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Original Article

GCMS BASED METABOLIC PROFILING OF ESSENTIAL OIL OF *CITRUS MACROPTERA* MONTRUZ. LEAVES AND PEEL, ASSESSMENT OF *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Objective: The present investigation was designed for Gas Chromatography Mass Spectrometry (GCMS) based metabolite profiling of *Citrus macroptera* Montruz. Leaves and peel oils followed by assessment of *in vitro* antioxidant and anti-inflammatory activity.

Methods: Essential oil was extracted from leaves and peels of *Citrus macroptera* Montruz. The oil samples were subjected to GCMS analysis using Shimadzu GCMS-QP_{2010} equiped with an AOC-2oi auto-injector and AOC-2os autosampler units. *In vitro* antioxidant activities were evaluated using DPPH radical scavenging, reducing power and nitric oxide reducing method. *In vitro* anti-inflammatory activity was evaluated using protease inhibitory assay, heat induced haemolysis and albumin denaturation assay.

Results: Both the peels and leaves of *Citrus macroptera* Montruz. Yielded good amount of essential oil. 57 compounds each were identified from leaves as well as peel of *C. macroptera*. 10 common compounds have been detected in both the oil samples. Peels oil showed IC_{50} at 118.07 µg/ml and that of leaves showed IC_{50} at 252.93 µg/ml in DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. In reducing assay, peel and leaves oil showed IC_{50} at 122.5 µg/ml and 208.24 µg/ml. In albumin denaturation, the peels showed IC_{50} at 73.91 µg/ml and that of leaves showed IC_{50} at 87.48 µg/ml.

Conclusion: The oil yield denotes peel as better source of volatile oil than leaves. Essential oil of peel showed more anti-oxidant and anti-inflammatory activity than that of leaves essential oil.

Keywords: GCMS, *Citrus macroptera* Montruz., Essential oil, Antioxidant activity and anti-inflammatory activity

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INTRODUCTION

Oxidative stress is a state of physiological condition, induced by overabundance of oxidants, including reactive oxygen species such as free radicals, oxygen ions and peroxide. Oxidative damage by free radicals is associated with many diseases; cancer and heart diseases is the most common [1-3]. The amount of the antioxidants generated in the body might be inadequate, particularly under conditions of oxidative stress or inflammation when free radicals production increases [4]. Thus, adequate amount of antioxidants is required to prevent building up of free radicals and oxidative damage in our body [5]. Inflammation is a biological response to noxious stimuli such as pathogens that cause tissue and cell damage [6]. It is classified as either acute or chronic, depending on whether it involves a short response or a prolonged one, respectively [7]. *Citrus macroptera* Montr. is a semi-wild species of the Rutaceae family and the Citrus genus [8]. The fruit (i.e., the peel) is used as an ingredient in different types of meat and chicken dishes, as well as in the preparation of pickles [9]. Traditionally, the fruit has been used to treat several diseases, such as hypertension, stomach pain and alimentary disorders [10-11]. It should be noted that Melanesian papeda, as well as other variations in several languages (e. g., English, Italian, French, Spanish, Chinese, and Japanese) may apply to varieties of *C. macroptera.*

Moreover, C. macroptera is now considered to be identical to other species (e. g., Citrus combara Raf. and *C. kerrii* (Swingle) Tanaka from Vietnam and Thailand) [12]. Safford, 1905 [13] gave a firsthand account describing the use of the fruit pulp of this species in Guam, not only for washing the hair, but also as a substitute for soap to wash clothes. This species is also used in complex remedies against various diseases (Yuanga region) including epilepsy-like symptoms (Nelemwa) [14].

Essential oils are natural volatile compounds that exhibit strong odors and are produced as secondary metabolites by aromatic plants [15]. They comprise complex mixtures of substances present in quite different concentrations, such as terpene and phenylpropanoid constituents. Historically, they have been used for various medicinal purposes, ranging from skin problems to cancer treatment and are known for their antimicrobial, anti-inflammatory, sedative and analgesic properties [16].

Citrus by-products release from processing plants represent rich source of naturally occurring flavonoids [17]. The peel contains the highest amount of flavonoids than in other parts [18] and those flavonoids represent in citrus fruits belong to six peculiar classes according to their structure. They are: flavones; flavanones; flavonols; isoflavones; anthocyanidins and flavanols [19].

In the past decades, the therapeutic potential of essential oils and their constituents has been the target of researchers in the pursuit of novel drugs of plant origin, particularly those exhibiting anti-inflammatory action, to be used in the prevention or treatment of diseases [20].

