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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS USING METHANOLIC EXTRACTS OF NARINGI CRENULATA (ROXB.) NICOLSON LEAVES.

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Abstract:

Herbs are very important for a researcher as most of the pharmaceutical companies depend on these plants for synthesizing various novel compounds. The crude plant extracts which is usually a complex mixture of various bioactive compounds including its secondary metabolites forms the effective herbal medicines. The aim of the current study is to determine the bioactive compounds from the methanolic extracts of the leaves of Naringi crenulata (Roxb.) Nicolson (MENC) *using GC-MS. This analysis was done by standard protocol using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II, the extract was matched with the National Institute of Standards and Technology (NIST) library. 26 components from leaves of the above said plant were identified. The GC-MS analysis disclosed the presence of various important phytoconstituents like carotene, lycopene, lycoxanthin, astaxanthin and vitamin D which is responsible for its antioxidant property. It was also observed that the presence of hexadecanoic acid revealed its anti-inflammatory activity, followed by stigmast has an anti- diabetic activity and cycloheptasiloxane has an emollient activity. These findings support the traditional use of Naringi crenulata in various disorders. Further studies are needed to isolate active principle of the extract as well as to elucidate their exact mechanism of action in various disorders.*

Keywords: *Naringi crenulata, bioactive compounds, GC-MS analysis,* MENC.

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INTRODUCTION:

Free radicals play a crucial role in the development of tissue damage in pathological events. Antioxidants are chemical compounds which have the ability to quench the free radicals and thereby it prevents the human body against various diseases. Plants are the rich sources of antioxidants which contain secondary metabolites such as phenolic and flavonoid compounds commonly which act as antioxidants with redox and metal chelating properties[1]. Antioxidants are chemical compounds extremely useful to humans, which has the ability to reduce free radicals and/or to decrease their rate of production and lipid peroxidation in human bodies that cause various human diseases and aging[2]. The active principles of many drugs found in plants are secondary metabolites[3]. Hence, for the purpose of scientific validation of traditional medicinal plants or the discovery of lead compounds for use as therapeutic drugs, the active principles in medicinal plants needs to be identified[4] **.**

Naringi crenulata (Roxb.) Nicols. belongs to the family Rutaceae. The trees are upto 8m tall , trunk with thorns ;bark dark grey, smooth; blaze yellowish. Young branchlets terete, glabrous, thorny. Leaves are compound, imparipinnate, 15cm long, alternate, spiral; rachis with oblanceolate wings, glabrous; leaflets 5-7, opposite, sessile, 2-4.5x1-1.5cm, elliptic to obovate, apex emarginated or obtuse, base acute, margin crenulate or irregularly serrulate, glandular punctuate, glabrous; secondary nerves 7-10 pairs, looped near margin; tertiary nerves ad medially ramified. All parts of this tree viz. root, stem, bark, leaf and fruit are used in several ailments. Leaves are used for curing dysentery and epilepsy[5]. The present study was aimed to evaluate the chemical constituents of methanolic extract of the leaves of *Naringi crenulata*. , using GC MS. It was already known that GC-MS is one of the best techniques for the identification of various bioactive compounds like long chain hydrocarbons, branched hydrocarbons etc. in a sample.

MATERIALS AND METHODS:

Collection and authentication of *naringi crenulata (roxb.) Nicolson:*

The leaves of *Naringi crenulata* (Roxb.) Nicolson was collected from Wayanad, Kerala and authenticated by Jithin. M. M Scientist in-charge. The Herbaria with the collection number 3989 was deposited in MS Swaminathan botanical garden herbarium with the accession numbers 654 & 655.

Preparation of plant extracts:

The leaves of *Naringi crenulata (*Roxb.) Nicolson was shade dried and powdered plant material (2Kg) was extracted in a Soxhlet apparatus using solvents of increased polarity like petroleum ether, chloroform and methanol. The solvents from crude extracts were recovered under reduced pressure using rotary vacuum evaporator. The methanol extract was further exposed to column chromatography.

Column chromatography:

The partial purification of methanol extract was carried out by the sequential purification through column chromatography. Activated silica gel (pore size 100-200) was used as a stationary phase , n-hexane, ethyl acetate and methanol in sequence were used as a mobile phase. The crude methanol residue partially dissolved in n-hexane and was triturated with silica and further column chromatography was carried out. First n-hexane fraction was collected followed by ethyl acetate fractions and finally the methanol fraction. Further GC MS analysis was carried out on MENC to confirm the presence of distinguishable phytochemicals.

