-, (S), is recommended to be an alkenes compound. Trans-2-Undecen-1-ol and 1, 14-Tetradecanediol are recommended to be an alcohol in nature[11]. Phytol is recommended to be a di-terpene compound and it might act as an antimicrobial, anticancer, anti-inflammatory and diuretic[11, 12, 13]. 1-Iodo-2-methylundecane is recommended to be an iodo compound and it might act as an antimicrobial and it improves sexual activities[12,14,15]. 3-Hexadecycloxycarbonyl-5-(2-hydroxyethyl)-4-methyl imidazolium ion is recommended to be a carbonyl compound. Octadecane, 1-(ethenyloxy) – and 2-[(2-methoxyphenyl)-(5-methyl-2-furyl)-methyl]-5-methyl-furan are recommended to be an alkane compound. Squalene is suggested to be a tri-terpene compound and it may act as an antibacterial, antioxidant, antitumor, immunostimulant, chemopreventive, lipoxygenase inhibitor and anti HIV[11, 12, 13]. Vitamin E is a vitamin compound and it may acts as an antiageing, analgesic, antidiabetic, anti inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, antiulcerogenic, antispasmodic, antibronchitic and anticoronary, hypocholesterolemic, vasodilator and hepatoprotective[12,13]. 2(1H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)- is recommended to be a ketone and act as an anti inflammatory. Alpha Amyrin is suggested to be a tri-terpenoid and acts as an antitumor. d- Norandrostane (5à, 14à) is suggested to be a steroid nucleus. Table 2 shows the nature of compound and biological activity of the predicted compounds.

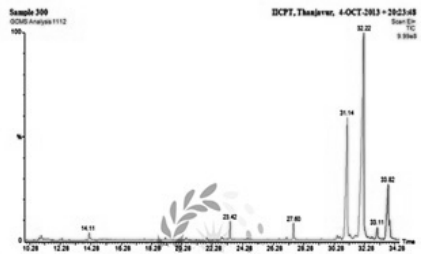
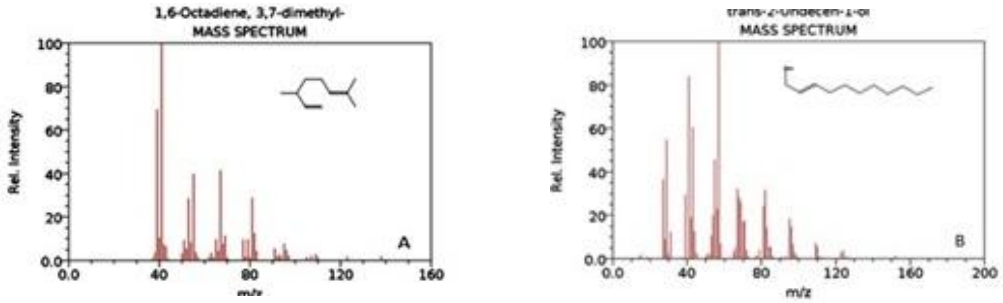