Thus, the present study was done to assess the volatile compounds in the essential oil of peel and leaves of *Citrus macroptera* Montruz.

MATERIALS AND METHODS

Collection

Fresh leaves and fruits of *Citrus macroptera* Montruz. Was collected from Kwatha village, Chandel district of Manipur. It was identified by scientists of IBSD, Imphal and faculty of Botany Department, Nagaland University (fig. 1A). A voucher specimen was deposited at IBSD with voucher number IBSD/M-1031A.

Fig. 1: Leaves, fruits and seeds of *Citrus macroptera* Montruz

Extraction

The peel of the collected fresh fruit was freshly grated using a fine grater. Flavedo the coloured portion of the peel was taken. Leaves were washed to remove dust and dirt. 560g of the finely grated peel and 1 kg leaves were separately extracted by hydro distillation with the help of Clevenger type apparatus for 3-4 h.

The collected oil droplets along with water was transferred to a conical flask and dried over anhydrous sodium sulfate (approx. 1g). The resulting solution was filtered through a funnel containing a cotton plug to enable complete removal of sodium sulfate. The essential oil yield were measured. The extracted oils were stored at 4 °C until gas chromatographic determination of its components and bioactivity assays were done.

Chemicals used

1, 1-Diphenyl-2-picryl hydrazil (DPPH), L-ascorbic acid, Trypsin were purchased from HiMedia, Bengaluru. Disodium hydrogen phosphate, sodium dihydrogen phosphate for preparing sodium phosphate buffer were purchased from Merck Millipore, Germany. Dimethylsulfoxide, Potassium ferricyanide, Griess reagent, Sodium nitroprusside, Casein, Tichloroacetic acid, diclofenac sodium, bovine serum albumin were purchased from Sigma Aldrich, USA.

Gas chromatographic analysis of essential oil extract

Gas chromatographic mass spectrometry analysis was carried out at Advanced Instrumentation Research Facility (AIRF), JNU, New Delhi on a Shimadzu GCMS-QP_{2010} equiped with an AOC-2oi auto-injector and AOC-2os autosampler units, column Rtx-5 MS (Restek Corporation). The column length was 30m, 0.25 mm diameter and 0.25 µm film thickness). Column temperature was initially kept at 50 °C, then gradually increased to 280 °C at 5 °C/min rate, column pressure was 69.0 KPa. Detector interface temperature was 270 °C, Carrier gas was helium. Purge flow was 3.0 ml/min. Injection temperature was 260 °C. 1 microlitre of sample was injected in split mode in a 1:10 split ratio by auto sampler attached to the instrument. Flow rate was 1.21 ml/min.

In mass spectrophotometry, ion source temperature was 230 °C, interface temperature was kept at 270 °C, solvent cut time at 2.50 min. threshold at 1000. Temperature program used was 2 min. hold at 50 °C followed by 3 °C/min ramp to reach temperature of 210 °C held for 0 min., and final ramp of 8 °C/min. to reach the final temperature of 280 °C held for 8 min. The total length of run was 72.07 min. with scan range of 40-650 m/z and scan speed 333 amu/sec.

Data acquisition and processing

Chromatograms and mass spectra recorded were acquired by GCMS 2010QP-PLUS (Shimadzu) and processed by GCMS Solution post run analysis software (ver. 2.5) provided with the instrument. The components were identified based on the comparison of their retention indices relative to n-alkanes series and mass spectra with those of authentic samples using commercially available mass spectral libraries NIST 05, NIST 08 with a similarity index (SI) higher than 75%.

Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of the essential oil were measured by DPPH method. 0.1 mmol solution of DPPH in ethanol was prepared. Then, 1 ml of this solution was added to 3 ml ofessential oil (prepared 1:1 in DMSO) and L-Ascorbic acid (positive control) solution at different doses (10–100µg/ml). The mixture were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in Thermo Multiscan Spectrum (Thermo Scientific). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [20].

Percentage DPPH free radical inhibition was calculated and this activity was expressed as an inhibition concentration 50 (IC_{50}). The percentage inhibition was calculated by using the formula.

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Test OD Control}}{\text{Control OD}} \text{ X } 100$$

Reducing power assay

The reducing power was determined according to the method of Das *et al.*, 2014[21] with little modification.100 µl of essential oil (prepared 1:1 in DMSO) with different concentrations (10-100µg/ml) were mixed with 100 µl of 0.2 M sodium phosphate buffer (pH 6.6) and 100 µl of 1 % Potassium fericyanide. The reaction mixture was incubated at 50 °C for 20 minute. After incubation, 100 µl of 10 % trichloro acetic acid (w/v) were added. It was then centrifuged at 5000 rpm for 10 min (Eppendorf centrifuge 5430 R). The upper layer (200 µl) was mixed with 200 µl deionized water and 40 µl of 0.1 % ferric chloride. The absorbance was read at 700 nm in a 96 well microplate reader (Thermo Scientific). Higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as mean values±standard error mean. Ascorbic acid was used as standard. Percentage inhibition was calculated and the activity was expressed as an inhibition concentration 50 (IC_{50}).