GC MC of MENC:

The separated methanol fraction of *Naringi crenulata* (Roxb.) Nicolson leaves obtained by column chromatography was subjected to GC-MS analysis. GC/MS analysis of these extracts were performed using the equipment Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm film. The carrier gas used is Helium with at low of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was mainly based on NIST libraries as Wiley, Mainlib, Replip and Tutorial. The constituents were identified after comparison with those available in the computer library attached to the GC-MS instrument and the results obtained have been tabulated.

Identification of phytoconstituents:

Interpretation of GC-MS mass-spectra were carried out using the database of National Institute Standard and Technology- 2008 (NIST-2008) having more than 62,000 patterns. The spectrums of the unknown components were compared with the spectrum of known components of NIST library and the parameters viz., R match, F match, probability, molecular formula, molecular weight , structure of the components, followed by retention time, peak name,

Res type, Peak area, amount and the R match of the targeted compounds were analyzed.

RESULT & DISCUSSION:

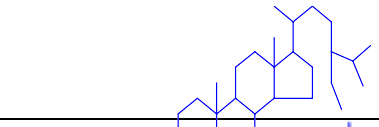
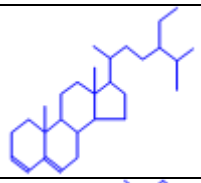
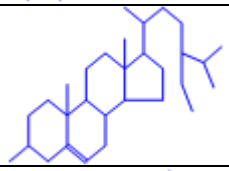
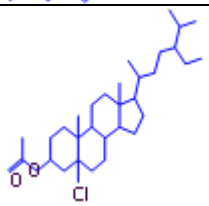
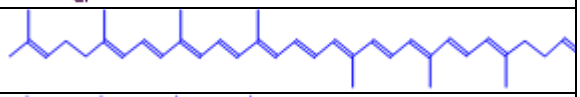
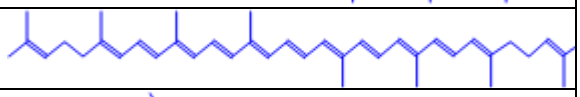
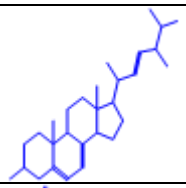
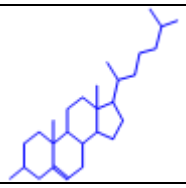
Medicinal plants are the resources of new drugs. Many of the modern medicines are produced indirectly from the medicinal plants. They have contributed many ingredients to fight against various diseases and illness. The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations.Studying of medicinal plants also facilitates to comprehend plant toxicity and also helps to protect human and animals from natural poisons. Hence the present study was undertaken to find out the bioactive compounds present in them methanolic extract of *Naringi crenulata* by using Gas chromatography and Mass spectroscopy.

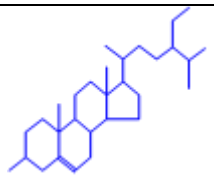
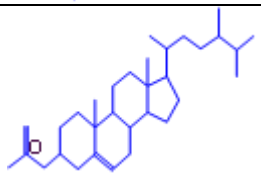
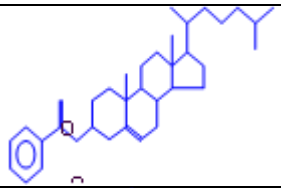
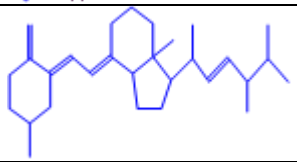
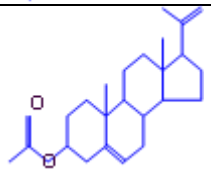
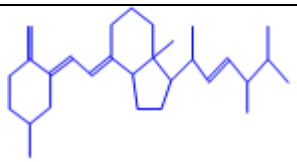
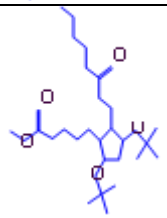
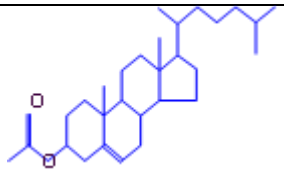
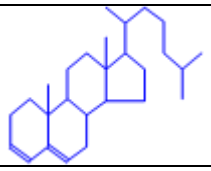
The active principles with their molecular formula, molecular weight (MW) and structures are presented in Table no. 1 and Fig.no.1 which shows the presence of 26 bioactive phytochemical compounds in the methanolic extract of *Naringi crenulata*. The activities identified in some of the bioactive compounds are also tabulated in Table no. 2. Among the identified compounds psi. psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy (R match: 611, F match:598, Probability: 2.39), lycopene(R match : 595, F match: 585, Probability : 1.40), lycoxanthin (R match :585, F match:577, Probability: 1.04.), astaxanthin (R match: 565, F match:569, Probability:0.76.),tocopherol (R match:996, F match: 824 , Probability:2.70) vitamin E(R match: 891, F match:867, Probability: 17.29.) and vitamin D(R match:664, F match: 515, Probability: 0.17.) have got the antioxidant property[6].

