Original Article

Screening Of Some Neuropharmacological Activities Of Naringi Crenulata (Roxb.)

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Abstract

Objective: The goal of this study is to examine the neuropharmacological effects of various extracts made from Naringi crenulata (Roxb.).

Materials and methods: Wayanad, Kerala was the source of the leaves used in the authentication of Naringi crenulata (Roxb.). Petroleum ether, chloroform, and methanol were used as solvents with higher polarity throughout the extraction process. To obtain ethyl acetate fractions, the methanol extract was also separated with ethylacetate. All of the extracts and the fraction underwent a phytochemicals screening to determine their phytoconstituent composition. For various in- vitro antioxidant assay methods, including the DPPH scavenging assay, hydroxyl radical scavenging assay, nitric oxide radical scavenging assay, different concentrations (5–640 g/mL) of chloroform, methanol, and ethylacetate fractions were used. The standard was ascorbic acid (AA).On higher and lower doses of NC, neuropharmacological screening techniques were carried out as sedative and hypnotic activity, anti-convulsant activity. Catalase, SOD, GPx,(enzymatic) and non-enzymatic antioxidants(GSH) were used in the in-vivo antioxidant research on isolated liver. In MENC and EAFNC, the levels of the brain neurotransmitters GABA and DA were also examined. Additionally, the methanol extract underwent partial purification using column chromatography and was then submitted to GC-MS to determine whether bioactive chemicals were present.

Results: The phytochemical analysis of Naringi crenulata (Roxb.) revealed the presence of alkaloids, phenolic compounds, carbohydrates, and flavonoids. According to in-vitro antioxidant data, as extract concentration increased, the scavenging property also improved. This was followed by an increase in percentage inhibition, which demonstrated lower IC_{50} values across the board for all extracts. With MENC, the greatest scavenging effect was discovered in testing techniques. Therefore, when compared to other antioxidants, this extract was thought to be beneficial. The increased dose of MENC was found to be significant when compared to other groups in in-vivo antioxidant activities, brain neurotransmitter estimate, and neuropharmacological screening procedures. A GC-MS analysis of the MENC partly purified column fractions revealed the presence of 26 bioactive chemicals, some of which had therapeutic potential.

Conclusion: The findings showed that Naringi crenulata (Roxb.) extracts can be employed as an antioxidant and in the treatment of some neurological illnesses.

Keywords: Antioxidant, MENC GC-MS, in-vivo, and Naringi crenulata (Roxb.), in-vitro.

INTRODUCTION

Neuropharmacology – the study of medications that impact the brain. It will briefly examine how chemicals and receptors are used by nerve cells to communicate with one another, gives an overview of the chemicals and receptors that are used, and discuss the nerve pathways and neuronal activities connected to various neurotransmitter systems [1]. It is also defined as the study of known exogenous and endogenous chemical agents' effects on neurobiological processes in the mammalian nervous system [2].

Due to the prevailing side effects, the efficacies of medications now available for treating these disorders are still quite restricted. To improve treatment effectiveness and lessen adverse effects, numerous solutions have been devised. In

order to prevent psychiatric disorders and cognitive impairment, research into innovative medication derived from medicinal plants has therefore greatly advanced and attracted a lot of attention.

According to data available, the development of psychiatric and neurological illnesses has been linked to oxidative stress. Recent research has shown that substances with antioxidant activity can improve cognitive performance and lessen mental symptoms [3].

People in contemporary culture experience a variety of psychiatric problems, particularly sadness, anxiety, and sleeplessness. In the complexity of mental health illnesses, prevalent comorbid psychiatric conditions include depression, anxiety, and sleeplessness. The immunological and cardiovascular systems are compromised by the association between significant depression, sleeplessness, and anxiety disorders [4].

Therefore, it is preferable to look for antidepressants that are quick acting, more tolerated, more effective, and have less adverse effects. Meanwhile, the most often utilised complementary and alternative medical treatment is herbal therapy [48]. Recently, it was shown that people with psychiatric conditions used herbs more frequently than those without [5]. Many natural products with neurological effects have been uncovered, and one such innovative medication that can be used for neurological issues is Naringi crenulata (Roxb.)

