Research Article

Phytochemical Profiling and GC-MS Analysis of Bioactive Constituents of Callus of *Naringi Crenulata* (Roxb.) Nicolson

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ABSTRACT

Naringi crenulata (Roxb.) Nicolson is a medicinally important tree species. Various parts of this plant have been employed in indigenous medicines. Several studies have proved that the phytocomponents present in a medicinal plant are widely responsible for the therapeutic potential of the plant. In the present study, preliminary phytochemical profiling and Gas chromatography- Mass Spectrum (GC-MS) analysis was carried out on the methanolic extract of callus of *Naringi crenulata* for identification of phytocompounds in the callus. The preliminary phytochemical profiling of callus showed the presence of bioactive components like terpenoids, flavonoids, steroids, glycosides, sugars, alkaloids, phenols, tannin and coumarin. GC- MS analysis of callus showed the presence of bioactive phytocompounds like methyl phenylacetate, estragole, Benzene,1-methoxy-4-1-propenyl, 1,3,4,5-Tetrahydroxycyclohexanecaboxylic acid, 1,3 Dicyclohexylpropane, Methyl1,4-methylpentadecanoate, Methyl cis-9-octadecenoate, 6-Methoxylfuro (2,3-h) chromen-2-one, Stearic acid, 4-t-Butoxy-3-hydroxy- butyric acid, Squalene, Vitamin E and Stigmasterol, all of which possessed a wide range of proven therapeutic uses. The highest peak area (%) of 16.31 was obtained by 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid (Retention time 17.709) and the lowest peak area (%) of 0.20 was obtained by Tetradecane (Retention time 14.809).The presence of various bioactive compounds confirms the application of *N. crenulata* for various ailments by traditional practitioners. Isolation of individual phytochemical constituents may pave the way to find a novel drug. This report is the first of its kind to analyze the chemical components of methanolic extract of callus of *Naringi crenulata.*

Keywords: Callus, GC-MS, *Naringi crenulata,* Phytochemical.

INTRODUCTION

In recent years, the interest for the study of the organic compounds from the plants and their activity has increased. A knowledge of the chemical constituents of plant is desirable not only for the discovery of therapeutic agents, but also helps in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies.[1] There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease.[2] The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC-MS an ideal technique for qualitative and quantitative analysis of volatile and semi volatile compounds. This technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids[3] and alkaloids.[4] There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity.[5-12] Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer[13] and Alzheimer's disease.[14]

Naringi crenulata (Roxb.) Nicolson is a tree 8-12 m tall, bark appeared dull brown yellow, smooth, spines are sharp, leaves compound, imparipinnate, alternate rachis with oblonceolate wings. Various parts of this plant have been employed in indigenous medicine and it is used as antiepileptic, purgative, sudoferic, colic trouble and cardialgia.[15] Leaves are used as a remedy for epilepsy. Bark is aromatic and cooling and is useful in vitiated conditions of Pitta.[16] Crenulatine along with twenty known indole alkaloids were isolated from the stem of the plant.[17] Sitosterol, Xanthotoxin and limonin diosphenol were isolated from the stem bark of the plant.[18] The powdered stem wood prepared by grinding the stem on the flat round stone, moistened with water has been used traditionally as a natural skin conditioner especially as facial cosmetics in Myanmar and some part of Northern Thailand. Burmese women paint their face yellow with the powder stem as sunscreen preventing sunburn, that consequently causes wrinkles, freckles and dry skin, in addition to its efficacy proven on anti aging, it prevents acne and provides soft and fresh skin texture.[19] A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. With this background, the present study was aimed to identify the phytoconstituents present in *N. crenulata* using GC-MS analysis.

MATERIALS AND METHODS

Plant material and explants preparation

Fresh twigs of *Naringi crenulata* (Roxb.) Nicolson were collected from Jaipur district of Rajasthan and its identity was confirmed by depositing the voucher specimen in the herbarium of University of Rajasthan, Jaipur. Nodal

explants of *Naringi crenulata* were excised and washed thoroughly in running tap water for about half an hour. They were then treated with 2% (v/v) Tween 20 (a commercial grade detergent) followed by several rinses in sterile distilled water. The disinfected explants were surface sterilized under aseptic conditions in a laminar flow chamber. The explants were treated with 70% ethanol for 30 seconds and washed thrice in sterile distilled water. The explants were then immersed in 0.1% mercuric chloride solution and again rinsed thrice in sterile distilled water.