The percent increase in reducing power was calculated using the following equation.

$$\% \text{ reduction} = [1-(1-As/Ac)] \text{ X } 100$$

As-maximum absorbance of max concentration of standard,

Ac-absorbance of sample

Nitric oxide reducing assay

Nitric oxide reducing assay was done following Gangwar *et al.* 2014[22] with minor modification. Under aerobic conditions, nitric oxide reacts with oxygen to produce stable products (nitrates and nitrite). The quantities of which can be determined using Griess reagent. 500 µl of test sample with different concentration (10-100 µg/ml) was mixed with 2 ml of 10 mmol SNP, 500 µl of 50 mmol phosphate buffer saline pH 7.4. They were incubated at 25 °C for 150 min. Griess reagent (500 µl) was added and incubated at 25 ° C for 30 minute. The absorbance was read at 540 nm. A phosphate buffer saline served as blank.

***In vitro* anti-inflammatory activity**

Protease inhibitory assay

1 ml of trypsin (0.5 mg. ml^{-1}) prepared in 0.1 M phosphate buffer, pH-7 was pre-incubated with 1 ml of sample with different concentration (10-100 ug/ml) at 37 °C for 15 minute. After incubation 2 ml of 1 % casein prepared in 0.1 M phosphate was added. It was then incubated at 37 °C for 30 minute. The reaction was terminated by adding 2.5 ml of 0.44 M Trichloroacetic acid. It was transferred to centrifuge tube and centrifuge at 10,000 rpm for 15 minute. Supernatant was taken and OD was measured at 280 nm [23].

Heat induced haemolysis assay

2 ml of reaction mixture consisting of 1 ml of test sample solution and 1 ml of 10% RBC suspension was taken in 2 ml micro centrifuge tube. It was incubated at 56 °C for 30 min. in water bath. The reaction mixture was cooled and centrifuged at 2500 rpm for 5 min. The supernatant was taken and absorbance was taken at 560 nm. Saline and Diclofenac sodium was taken as control and standard reference respectively [24].

Albumin denaturation assay

The *in vitro* anti-inflammatory activity was assessed using inhibition of albumin denaturation method. Reaction mixture of 1 % aqueous solution of bovine serum albumin (Sigma) and an essential oil at different concentration (10-100 µg/ml) was taken in a centrifuge tube and pH was adjusted to 6.8 using 1N HCl. It was incubated at 37 °C for 20 min followed by heating at 57 °C for 20 min. The solution was cooled and absorbance was read at 660 nm [25].

Statistical analysis

The results were expressed as the mean±SEM (n=3). Linear regression was used to calculate IC50. Results were considered significant at ***P<0.001, or **P<0.01 or * P<0.05 when compared test groups v/s control group. For numerical results, one-way analysis of variance (ANOVA) with Tukey-Kramer Multiple Comparisons post tests were performed using GraphPad InStat Version 3 (GraphPad Software). All the graphs and fig. were drawn using GraphPad Prism.

RESULTS AND DISCUSSION

Hydrodistillation of peel and leaves of *C. macroptera* yielded 8 ml for peel and 4.54 ml for leaves which represent 1.43 % and 0.46 % respectively. Hydrodistillation of the leaves of *C. macroptera* yielded 1.67 % of essential oil, GC/MS Analysis of the *C. macroptera* essential oil allowed the identification of 35 compounds accounting for 99.1% of the total composition [25]. 57 compounds each were identified from leaves and peel of *C. macroptera*. Identified compounds in leaves are presented accounting to 96.79 % of total composition negating the trace amount (<0.1%), and that of peels accounting to 99.18% of total composition. Relative percentage composition of essential oil of the leaves (table 1) and peel (table 2) are presented.