To the best of our knowledge, no research has been done on the neuropharmacological effects of Naringi crenulata. An attempt could be made to assess Naringi crenulata's neuropharmacological effects in experimental animals using various scientific animal models in light of literature that examined the presence of flavonoids and saponins in this plant as well as based on knowledge of its traditional use as an anticonvulsant in folk medicine.

MATERIALS AND METHODS

PLANT MATERIAL

Table no: 1 Plant material

SL. NO	PLANT NAME	PART USED	FAMILY	COLLECTION PERIOD	PLACE OF COLLECTION
1.	NaringicrenulataRoxb. Nicolson	Leaves	Rutaceae	November	MS Swaminathan Botanical Garden, Wayanad, India

COLLECTION AND AUTHENTICATION OF NARINGI CRENULATA (ROXB.)

The herb was bought as follows.

Jithin.M. M. is the lead scientist who certified the leaves of Naringi crenulata, which were procured from Wayanad district in Kerala. MS Swaminathan Botanical Garden herbarium received the herbaria with the collection number 3989 and the accession numbers 654 and 655.

Wide spread leaves & flowers of Naringi crenulata (Roxb) Nicolson

PREPARATION OF PLANT EXTRACTS

The leaves for the study were prepared as such. Firstly the leaves of Naringi crenulata (Roxb.) were shade dried and ground up was extracted in a Soxhlet device using polarity of increasing chemicals, starting with petroleum ether,

moving up to chloroform, and ending with methanol. Using a rotating vacuum evaporator, the lower pressure, liquids from the crude extracts were recovered. [8]

The methanol extract of 20 g of plant material was diluted uniformly in water, transferred to a flask with a flat bottom, separated with ethyl acetate, and heated at 50 ^{o}C for 30 min while being swirled till a fixed point .Thereafter the partitioning with ethyl acetate procedure required ten iterations to be successful.

A screening approach was used on the methanol extract and EAF of Naringicrenulata (Roxb.) Nicolson.

CALCULATION OF PERCENTAGE YIELD

Using the formula shown below, the percentage yield regarding the raw sample was established for the isolates and primary components.

$$\text{\% yield in relation to the raw plant component} = \frac{\text{Weight in grams of isolate achieved}}{\text{Weight in grams of plant content reserved}} \times 100$$

PRELIMINARY PHYTOCHEMICAL (QUALITATIVE) ANALYSIS OF EXTRACTS OF NARINGI CRENULATA (ROXB.)

The chemical experiments were performed individually on the extracts of leaves from Naringi crenulata (Roxb.) Nicolson in order to identify the distinct active ingredients [6].

ASSESSMENT OF THE IN-VITRO ANTIOXIDANT ACTIVITY OF LEAF EXTRACTS FROM NARINGI CRENULATA (ROXB.) NICOLSON

There is a need to estimate the antioxidant property of the herbal extract. Thus different in-vitro anti-oxidant parameters were evaluated

A) **DPPH (1, 1 diphenyl 2, picrylhydrazyl)**
B) **HYDROXYL RADICAL SCAVENGING ACTIVITY(HRSA)**
C) NITRIC OXIDE RADICAL SCAVENGING(NORS)

TOXICITY STUDIES OF EXTRACTS OF LEAVES OF Naringi crenulata (Roxb.)

ACUTE TOXICITY STUDIES

The examination and evaluation of all substances with hazardous properties often begin with a determination of their acute oral toxicity. Acute, sub-acute, and chronic toxicity are the sorts of toxicity tests that are frequently carried out by pharmaceutical firms in the examination of a new medicine.

Estimating LD_{50}—the dose that has been shown to be deadly (producing death) in 50% of the tested group of animals—involves consideration of acute toxicity. [9]

The Committee for the Purpose of Control and Supervision of Experimental Animals provided the (OECD) draught guidelines 423, which were used for the acute toxicity tests of Naringi crenulata (Roxb.) leaves.

ACCLIMATIZATION OF ANIMALS

Female Swiss Albino mice weighing 22–25g and Sprague Dawley rats weighing 150-200gm were kept in a regular laboratory setting at the Cape Bio-Lab & Research Centre, CSI Complex, Marthandam for experimental studies.