Callus induction

For callus induction, nodal segment were placed on full strength Murashige and Skoog's, (1962) basal medium (MS medium) supplemented with different concentrations and combinations of growth regulators such as NAA (0.5-5.0 mg/l), IBA (0.5-5.0 mg/l), IAA(0.5-5.0 mg/l) and 2,4-D (0.5-5.0 mg/l) containing 3% (w/v) sucrose and 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 and it was autoclaved at 121ºC under 15 psi for 20 minutes. Cultures were incubated at 26 ± 2ºC under 16 hours photoperiod illuminated by fluorescent light of 2000-3000 lux intensity and 55±5% relative humidity. Each experiment was repeated thrice with 5 replicates per treatment. Periodic observations were recorded.

Preparation of the extracts

The callus of *Naringi crenulata* was shade dried at room temperature (28± 2°C). The dried callus was powdered by electrical blender. Methanol was used for the extraction of 5.0 g callus in the Soxhlet apparatus .The plant material was loaded in the inner tube of the Soxhlet apparatus and then fitted into a round bottomed flask containing methanol. The solvent was boiled gently (40ºC) over a heating mantle using the adjustable rheostat. The extraction was continued until complete extraction was effected (8 h) and the solvent was removed at the reduced pressure with the help of vacuum evaporator to yield a viscous dark brown colour residue of methanolic extract. This methanolic extract was subjected to phytochemical and GC-MS analysis.

Phytochemical Profiling

Phytochemical profiling of the callus extract was carried out as per the methods given by[20-21] to decipher the presence or absence of various phytocompounds.

Phytochemical screening procedure

Test for steroids

One gram of the test substance was dissolved in a few drops of acetic acid, acetic anhydride, warmed and cooled under tap water and a drop of concentrated sulphuric acid was added along the sides of the test tube. Presence of green colour indicated the presence of steroids.

Test for alkaloids

Test substance shaken with few drops of 2N HCL. Aqueous layer formed, decanted and to which one or two drops of Mayer's reagent added. Formation of white turbidity or precipitate indicated the presence of alkaloids.

Test for flavonoids

Shinado's test: To the substance in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red coloration showed the presence of flavonoids.

Test for triterpenoids

Noller's test: The substance was warmed with Tin and Thionyl chloride. Purple coloration indicated the presence of triterpeniods.

Test for tannins

The substance mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of tannins.

Test for saponins

The substance shaken with water, foamy lather formation indicated the presence of saponins.

Test for quinones

To the test substance, sodium hydroxide was added. Blue green or red colour indicated the presence of quinone.

Test for coumarin

To the test sample 10% of sodium hydroxide and chloroform were added. Formation of yellow colour indicated the presence of coumarin.

Test for protein

To the test solution the Biuret reagent was added. The blue reagent turned violet in the presence of proteins.

Test for sugars

The substance was mixed with equal volume of Fehling's A and B solutions, heated in water bath. Formation of red colour indicated the presence of sugar.

Gas chromatography – Mass Spectrum analysis

Before analyzing the extract using Gas Chromatography and Mass Spectrometry, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane).

The GC-MS analysis of the callus extract was done in a Shimadzu QP 5000 (Japan make) instrument under

computer control at 70 e. About 1µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the Retention time (RT).While the instrument ran, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The M/Z (Mass / Charge) ratio obtained was calibrated from the graph obtained which was called as the Mass spectrum graph which is the fingerprint of a molecule. The identified compounds were compared with standards run under the same conditions. These data were also compared with the compounds already stored in a compact library of chemical substances (NIST Library).