In both the essential oil, 10 same compounds have been detected (table 3). However, the percentage oils components calculated as the percent peak area were different. Chromatogram of leaves and peel essential oil were presented in fig.1 and fig.2. The major volatile component present in peels were Bicyclo [4.1.0] heptane, 7-(1-methylethylidene)-(60.03 %), Mentha-2, 8-dien-1-ol<trans-,para->(4.0%), Limonene oxide (3.53%), trans Carveol (2.67%) whereas in leaves essential oilmajor components were 2-methylaminobenzoic acid methyl ester (57.16 %), bicyclo [4.1.0] heptane, 7-(1-methylethylidene)-(23.23 %), β-pinene (8.79 %), ocimene<(E)-BETA->DB5-519 (3.29 %). *Citrus macroptera* has been reported to contain lupeol, stigmasterol, beta-pinene, limonene, beta-caryophyllene, geranial edulinine, ribalinine and isoplatydesmine [26-28].

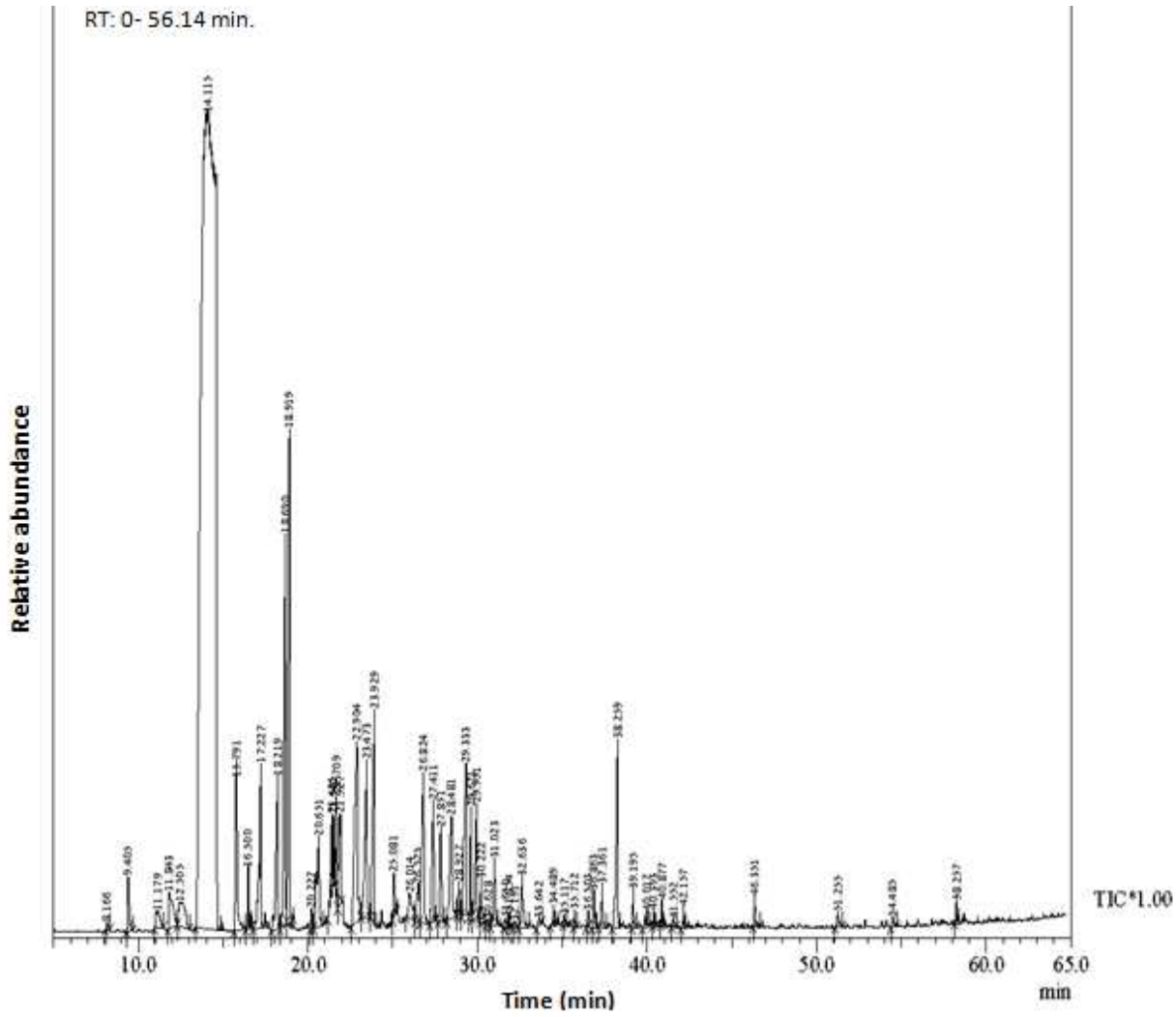

Fig. 1: Chromatogram of leaves essential of *Citrus macroptera* Montruz., RT-retention time