ADMINISTRATION OF DOSES

The study involved three animals for each phase. Following the period of fasting, animals were forced to go without food and water for three hours before to medication delivery. Starting at a concentration of five milligram per kilo gram, it was subsequently raised to 50, 300, and 2000 mg/kg body composition for the study.

EVALUATION OF IN-VIVO NEUROPHARAMCOLOGICAL SCREENING OF Naringi crenulata (Roxb.) Nicolson leaves.

Different activities and under each activity two studies were performed in order to analyze the in-vivo neuropharmacological screening with precession.

SEDATIVE & HYPNOTIC ACTIVITY

A. Phenobarbitone sodium induced sleep latency and sleeping time

Six divisions were created for the mice of six in each groups. Mice from all groups were caged on the experimentation day. The two methanolic and ethylacetate fractions of Naringi crenulata (Roxb.) Nicolson were administered in varying amounts to the four test groups. After being given Naringi crenulata (Roxb.) Nicolson for thirty minutes, it was given Phenobarbitone sodium 40 mg/kg (i.p), to induce sleep.

Treatment protocol

Animals : Swiss Albino mice weighing 20-25g, (both sex)
Total number of animals : 30
Group 1 : Phenobarbitone sodium40mg/kg,(i.p), control group (n=6)
Group 2 : Methanol extract (400mg/kg,(p.o),(n=6)
Group 3 : Methanol extract (200mg/kg,(p.o), (n=6)
Group 4 : Ethyl acetate fraction (400mg/kg,(p.o), (n=6)
Group 5 : Ethyl acetate fraction (200mg/kg,(p.o), (n=6)[12].

HISTOPATHOLOGICAL STUDIES:

A portion of the brain was preserved in 10% formalin, haemotoxylin and eosin-stained specimens that were examined under a light microscope to determine the degree of brain damage. A different portion of the brain was used to estimate the levels of neurotransmitters GABA and dopamine (DA).

B. Motor coordination activity

The performance time was measured as the amount of time between the animal being mounted on the rod and falling off of it. Following administration of the conventional medication and extract, the performance time was recorded every 15 minutes for a total of 90 minutes and 300 seconds (cut-off time)

Treatment protocol

Animals : Swiss Albino mice weighing 20-25g, (both sex).
Total number of animals : 36
Group 1 : Normal control group(n=6)
Group 2 : Standard drug Diazepam 4mg/kg (i.p),(n=6)
Group 3 : Methanol extract (higher dose (p.o),(n=6)
Group 4 : Methanol extract (lower dose (p.o), (n=6)
Group 5 : Ethylacetate fraction (higher dose (p.o), (n=6)
Group 6 : Ethylacetate fraction (lower dose (p.o), (n=6)[13].

ANTIANXIETY ACTIVITY

A. Elevated plus -maze (EPM)

The EPM has received extensive validation for measuring anxiety in rodents. 2 open arms (thirty cm x five cm) with 30 cm walls make up this device. Each animal was then put in the centre of the maze, facing one of the enclosed arms, from a central platform that is 5 cm x 5 cm and positioned forty cm above the floor of the room. .

Treatment protocol

Animals : Swiss Albino mice weighing 20-25g, (both sex)
Total number of animals : 36
Group 1 : Normal control group(n=6)
Group2 : Standard Diazepam 4mg/kg (i.p), (n=6)
Group 3 : Methanol extract (higher dose (p.o), (n=6)
Group 4 : Methanol extract (lower dose (p.o), (n=6)
Group 5 : Ethyl acetate fraction (higher dose (p.o), (n=6)
Group 6 : Ethyl acetate fraction (lower dose (p.o), (n=6)[14].

COLLECTION OF TISSUE ISOLATE

At the culmination of the trial, the animals were slaughtered using extra carbon dioxide. Every animal's liver was taken out, scraped, cut, and bathed in cooled sodium chloride mixture. A 10% w/v mixture of the homogenised tissue, referred to as the homogenate, was then produced using 0.1 M tris buffer, pH 7.0, and separated at 10,000 x gram for 20 minutes at 4 degrees Celsius [7].