RESULTS AND DISSCUSSION

Studies on the native or folk medicinal use of medicinal plants are important from the scientific point of view in that it enables rapid scientific studies towards finding and development of newer drugs from centuries old practical use-derived knowledge of medicinal plants. Green plants represent a reservoir of effective chemicals, therapeutants and can provide valuable sources of natural pesticides. Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. In the present study, it was observed that the *Naringi crenulata* on which scientific studies have been conducted are validated in their uses in various parts of India. The medicinal value of this plant lies in some chemical substances that have a definite physiological action on the human body. Different auxin concentrations had a significant effect on callus regeneration (Table 1). Explants cultured on growth regulator free medium (control) did not produce any callus. Lower concentration of IAA (0.5 - 1.0 mg/l) resulted in moderate amount of callus which was dark brown in colour (Table 1). IBA (1.0 mg/l) induced profuse callusing. Callus was initially white to green in colour, soft and fast growing but later on showed rhizogenic response. 2,4-D at lower concentrations (0.5 mg/l) showed yellowish brown, slow growing and hard callus. NAA (0.5 to 2.0 mg/l) induced green, hard and fast growing callus (Table 1). The phytochemical profiling of *Naringi crenulata* callus extract showed the presence of bioactive components like Terpenoids, Flavonoids, Glycosides, Alkaloids, Phenols, Tannins, Saponins and Coumarin (Table 2). Several phytochemical screening studies have been carried out in different parts of the world using GC-MS.[22-25] In the present study we characterized the chemical profile of *N. crenulata* using GC- MS.

Table 1: Effect of different concentration of auxins on callus induction from nodal explants of *Naringi crenulata* (Roxb.) Nicolson

Auxin conc.(mg/l)	Callus response	Type of callus
	Nodal segment	
Control : MS basal medium	NIL	NIL
NAA		Green hard and fast growing callus
0.5	C+	
1.0	C++	
2.0	C++	
3.0	C+	
4.0	C+	
5.0	C+	
IBA		White to green in colour, fast growing
0.5	C+	
1.0	C+++	
2.0	C+	
3.0	C+R+	
4.0	C+R++	
5.0	C+	
IAA		Dark brown
0.5	C++	
1.0	C++	
2.0	C+	
3.0	C+	
4.0	C+	
5.0	C-	
2,4-D		Yellowish brown, slow growing and hard.
0.5	C+	
1.0	C+	
2.0	C+	
3.0	NIL	
4.0	NIL	
5.0	NIL	

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and

structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of methanolic extract of callus of *N. crenulata*. The highest peak area (%) of 16.31 was obtained by 1,3,4,5-Tetrahydroxycyclohexane carboxylic acid (Retention time 17.709) and the lowest peak area (%) of 0.20 was obtained by Tetradecane (Retention time 14.809). The detailed tabulation of GC-MS analysis has been given in Table 3. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been given in Figure 1. Different phytochemicals which have been identified from methanolic extract have been found to possess a wide range of activities, which may help in protection against chronic diseases. Alkaloids protect against chronic disease, saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show analgesic properties. The steroids and saponins were responsible for central nervous system activity. The identification and isolation of these active compounds could lead to the new drug discovery at a cheaper cost which would be useful in medicine.

Table 2: Phytochemical Profiling of Methanolic Extract of callus of *Naringi crenulata*

Name of compound	Result
Terpenoids	Present
Flavonoids	Present
Steroids	Present
Anthroquinone	Absent
Glycosides	Present
Sugars	Present
Alkaloids	Present
Quinines	Absent
Phenols	Present
Tannins	Present
Saponins	Present
Coumarins	Present

Table 3: Compounds identified from methanolic extract of *Naringi crenulata* callus using Gas Chromatography – Mass Spectrum analysis