Table 1: Relative percentage chemical composition of essential oil of leaves of *citrus macroptera* montruz

Peak	Compound	R. time	Area %
1	Nonane<n->	8.062	tr
2	Thujene<alpha->	9.141	tr
3	BICYCLO[3.1.1]HEPT-2-ENE, 2,6,6-TRIMETHYL-	9.429	tr
4	BICYCLO[2.2.1]HEPTANE, 2,2-DIMETHYL-3-METHYL	10.013	tr
5	BICYCLO[3.1.1]HEPTANE, 6,6-DIMETHYL-2-METHYL	11.420	8.79
6	1,6-OCTADIENE, 7-METHYL-3-METHYLENE-	11.895	tr
7	OCTANAL	12.423	tr
8	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	14.022	23.23
9	1,3,7-OCTATRIENE, 3,7-DIMETHYL-, (E)-	14.149	0.17
10	OCIMENE<(E)-BETA->DB5-519	14.675	3.29
11	1,4-CYCLOHEXADIENE, 1-METHYL-4-(1-METHYLET	15.040	0.28
12	BENZENAMINE, N-METHYL-	15.348	0.36
13	2-FURANMETHANOL, 5-ETHENYLTETRAHYDRO-. AL	15.666	tr
14	2,7,7-TRIMETHYL-3-OXATRICYCLO[4.1.1.0~2,4~]OCT	15.880	tr
15	CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHYLIDE	16.339	tr
16	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-	16.987	0.76
17	NONANAL	17.109	tr
18	1H-PYRAZOLE, 3-METHYL-	17.531	tr
19	Mentha-2,8-dien-1-ol<trans-, p->	17.897	tr
20	Limonene oxide<cis->	18.468	tr
21	P-MENTHA-E-2,8(9)-DIEN-1-OL	18.575	tr
22	(Z)-2,2-Dimethyl-3-(3-methylpenta-2,4-dien-1-yl)oxirane	18.892	tr
23	Pinocarvone	19.848	tr
24	1-ISOPROPYL-4-METHYL-3-CYCLOHEXEN-1-OL	20.612	0.56
25	Heptanedinitrile, 4-acetyl-4-methyl-	20.990	tr
26	(+)-ALPHA-TERPINEOL (P-MENTH-1-EN-8-OL)	21.257	tr
27	2,4,6-Trimethyl-1,3,6-heptatriene	21.442	tr
28	DECANAL	21.853	tr
29	2,6-Dimethyl-3,5,7-octatriene-2-ol,,E,E-	22.034	tr
30	Carveol<trans->	22.546	tr
31	2-CYCLOHEXEN-1-OL, 2-METHYL-5-(1-METHYLETHE	23.078	tr
32	2-CYCLOHEXEN-1-ONE, 2-METHYL-5-(1-METHYLET	23.659	tr
33	Dihydro carveol<neoiso->	25.061	tr
34	Formamide, N-methyl-N-phenyl-	25.486	0.11
35	2H-PYRAN-2-CARBOXALDEHYDE, 3,4-DIHYDRO-2,5-	27.630	tr
36	Copaene<alpha->	29.543	tr
37	Elemene<beta->	30.251	tr
38	2-METHYLAMINOBENZOIC ACID METHYL ESTER	32.222	57.16
39	1,4,8-CYCLOUNDECATRIENE, 2,6,6,9-TETRAMETHYL	33.323	tr
40	Anthranilate<methyl-, N,N-dimethyl->	34.237	tr
41	Bicyclogermacrene	34.881	0.12
42	Farnesene<(E,E)-, alpha->	35.197	tr
43	NAPHTHALENE, 1,2,3,5,6,8A-HEXAHYDRO-4,7-DIME	35.849	tr
44	Methyl anthranilate	37.212	0.17
	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	37.459	0.87
46	(-)-Spathulenol	38.097	0.39
47	1,1,4,7-TETRAMETHYLDECAHYDRO-1H-CYCLOPROP	38.289	0.18
48	Globulol	38.607	0.10
49	Cryptomeridiol	38.973	tr
50	GLOBULOL DB5-1841	39.822	tr
51	T-Muurolol	40.415	tr
52	. alpha.-Cadinol	40.905	0.10
53	Farnesol<(2Z,6Z)->	43.329	tr
54	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-	44.679	tr
55	HEXADECANOIC ACID	51.407	tr
56	Benzoic acid, 2-(2-methoxycarbonylphenylaminomethylami	53.282	tr
57	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R	56.143	0.15
			96.79