Fig 1- The GC-MS chromatogram of ethanolic extract of *C. retusa*



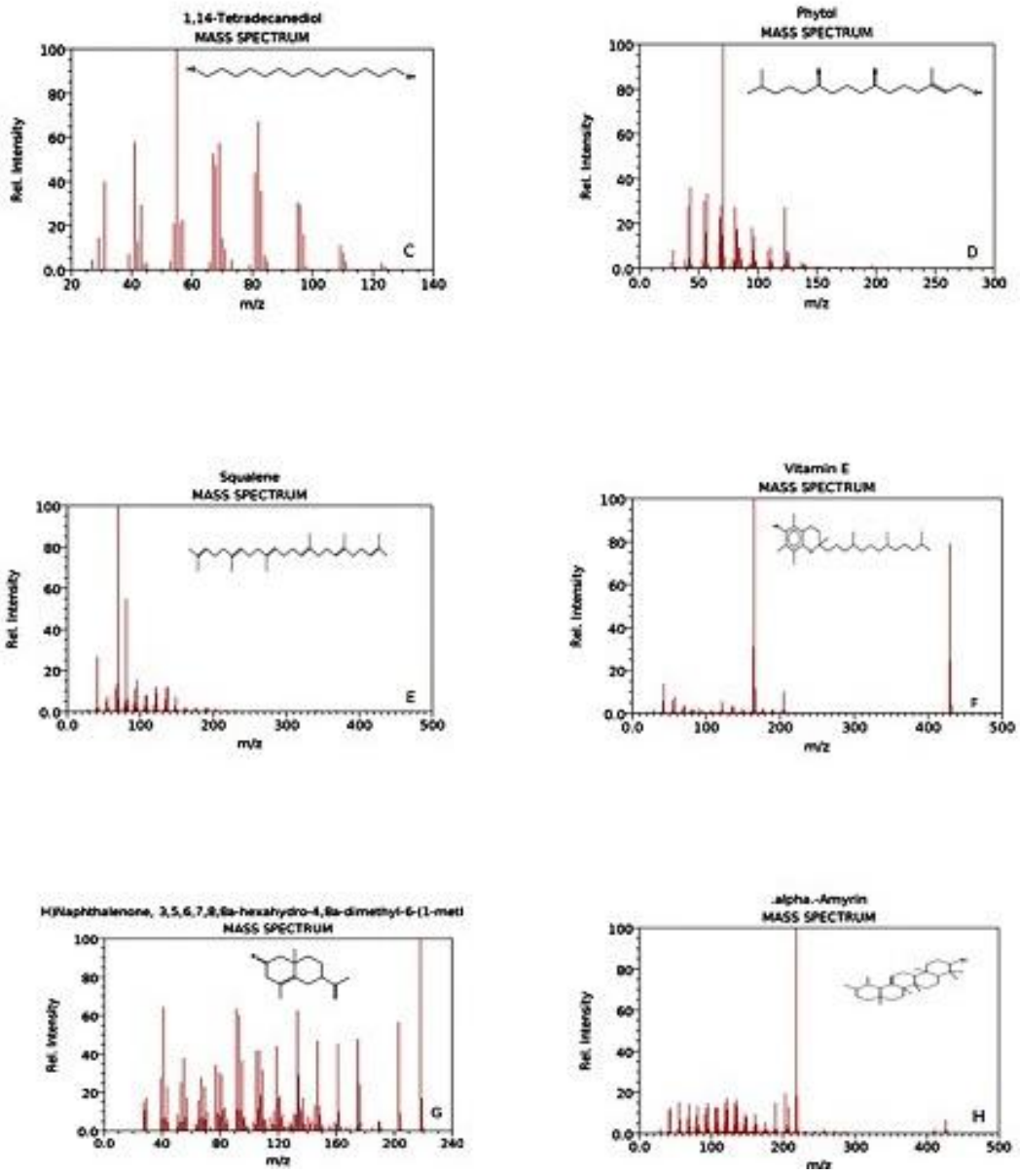


Fig A-H: The individual fragmentation pattern of the important compounds

Table1- Components identified in ethanol extract of aerial parts of C. retusa

S. No	R_t	*Name of the compound*	*Molecular Formula*	*Mol.Weight*	*Peak Area %*
1	10.82	1,6-Octadiene, 3,7-dimethyl-, (S)-	$C_{10}H_{18}$	138	0.26
2	10.97	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170	0.57
3	11.41	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230	0.13
4	14.11	Phytol	$C_{20}H_{40}O$	296	1.04
5	17.76	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	0.07
6	19.14	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409	0.17
7	21.88	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	0.17
8	22.85	2-[(2-methoxyphenyl)-(5-methyl-2-furyl)-methyl]-5-methyl-furan	$C_{18}H_{18}O_3$	282	0.52
9	23.42	Squalene	$C_{30}H_{50}$	410	1.24
10	27.6	Vitamin E	$C_{29}H_{50}O_2$	430	1.82
11	31.14	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methyl ethenyl)-	$C_{15}H_{22}O$	218	21.54
12	32.22	α-Amyrin	$C_{30}H_{50}O$	426	59.24
13	33.82	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3á,4à,5à)-	$C_{32}H_{52}O_2$	468	11.03
14	33.92	d-Norandrostane (5à,14à)	$C_{18}H_{30}$	246	2.19

Table 2- Activity of phyto-components identified in ethanol extract of Aerial parts of *C.retusa*

S.No	*Name of the compound*	*Compound nature*	*Activity*
1	1,6-Octadiene, 3,7-dimethyl-, (S)-	Alkene	No activity reported
2	trans-2-Undecen-1-ol	Alcohol	No activity reported
3	1,14-Tetradecanediol	Alcohol	Antimicrobial
4	Phytol	Diterpene	Anti microbial, Antinflammatory, Cancer preventive, Diuretic
5	1-Iodo-2-methylundecane	Iodo	Antimicrobial, Enhance reproductive activities
6	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	Carbonyl	No activity reported
7	Octadecane, 1-(ethenyloxy)-	Alkane	No activity reported
8	2-[(2-methoxyphenyl)-(5-methyl-2-furyl)-methyl]-5-methyl-furan	Alkane	No activity reported
9	Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemopreventive, Lipoxygenase inhibitor, Anti HIV.
10	Vitamin E	Vitamin	Antiageing, Analgesic, Antidiabetic, Anti inflammatory, Antioxidant, Antileukemic, Antitumor, Anticancer, Anti coronary, Vasodilator, Hepatoprotective
11	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	Ketone	Anti inflammatory
12	α-Amyrin	Triterpenoid	Antitumor
13	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3á,4à,5à)-	Alkene	No activity reported
14	d-Norandrostane (5à,14à)	Steroid	No activity reported

4. DISCUSSION

Gas Chromatography- Mass Spectrometry (GC-MS) is a precious tool for reliable detection of bioactive constituents. This study results were interpreted. By interpreting these compounds, it is found that *C. fruticosa* possesses various therapeutical applications.

5. CONCLUSION

Copious phytochemical transmission studies have been carried out in different parts of world using GC-MS. The current study characterized the chemical profile of *C. retusa* using GC-MS. The gas chromatogram shows the comparative concentration of various compounds getting eluted as a purpose of retention time. The heights of the peak point toward the relative concentration of the components exist in the plant extract. The Mass analyzer analyzes the compounds eluted at different times to recognize the nature and structure of the compounds. These mass spectra are figure print of that compound which can be recognized from the data library. Occurrence of various bioactive compounds confirms the application of *C.retusa* for a variety of ailments. A quantity of compounds has previously been reported from a number of other plant species. Thus the detection of a good number of compounds from *C. retusa* might have some biological connotation. Further research is in progress for the isolation of individual phytochemical constituents which may act as templates for novel drug molecules.

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