Peak	R.Time	Area%	Compound Name	Molecular Formula	RI
1	8.724	1.05	Methyl Phenylacetate	$C_9H_{10}O_2$	1160
2	8.915	0.29	Hexadecane	$C_{16}H_{34}$	1612
3	**9.122**	**12.55**	**Estragole**	**$C_{10}H_{12}O$**	**1172**
4	9.402	1.00	1,4,3,6-Dianhydro-alpha-d glucopyranose	$C_6H_8O_4$	916
5	10.850	4.52	Benzene, 1-methoxy-4-(1-propenyl)	$C_{10}H_{12}O$	1190
7	12.673	0.82	3-Hexadecene	$C_{16}H_{32}$	1620
8	12.820	0.97	Tetradecane	$C_{14}H_{30}$	1413
9	13.701	0.68	Caryophyllene	$C_{15}H_{24}$	1494
10	13.935	0.28	Octylcyclohexane	$C_{14}H_{28}$	1476
11	14.809	0.20	Tetradecane	$C_{14}H_{30}$	1413
12	15.018	1.79	Beta.-D-Allose	$C_6H_{12}O_6$	1698
13	16.610	1.33	9-Eicosene,	$C_{20}H_{40}$	2017
14	16.742	0.99	Pentadecane	$C_{15}H_{32}$	1512
15	**17.709**	**16.31**	**1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid**	**$C_7H_{12}O_6$**	**1852**
16	17.939	2.31	1,3-Dicyclohexylpropane	$C_{15}H_{28}$	1639
17	18.171	1.51	8-Pentadecanone	$C_{15}H_{30}O$	1648
18	19.061	2.10	3-Oxo-7,8-dihydro-.alpha.-ionol	$C_{13}H_{22}O_2$	1619
19	20.094	1.11	1-Heptadecene	$C_{17}H_{34}$	1701
20	20.190	0.43	Octadecane	$C_{18}H_{38}$	1810
21	20.265	0.32	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	$C_{11}H_{20}O$	1183
22	20.802	2.81	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	2045
23	20.892	0.42	3,7,11,15-Tetramethyl-2-hexadecene	$C_{20}H_{40}$	1802
24	21.158	0.29	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	2336
25	21.329	1.43	8-Octadecanone	$C_{18}H_{36}O$	1946

Table 3: Compounds identified from methanolic extract of *Naringi crenulata* callus using Gas Chromatography – Mass Spectrum analysis (Continued.....)

Peak	R.Time	Area%	Compound Name	Molecular Formula	RI
26	21.410	1.69	5-Tetradecen-1-ol, acetate,	$C_{16}H_{30}O_2$	1787
27	21.983	7.39	Methyl 1,4-methylpentadecanoate	$C_{17}H_{34}O_2$	1814
28	22.340	0.57	Methyl 3-(3,5-di-tert-butyl-4 hydroxyphenyl)propionate	$C_{18}H_{28}O_3$	2134
29	22.604	1.94	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	2037
30	22.811	0.78	Hexahydroaplotaxene	$C_{17}H_{34}$	1701
31	22.883	0.30	1-Iodotridecane	$C_{13}H_{27}I$	1728
32	22.973	0.79	Methyl 8-(2-hexylcyclopropyl)octanoate	$C_{18}H_{34}O_2$	1941
33	23.218	0.78	Margaric acid methyl ester	$C_{18}H_{36}O_2$	1978
34	23.810	0.48	Di-n-nonyl ketone	$C_{19}H_{38}O$	2046
35	24.086	7.22	Methyl cis-9-octadecenoate	$C_{19}H_{36}O_2$	2085
36	24.245	4.99	6-Methoxyfuro[2,3-h]chromen-2-one	$C_{12}H_8O_4$	1901
37	24.328	2.55	Stearic acid, methyl ester	$C_{19}H_{38}O_2$	2077
38	24.668	0.36	1-Tert-buthoxy-6-trimethylsilyloxyhexane	$C_{13}H_{30}O_2Si$	1291
39	24.992	0.36	cis-1-Chloro-9-octadecen	$C_{18}H_{35}Cl$	2044
40	25.365	0.28	5-Methyl-2-hexanone oxime	$C_7H_{15}NO$	985
41	27.767	1.01	4-t-Butoxy-3-hydroxy-butyric acid, ethyl ester	$C_{10}H_{20}O_4$	1336
42	27.981	0.51	Octacosanic acid methyl ester	$C_{29}H_{58}O_2$	3071
43	28.272	1.25	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	2704
44	30.764	0.36	Squalene	$C_{30}H_{50}$	2914
45	31.341	0.52	Heneicosane	$C_{21}H_{44}$	2109
46	31.483	0.36	4,5-Diethyl-2,3-dimethyl-2,3-dihydrofuran	$C_{10}H_{18}O$	1071
47	33.842	0.44	Tetracontane	$C_{40}H_{82}$	3997
48	34.093	0.51	Stigmasta-5,22-dien-3.beta.-ol, acetate	$C_{31}H_{50}O_2$	2879
49	35.016	4.93	alpha-Tocopherol , Vitamin E	$C_{29}H_{50}O_2$	3149
50	37.362	0.73	Campesterol	$C_{28}H_{48}O$	2632
51	37.981	1.73	Stigmasterol	$C_{29}H_{48}O$	2739
52	39.279	0.41	Beta-sitosterol	$C_{29}H_{50}O$	2731