R. Time-retention time, tr–trace (<0.1 %)

The antioxidant activity of essential oil extracted from leaves and peel of *Citrus macroptera* were investigated. Peels oil showed IC_{50} at 118.07 µg/ml and that of leaves showed IC_{50} at 252.93 µg/ml in DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay (table 4). In reducing assay, peel and leaves oil showed IC_{50} at 122.5 µg/ml and 208.24 µg/ml (table 4). In nitric oxide assay essential oil of peel and leaves showed 236.71 and 135.43 as their respective IC_{50s}. Thus, the peels proved to be more potential candidate of antioxidant as compared to leaves essential oil. We are reporting the antioxidant potential of the essential oil of *Citrus macroptera* Montruz. Although there was a report that the oil did not exhibit any *in vitro* free-radical-scavenging (DPPH) [29].

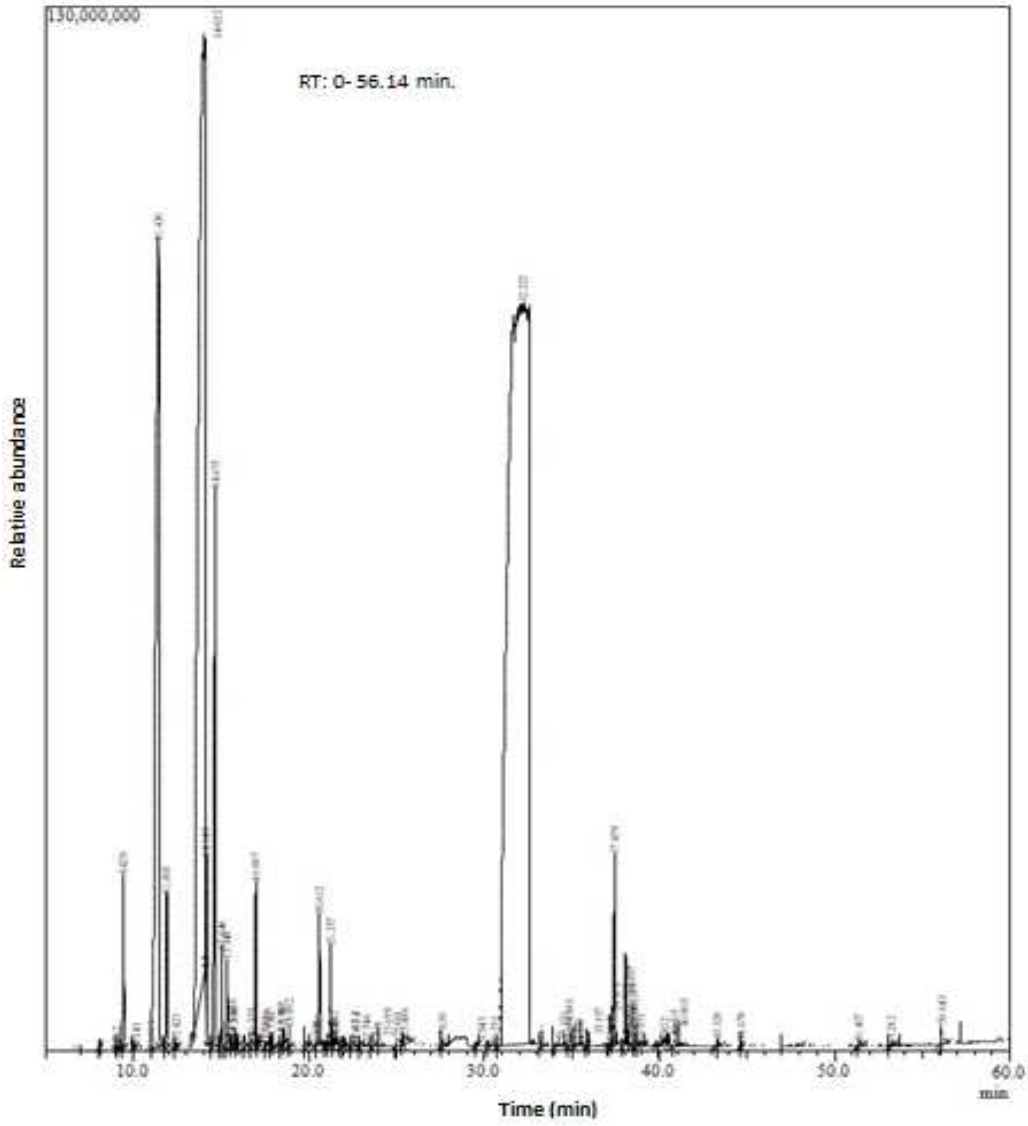

Fig. 2: Chromatogram of peel essential oil *Citrus macroptera* Montruz, RT-retention time