SECTIONS OF THE EXCISED TISSUES

A section of the brains was maintained in 10% formalin, stained with haemotoxylin and eosin, and then evaluated underneath a fluorescent microscope to assess the severity of concussion. The concentrations of the neurotransmitters GABA and dopamine(DA) were measured in a distinct brain region.[10]

STATISTICAL ANALYSIS

Without a statistical analysis proper justification cannot be made with the result obtained. Thus in order to obtain a reasonable outcome statistical analysis must be conducted.
Data from animal studies, where all variables are presented as mean±SEM from any group, were analysed using graph pad software. Dunnett's multiple comparison test was used after one-way ANOVA for statistical analysis of the results. Tests were conducted in triplicate for the assessment of in-vitro antioxidants and brain neurotransmitters, and the data was expressed as mean±SD. $P < 0.05$ was chosen as the threshold for statistical significance.[11]

RESULTS

PERCENTAGE YIELDS

Table no: 2 Percentage yields of various extracts of NC

PLANT NAME	PART USED	METHOD OF EXTRACTION	SOLVENT SYSTEM	PERCENTAGE YIELD(%W/W)
Naringi crenulata	Leaves	Soxhlet apparatus	Petroleum ether	**0.140 (**0.7g/500g*100)
			Chloroform	**0.578** (2.89g/500g*100)
			Methanol	**5.74 (**28.7g/500g*100**)**

(Roxb.) Nicolson			Ethylacetate fraction	**24 (**4.8g/20g*100)

Table no: 3 Phytochemical analysis of extracts of leaves of Naringi crenulata (Roxb.) Nicolson

Sl. No:	TEST	PEENC	CENC	MENC	EAFNC
I	**Alkaloids** • Mayers test	-	++	+++	++
	• Wagners test	-	++	+++	+
	• Hagers test	-	++	+++	++
II	**Phenolic compounds** $FeCl_3$ test	+	++	+++	+++
	• Lead acetate test	+	++	+++	+++
	• $K_2Cr_2O_7$ test	-	++	+++	+++
III	**Flavanoids** Shinoda test	+	+++	+++	+++
IV	**Proteins** Ninhydrin test	+	+	+++	++
	• Biuret test	+	+	+++	++
V	**Carbohydrates** Molisch's test	+	++	+++	+++
	• Fehling's test	+	++	+++	+++
	• Barfoed's test	-	++	+++	+++
VI	**Fats/oil** Sudan IV test	+++	+	-	-
VII	**Steroids** Salkowski test	-	++	+	+
VIII	**Saponins** Foam test	+++	+	+	-
IX	**Cardiac glycosides** Keller killiani test	-	++	+	++
X	**Anthraquinone** Borntragers test	-	-	++	++

+++ = Abundantly present
++ = SlightLy present
+ = Merely present.
- = Not present

PEENC : Petroleum ether extract of Naringi crenulata (Roxb.) Nicolson
CENC : :Chloroform extract of Naringi crenulata (Roxb.) Nicolson
MENC : :Methanol extract of Naringi crenulata (Roxb.) Nicolson
EAFNC : Ethylacetate fractions of Naringi crenulata (Roxb.) Nicolson

IN-VITRO ANTIOXIDANT ACTIVITIES

DPPH (1, 1 diphenyl 2, picrylhydrazyl)

Table no: 4 Effect of extracts of Naringi crenulata (Roxb.) Nicolson leaveson DPPH assay

Sl. No:	Concentration (µg/ml)	Percentage inhibition (%)			
		Extracts			
		CENC	MENC	EAFNC	Standard (Ascorbic acid)
1.	5	72.83±0.24	126.51±2.54	99.84±0.49	122.84±1.34
2.	10	85.19±0.62	133.19±4.49	105.12±1.12	130.1±1.79
3.	20	91.18±2.99	244.36±1.60	126.55±0.59	246.8±1.29
4.	40	123.87±1.70	254.13±0.62	170.72±1.24	255.54±1.08
5.	80	133.72±1.96	258.23±1.21	188.37±0.59	256.81±1.12
6.	160	137.11±1.02	266.18±0.94	200.49±1.18	268±1.17
7.	320	147.20±5.11	268.23±0.13	203.68±1.29	270.39±0.24
8.	640	157.20±1.25	271.23±0.36	206.68±0.36	271.39±1.44

Hydroxyl radical scavenging assay (HRSA)

Table no: 5 Effect of extracts of Naringi crenulata (Roxb.) Nicolson leaves on HRSA assay