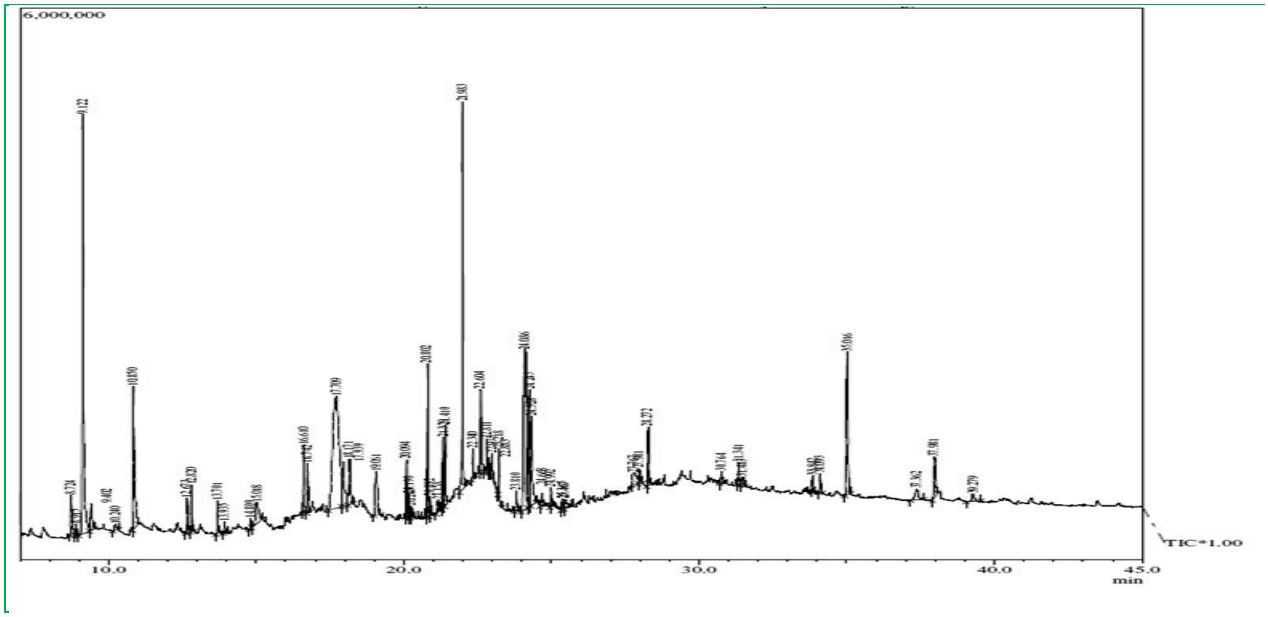

Figure 1: GC-MS Spectrum of *Naringi crenulata*

CONCLUSION

The experimental plant *Naringi crenulata* studied here can be a potential source of useful drugs exploiting the anti-inflammatory, anti-cancer and immunomodulatory activities of this plant. This type of study provides health application at affordable cost. Further research needs to be done on the utility of these phytochemicals in treating other dreadful diseases. Advanced studies are being conducted on this plant in order to isolate, identify, characterize and elucidate the structure of these bioactive compounds.

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