Table 2: Relative percentage chemical composition of essential oil of peels of *Citrus macroptera* montruz

Peak	Compound	R. Time	Area%
1	HEPTANAL	8.166	tr
2	BICYCLO[3.1.1]HEPT-2-ENE, 2,6,6-TRIMETHYL-	9.405	0.46
3	Pinene oxide<beta->	11.179	0.36
4	1,6-OCTADIENE, 7-METHYL-3-METHYLENE-	11.843	0.75
5	OCTANAL	12.505	0.82
6	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	14.115	60.03
7	1-OCTANOL	15.791	1.43
8	2-FURANMETHANOL, 5-ETHENYLTETRAHYDRO-. AL	16.500	0.31
9	NONANAL	17.227	1.45
10	Mentha-2,8-dien-1-ol<trans-, para->	18.219	1.49
11	Limonene oxide<cis->	18.690	3.53
12	Mentha-2,8-dien-1-ol<cis-, para->	18.919	4.00
13	3-METHYL-3,3A,4,6A-TETRAHYDRO-2(1H)-PENTALE	20.227	0.11
14	2-ISOPROPENYL-5-METHYL-HEX-4-ENAL	20.651	1.26
15	3-CYCLOHEXENE-1-METHANOL,. ALPHA.. ALPHA.,4-	21.425	0.45
16	1,3,6-Heptatriene, 2,5,5-trimethyl-	21.540	0.34
17	1,3,6-Heptatriene, 2,5,6-trimethyl-	21.709	0.45
18	DECANAL	21.927	0.92
19	Carveol<trans->	22.904	2.67
20	2-CYCLOHEXEN-1-OL, 2-METHYL-5-(1-METHYLETHE	23.473	1.79
21	2-CYCLOHEXEN-1-ONE, 2-METHYL-5-(1-METHYLET	23.929	2.07
22	2-CYCLOHEXEN-1-ONE, 3-METHYL-6-(1-METHYLET	25.081	0.28

23	Limonen-10-ol	26.014	0.47
24	Undecanal	26.525	0.53
25	(3R,6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohe	26.824	1.71
26	(3R,6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohe	27.411	1.14
27	P-MENTHA-1,8-DIEN-4-HYDROPEROXIDE	27.851	0.79
28	1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	28.481	1.23
29	3-Heptadecen-5-yne, (Z)-	28.927	0.24
30	2(5H)-Furanone, 4-methyl-3-(2-methyl-2-propenyl)-	29.333	1.71
31	Copaene<alpha->	29.621	0.52
32	Sinensal<alpha->	29.951	1.03
33	. beta.-copaene	30.222	0.29
34	Carvone oxide<cis->	30.628	tr
35	Limonene oxide<cis->	31.023	0.58
36	1-CYCLOHEXENE-1-METHANOL, 4-(1-METHYLETHE	31.650	tr
37	. beta.-copaene	31.854	tr
38	2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL-	32.191	tr
39	Limonene oxide<cis->	32.636	0.52
40	Heptan-2-one	33.642	tr
41	2,7-Octadiene-1,6-diol, 2,6-dimethyl-	34.489	0.13
42	Thujyl acetate	35.117	tr
43	CADINENE<DELTA->DB5-1700	35.712	tr
44	1-ISOPROPYL-4,7-DIMETHYL-1,2-DIHYDRONAPHTH	36.503	tr
45	Hedycaryol	36.863	0.27
46	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	37.361	0.30
47	(-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRIM	38.259	1.63
48	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]do	39.195	0.22
49	Eudesmol<epi-gamma->	40.017	0.16
50	T-Muurolol	40.393	tr
51	. alpha.-Cadinol	40.877	0.14
52	Caryophyllene oxide	41.552	tr
53	2-Propenoic acid, tridecyl ester	42.157	0.12
54	2(3H)-NAPHTHALENONE, 4,4A,5,6,7,8-HEXAHYDRO-4	46.351	0.21
55	3-Methyl-hepta-1,6-dien-3-ol	51.255	0.14
56	Isophytol	54.485	tr
57	4,8-DIMETHYLNONA-3,7-DIEN-2-OL	58.257	0.13
			99.18

R. Time-retention time, tr–trace (<0.1 %)

Table 3: Common compound found in essential oil of leaves and rind of *Citrus macroptera* montruz with their area %