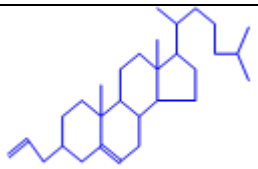
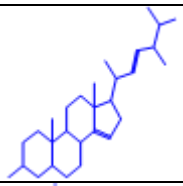
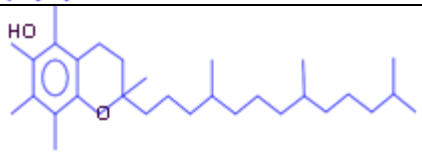
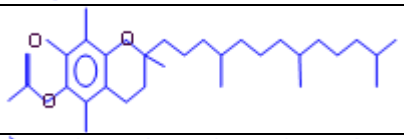
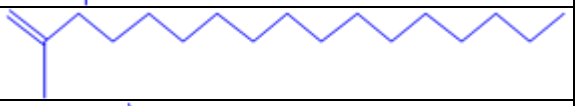
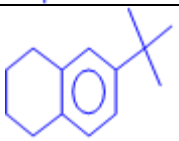
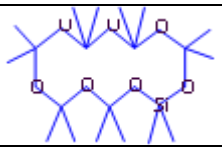
Hexadecanoic acid(R match: 815, F match: 815, Probability:58.2) have the property of antioxidant and anti-inflammatory [7], followed by stigmasterol (R match:601, F match: 541, Probability:0.26.) which has been investigated for its pharmacological prospects such as antiosteoarthritic, anti-hypercholestrolemic, antitumor, hypoglycemic, antioxidant, anti-inflammatory and CNS effects[8].

Stigmast-5-en-3-ol (R match:662,Fmatch:637, Probability:11.18) is proven to be used for Diabetes Mellitus.[9],cycloheptasiloxane (R match:879, F match: 879, Probability:97.7) has an emollient activity[10], Naphthalene (R match:605, F match:524, Probability:0.08) also having good antimicrobial activity[11].Moreover the target compounds identified includes hexadecanoic acid and cycloheptasiloxane, tetradecamethyl with the retention time of 36.758 and 26.657 and is mentioned in figure no. 2. The target spectrum of MENC is also depicted in Fig.no.3.

Table no. 1 Bioactive compounds in the leaves of methanolic extract of *Naringi crenulata* (Roxb.) Nicolson.

Sl.No.	Name of the compound	Molecular formula	Molecular weight	Structure
1.	Stigmast-5-en-3-ol	C29H50O	414	
2.	psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	C42H64O2	600	
3.	Stigmastan-3,5-diene	C29H48	396	
4.	Gamma.-Sitosterol	C29H50O	414	
5.	Stigmastan-3-ol, 5-chloro-, acetate	C31H53ClO2	492	
6.	Lycopene	C40H56	536	
7.	Lycoxanthin	C40H56O	552	
8.	Ergosterol	C28H44O	396	
9.	Astaxanthin	C40H52O4	596	
10.	Cholesterol	C27H46O	386	

11.	Stigmasterol, 22,23-dihydro-	C29H50O	414	
12.	Ergost-5-en-3-ol, acetate	C30H50O2	442	
13.	Cholesteryl benzoate	C34H50O2	490	
14	Ergocalciferol	C28H44O	396	
15	Pregn-5-en-20-one, 3-acetyloxy	C23H34O3	358	
16	Vitamin D	C28H44O	396	
17	Cyclopentanepentanoic acid, 2-(3-oxooctyl)-3,5-bis[(trimethylsilyl) oxy]-, methyl ester	C25H50O5Si2	486	
18	Cholest-5-en-3-ol - acetate	C29H48O2	428	
19	Cholesta-3,5-diene	C27H44	368	

20	Cholesteryl formate	C28H46O2	414	
21	Ergosta-14,22-dien-3-ol	C28H46O	**398**	
22	Vitamin E	C29H50O2	430	
23	Alpha tocopherol acetate	C31H52O3	472	
24	Hexadecanoic acid	C16H32O2	256	
25	Naphthalene, 6-(1,1-dimethylethyl)-1,2,3,4-tetrahydro	C 1 4 H 2 0	188	
26	Cycloheptasiloxane, tetradecamethyl-	C14H42O7Si7	518	

Due to the presence of above-mentioned compounds in the leaves of methanolic extract of *Naringi crenulata* (Roxb.) Nicolson may be used in various pharmaceutical and industrial applications.