Sl. No:	Concentration (µg/ml)	Percentage inhibition (%)			
		Extracts			
		CENC	MENC	EAFNC	Standard (Ascorbic acid)
1.	5	155.06±0.25	322.88±2.15	243.93±0.84	321.36±0.24
2.	10	158.54±0.49	333.85±6.89	246.73±0.11	324.89±0.74
3.	20	162.5±1.04	355.22±0.32	249.61±0.38	338.05±0.12
4	40	165.58±0.18	382.83±0.43	268.46±0.14	354.66±0.45
5.	80	169.5±0.12	387.91±0.54	271.74±0.11	365.74±2.18
6.	160	172.66±0.45	401.68±0.12	274.1±0.21	383.31±1.56
7.	320	177.03±0.68	406.52±0.14	285.95±0.30	403.68±0.77
8.	640	220.01±8.49	408.4±0.12	299.75±0.73	409.64±0.52

Nitric oxide radical scavenging Assay(NORSA)

Table no: 6 Effect of extracts of Naringi crenulata (Roxb.) Nicolson leaves on NORS assay

Sl. No:	Concentration (µg/ml)	Percentage inhibition (%)			
		Extracts			
		CENC	MENC	EAFNC	Standard (Ascorbic acid)
1.	5	23.67±0.68	112.3±1.19	53.29±0.71	110.26±0.65

2.	10	29.04±0.06	116.56±1.28	57.48±1.05	116.05±0.82
3.	20	34.8±2.35	117.90±0.50	62.71±0.611	124.24±1.02
4	40	39.44±1.12	119.98±1.01	69.83±1.12	140.67±1.19
5.	80	46.49±2.05	138.54±0.61	72.85±1.32	141.93±1.11
6.	160	53.40±1.57	146.67±1.19	81.53±1.47	144.38±0.11
7.	320	57.23±1.19	154.08±1.46	93.3±7.80	158.09±0.61
8.	640	63.21±0.58	164.29±0.11	105.47±5.72	162.43±1.51

ACUTE TOXICITY STUDIES OF Naringi crenulata (Roxb.) Nicolson LEAVES

Table no: 7 Results of acute toxicity study at 2000mg/kg of MENC and EAFNC

SL.NO:	PARAMETERS	RESULT
1	Motor functions	Not present
2	Twitching	Not present
3	Seizures	Not present
4	Straub reaction(restlessness)	Not present
5	Pile stiffness	Not present
6	Decreased light reflex	Not present
7	Sleepiness	Not present
8	Skeletal muscle stretching	Not present
9	Hypnosis	Not present
10	Pain	Not present
11	Ptosis(eye problems)	Not present
12	Excessive tears	Not present
13	Dysentry	Not present
14	Variation in skin tone	No change

IN-VIVO NEUROPHARMACOLOGICAL SCREENING

SEDATIVE & HYPNOTIC ACTIVITY

Sleep latency and sleeping time prompted by Phenobarbitone sodium

Table no: 8 Effect of MENC on sleep latency and sleeping time prompted by Phenobarbitone sodium

SL.NO:	TREATMENT GROUP	DURATION OF TIME(Min)	
		SLEEP LATENCY	SLEEP TIME
1	Phenobarbitone sodium(40mg/kg)(i.p)	55.16±6.27	87.16±3.71
2	MENC(400mg/kg)(p.o)	14.83±2.56[b]	175.66±15.11[b]
3	MENC(200mg/kg)(p.o)	20.33±1.63[b]	146.66±5.57[b]
4	EAFNC(400mg/kg)(p.o)	24.33±2.58[b]	142.5±8.14[b]
5	EAFNC(200mg/kg)(p.o)	31.83±1.72[b]	103±8.64[c]