Component	Area % leaves essential oil	Area % peels essential oil
BICYCLO[3.1.1]HEPT-2-ENE, 2,6,6-TRIMETHYL-	0.64	0.46
Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	23.23	60.03
NONANAL	tr	1.45
Limonene oxide<cis->	tr	3.53
P-MENTHA-E-2,8(9)-DIEN-1-OL	tr	1.49
Carveol<trans->	tr	2.67
Copaene<alpha->	tr	0.52
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	0.87	0.30
T-Muurolol	Tr	tr
alpha.-Cadinol	0.10	0.14

Tr-trace (<0.1%)

Table 4: *In vitro* antioxidant activity of essential oil of peels and leaves of *Citrus macroptera* montruz

Essential oil of	Oil yield %	DPPH (IC_{50}) µg/ml	Reducing power assay (IC_{50}) µg/ml	Nitric oxide reducing assay (IC_{50}) µg/ml
leaves	0.46 %	252.93±0.004	208.24±0.08	236.71±0.01
Rind	1.43 %	118.07±0.007	122.5±0.12	135.43±0.09
Ascorbic acid	-	5.62±0.001	8.1±0.04	7.6±0.67

Results are mean±SD-standard deviation, *n*= 3, DPPH-1, 1-Diphenyl-2-picryl hydrazil

The IC_{50} of the *in vitro* anti-inflammatory assay (protease inhibitory, heat induced haemolysis and albumin denaturation) of the essential oil sample are presented in (table 5). In albumin denaturation assay, the peels showed IC_{50} at 73.91 µg/ml and that of leaves showed IC_{50} at 87.48 µg/ml. The IC_{50} of the other assay are also presented in the table 5. The oil yield denotes peel as better source of volatile oil than leaves. Essential oil of peel showed more anti-oxidant and anti-inflammatory activity than leaves oil. Plant essential oils and their components have been known to have biological activities, especially antimicrobial [30], antifungal [31], insecticidal [32], antiparasitic, spasmolytic and antioxidant activities [33].

Table 5: *In vitro* anti-inflammatory activity of peels and leaves of *Citrus macroptera* montruz

S. No.	Essential oil sample	Protease inhibitory assay (IC_{50}) µg/ml±SD	Heat induced haemolysis (IC_{50}) µg/ml±SD	Albumin denaturation assay (IC_{50}) µg/ml±SD
1	*C. macroptera* leaves	106.71±0.11	187±0.33	87.48±0.32
2	*C. macroptera* rind	96.4±0.25	124.89±0.07	73. 91±0.05
3.	Diclofenac sodium	-	11.79±0.01	55.8±0.16
4.	Protease inhibitor cocktail (Sigma)	11.49±0.008	-	-

IC-Inhibition Concentration, SD-standard deviation, no. of replicate, *n=3*

In many inflammatory disorders there is excessive activation of phagocytes, production of O_2. OH radicals as well as non-free radicals species (H_2O_2), which can harm severely tissues either by powerful direct oxidizing action by activating matrix metello protinase damage seen in various arthritic tissues [34, 35]. Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research, more so, since the currently used anti-inflammatory agents both steroidal and non-steroidal are prone to evoking serious adverse reactions [36, 37]. Recently fruits and vegetables have played a significant part in the chemoprevention of diseases and aging are recognized as natural antioxidants; antioxidant compounds play a crucial role in the treatment of various diseases related to degenerative disorders namely cardiovascular and brain diseases, arthritis, diabetes, cancer, immune system decline by acting as free radical scavengers, and thus decreasing the extent of oxidative damage [38]. Several factors related to dietary antioxidants such as poor solubility, inefficient permeability, instability, extensive first pass metabolism and rapid gastro-intestinal degradation, which have limited their extensive use; hence a therapeutic strategy may be formulated where antioxidant capacity of the cells may be used for long term effective treatment [39]. GC-MS analysis provides the idea about the chemical structure, molecular formula and idea about the functional group present in the compound [40].

CONCLUSION

Components of essential oil of peel and leaves of *Citrus macroptera* Montruz. Were identified. Oil yield were found to be 1.43 % and 0.46 % in peel and leaves respectively. The oil yield % denotes peel as better source of volatile oil than leaves. The same compounds has also been detected in both the essential oil but the relative percentage composition of the essential oil were different. Essential oil of peel as well as leaves exhibit antioxidant and anti-inflammatory activity. From the calculated activity based on IC_{50} values, essential oil of peel showed more anti-oxidant and anti-inflammatory activity than leaves oil.

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AUTHORS CONTRIBUTION

The study concept was designed by Dr. Ch. Brajakishore Singh, Data acquisition, data analysis and manuscript preparation was done by Kh. Nongalleima. Final approval and overall checking was done by Dr. T. Ajungla.

CONFLICT OF INTERESTS

Declared none

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