CONCLUSION:

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. Thus, the identification of bioactive compound in *Naringi crenulata* was done by GC-MS analysis which shows the presence of 26 compounds. Among the identified compounds astaxanthin, lycopene, carotene, vitamin indicated its anti-oxidant activity and hexadecanoic acid showed the anti-inflammatory activity. From this study it can be concluded that the *Naringi crenulata(Roxb.) Nicolson leaves* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds.

Fig.no:1 GC MS Chromatogram of leaves of MENC

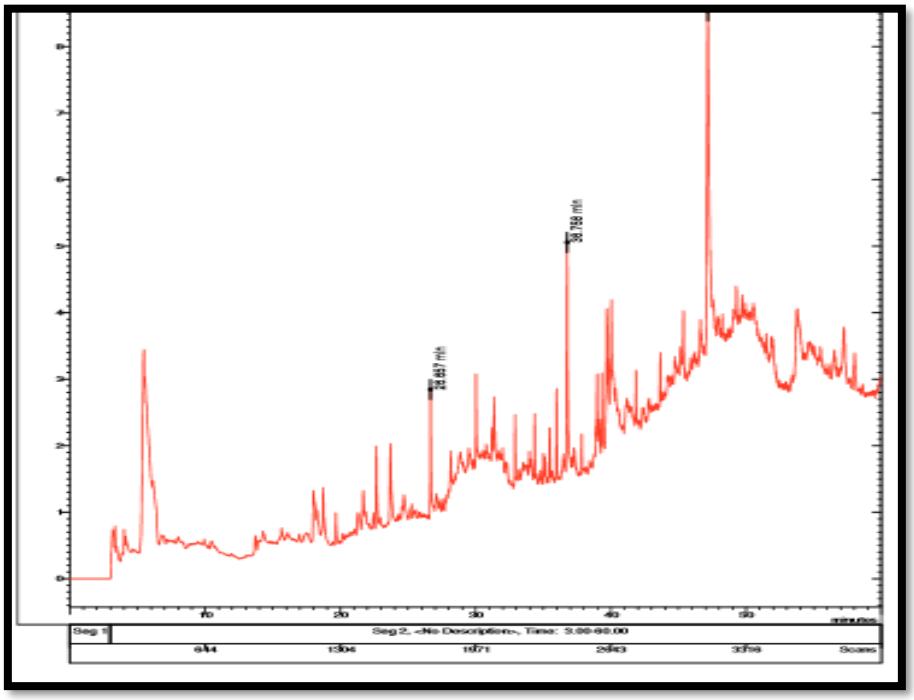
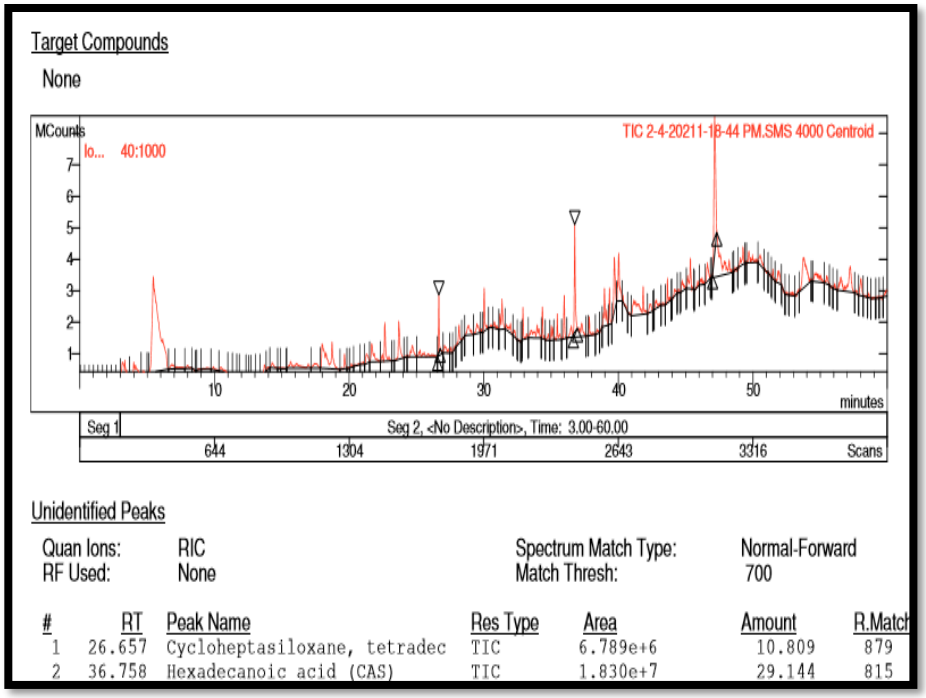


Fig.no: 2 Target compounds of leaves of MENC.