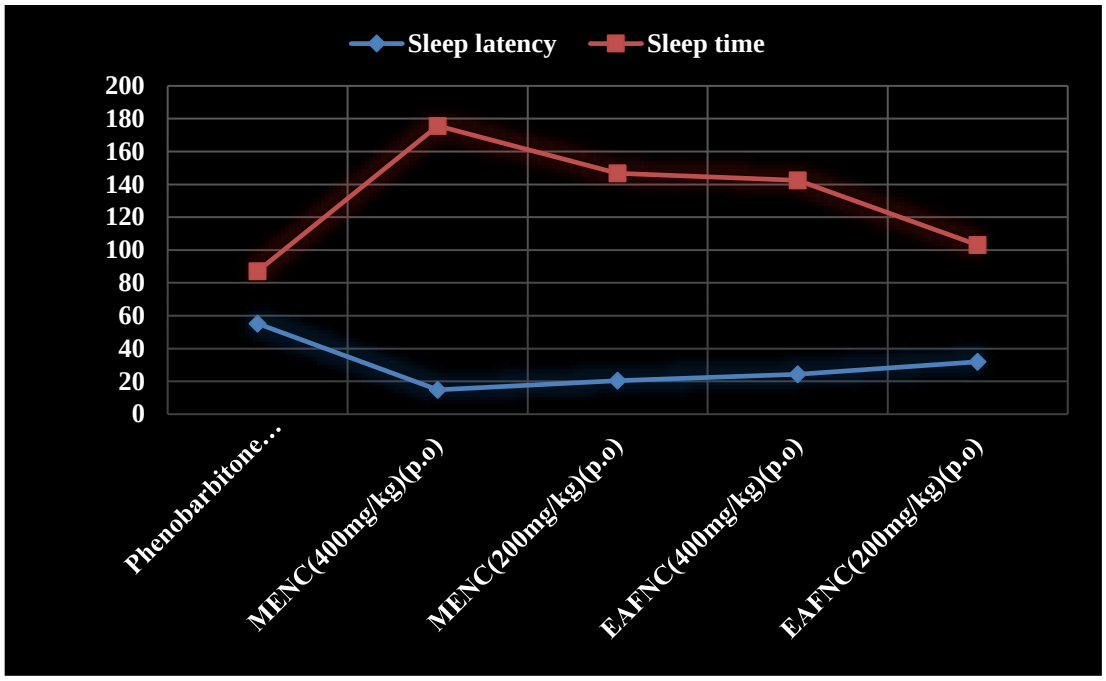

Fig.no:1. Phenobarbitone sodium induced sleep latency and sleep time on MENC and EAFNC

ANTIANXIETY ACTIVITY

Elevated plus maze(EPM)

Table no: 9 Effect of MENC & EAFNC on locomotor activity in EPM

SL. NO:	TREATMENT GROUP	TIME SPENT(sec)		NUMBER OF ENTRIES	
		OPEN ARM	CLOSED ARM	OPEN ARM	CLOSED ARM
1	Control	17.33±0.61	318.16±0.87	3.5±0.22	17.5±0.22
2	Diazepam(4mg/kg)(i.p)	26.83±0.74^{b}	391.83±2.94^{b}	2±0.36^{c}	16.33±0.49^{c}
3	MENC(400mg/kg)(i.p)	23.83±0.90^{b}	349.5±0.76^{b}	2±0.56^{c}	13.5±0.34^{b}
4	MENC(200mg/kg)(p.o)	13.16±0.47^{b}	290.1±2.96^{b}	1.33±0.55^{b}	8.33±0.33^{b}
5	EAFNC(400mg/kg)(p.o)	8.33±0.91^{b}	269.6±1.56^{b}	1±0.36^{b}	4.5±0.42^{b}
6	EAFNC(200mg/kg)(p.o)	3±0.36^{b}	198.6±2.12^{b}	0.33±0.21^{b}	2.5±0.34^{b}