Target Compounds

None

Unidentified Peaks

Quan Ions: RIC
RF Used: None
Spectrum Match Type: Normal-Forward
Match Thresh: 700

#	RT	Peak Name	Res Type	Area	Amount	R.Match
1	26.657	Cycloheptasiloxane, tetradec	TIC	6.789e+6	10.809	879
2	36.758	Hexadecanoic acid (CAS)	TIC	1.830e+7	29.144	815

Fig no. 3 Target spectrum of leaves of MENC.

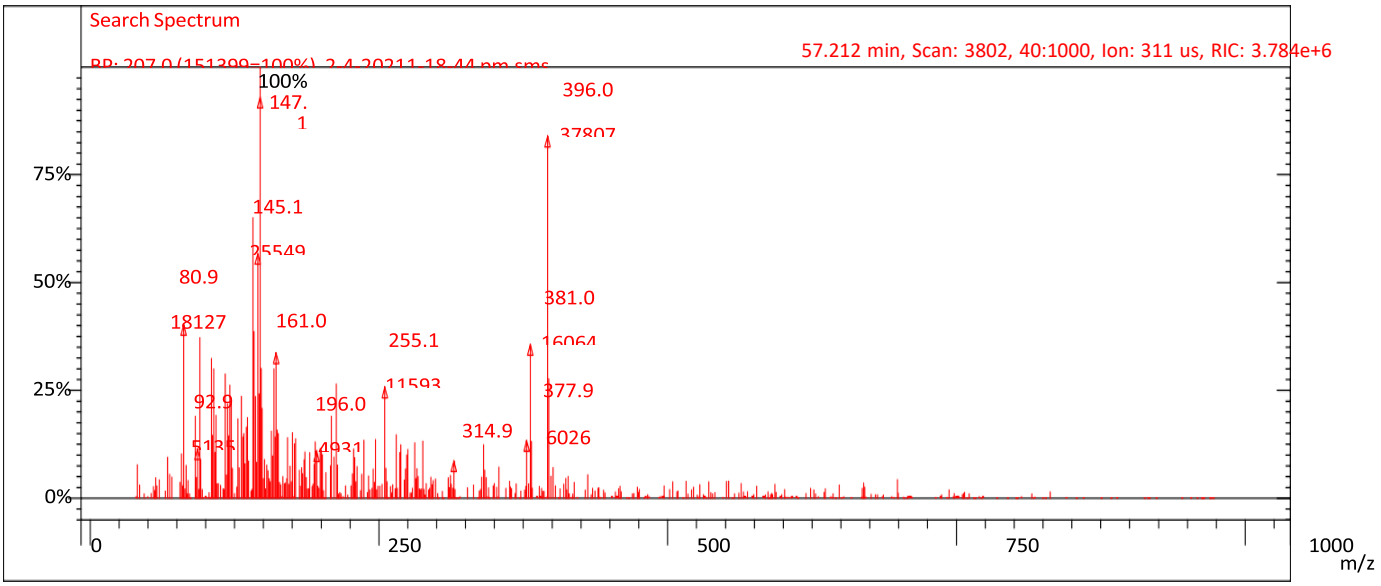


Table no. 2. Activity of various phytoconstituents which is identified in MENC

Sl.No.	Name of the compound	Activity
1.	Carotene	Antioxidant
2.	Lycopene	Antioxidant
3.	Lycoxanthin	Antioxidant
4.	Astaxanthin	Antioxidant
5.	Tocopherol	Antioxidant
6.	Vitamin E	Antioxidant
7.	Vitamin D	Antioxidant
8.	Hexadecanoic acid	Anti-inflammatory and anti-oxidant
9.	Stigmasterol	Anti-tumor, antiangiogenic and CNS effects
10.	Stigmast	Antidiabetic
11.	Cycloheptasiloxane	Emollient
12.	Naphthalene	Antimicrobial activity
13.	Gamma.-Sitosterol	Anti-diabetic activity
14.	Stigmastan-3-ol	Cholesterol lowering agent
15.	Ergosterol	Anti-fungal agent
16.	Ergocalciferol	Hypoparathyroidism

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