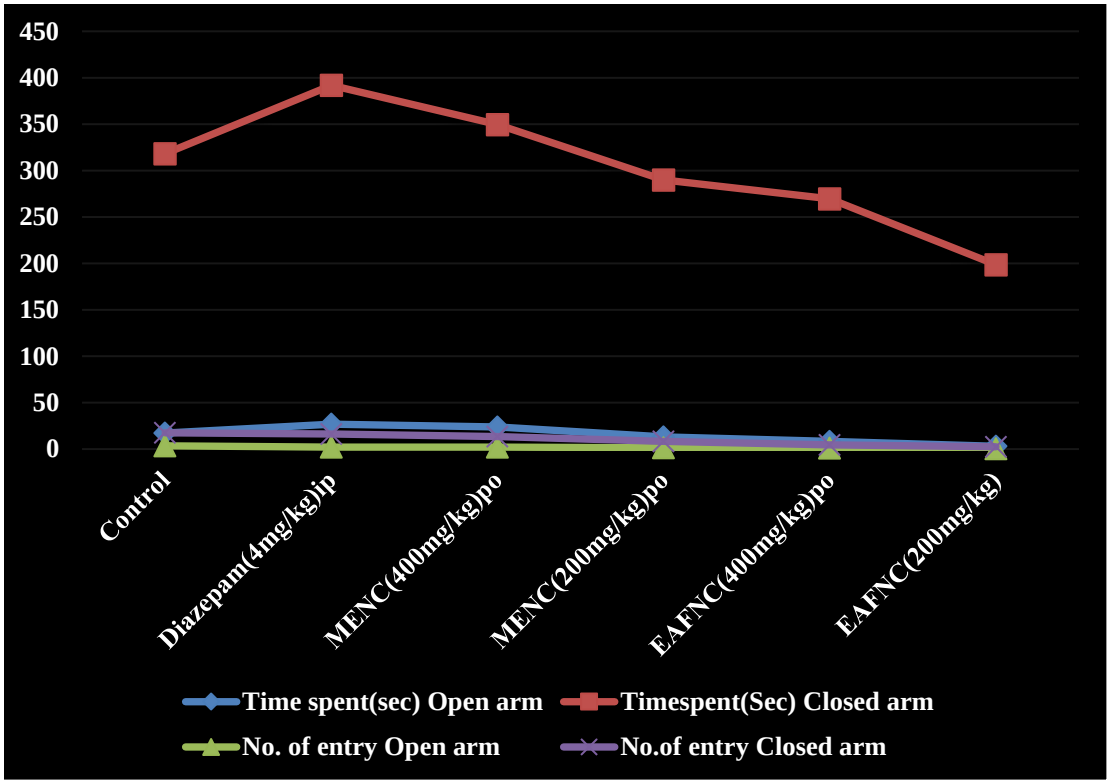

Fig.no: 2 Locomotor activity in EPM on MENC and EAFNC

GC MS OF MENC

With the inclusion of 26 distinct major recognisable chemicals in the methanolic preparation of Naringi crenulata, the GC-MS investigation has shown impressive results (Roxb.)

The existence of 26 functional phytoconstituents in the methanolic extract of Naringi crenulata is shown in Table no: ,along with the bioactive components' molecular formulas, molecular weights and structural details.The listed activities in some of the bioactive compounds are also tabulated in Table no:.Among the identified compoundspsi. 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.

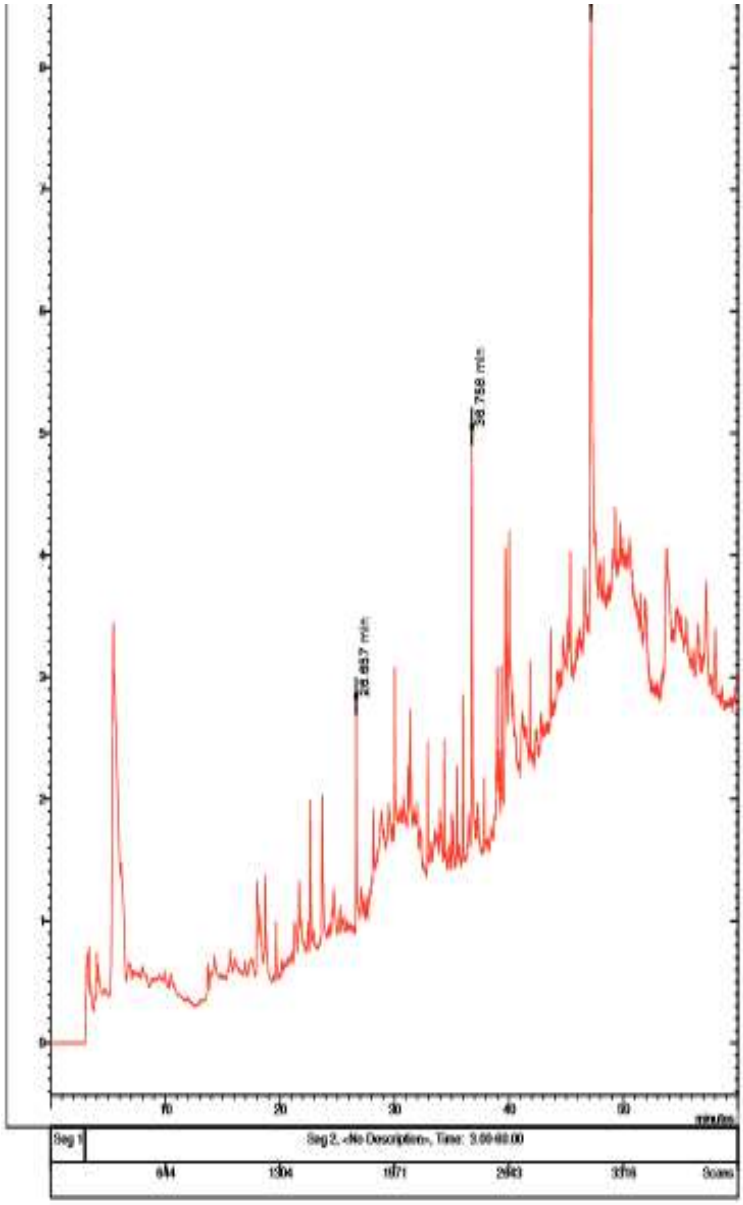

Fig.no: 3 GC MS Chromatogram of leaves of MENC

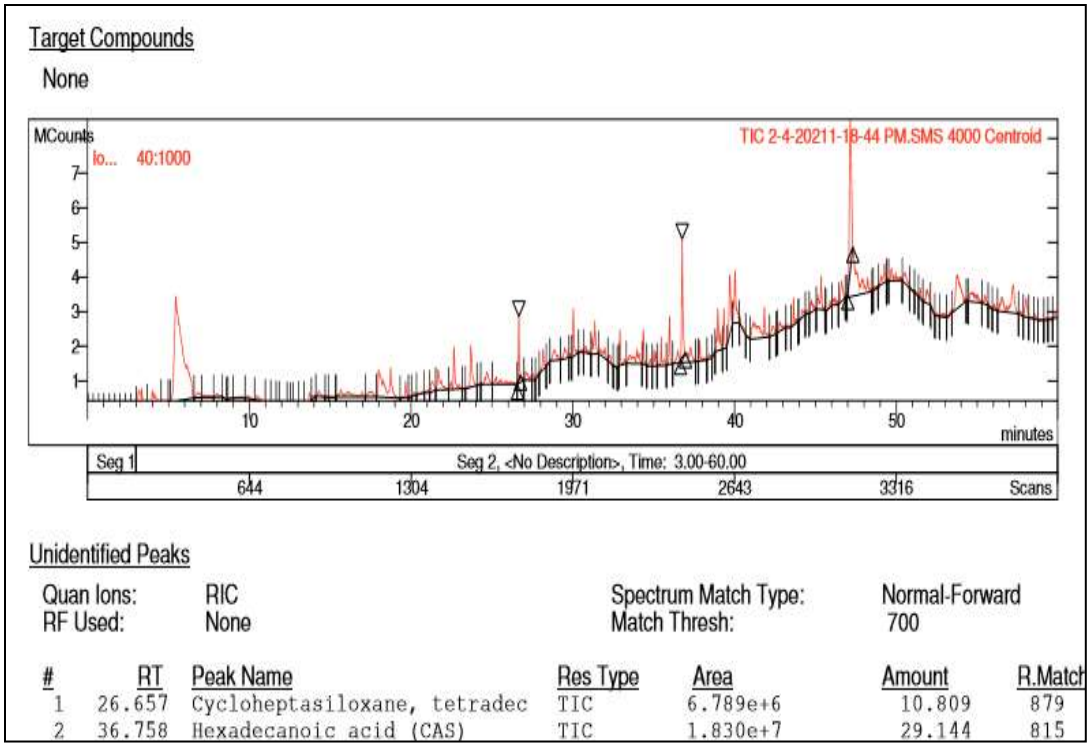

Fig.no: 4 Target compounds of leaves of MENC.

Conclusion: The findings showed that Naringi crenulata (Roxb.) extracts can be employed as an antioxidant and in the treatment of some neurological illnesses.

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