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Bioactive Compounds from Fruit and Seeds of *Citrus medica* L.

Dr. Mrunalini N. Desai[1]; Dr. Priyanka P. Jadhav[2]; Girija A. Ghorpade[3]

[1;2;3]Vivekanand College, Kolhapur (An Empowered Autonomous Institute)

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Abstract: The bioactive volatile compounds from plant samples are analyzed by using GC-MS technique. Plant extract of *Citrus medica* L. are analysed for bioactive compounds. 40 major bioactive volatile compounds have been identified in the fruit and 16 in seeds viz, β-Myrene, β- Bisabolene, Octadecanoic acid, ethyl ester, Acetyl betulinaldehyde, D-Limonene,Citronellal, Geranyl acetate, 2,4-Heptadienal, (E,E)- Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 2-Decenal(E).These compounds are found to be useful as anti-cancerous, antimicrobial , anti-inflammatory, anti-aging and analgesic, nematicide. *Citrus medica* fruit and seeds are thus rich in volatile compounds.

Keywords: *Citrus Medica, Bioactive Volatile Compounds, GC-MS Technique, Mhalung, Bioresource,*

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I. INTRODUCTION

Citron is a small evergreen tree or shrub in the family of Rutaceae found in the western ghats of Maharashtra. The mythological importance of this fruit in Kolhapur is famous for Ambabai temple is considered as one of the Shakti peethas in India. This supreme deity is believed to be the mother of three different forms i.e Mahakali, Mahalaxmi and Mahasaraswati. The deity of Mahalaxmi holds objects of symbolic value. Goddess Ambabai holds a mhalunga i.e *Citrus medica* in her lower right hand. Fruit is used by local communities for their ethno medicinal potential. It is a short, medium-sized evergreen tree, reaches 4–8 m in height. Leaves are up to 20 cm long and flowers grow in groups of three to twelve. The fruit size 20–30 cm varies according to the state of maturation from green to yellow (Fig-3). Most citron varieties contain a large number of monoembryonic seeds. The seeds are white with dark inner coats and red-purplish chalazal spots for the acidic varieties, and colorless for the sweet ones (Fig-4). Mātuluṅga (मातुलुङ्ग) is a Sanskrit word referring to the "Citron", a species of citrus fruit from the Rutaceae family. It is also known as Mātuliṅga or Mātuliṅgī. It is used throughout Ayurvedic literature such as the Caraka-saṃhitā and the Suśruta-saṃhitā. Chemical composition and the Antioxidant, anti-inflammatory and hypoglycemic potential of flowers, leaves and fruits of *Citrus medica* L. was studied by Menichini et al.(2011)[1]. The beneficial phytochemicals, antioxidant activities, and health benefits were discussed by K Panera, et. al. (2012)[2]. Nutritional, phytochemical composition and health benefits were discussed by N Chhikara, et. al. (2018)[3]. The antifungal activity of limonin, limonol from Citrus medica against Puccinia arachidis, a groundnut rust pathogen was shown by Govindachari et al. (2000)[5]. In this study researchers determined the cytotoxicity of essential oils of Iranian *Citrus lemon* L *Citrus medica* L peels on cancer cell was tested by Monajemi et al. (2005)[4]. The present attempt was determine the possible chemical constituents from *Citrus medica* fruit and seeds by using GC-MS technique.

II. MATERIAL AND METHOD

The freshly collected fruits and seeds were dried in the oven at 60^0 and coarsely powdered in an electric grinder. The ethanolic extracts obtained from fruit and seeds of *Citrus medica* were subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds.

GC-MS analysis of the samples were carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5 MS column-Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40°C and held for 3 min. and the final temperature of the oven was 480°C with rate at 10°C [min. sup. 1].

2 ml sample was injected with split less mode. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 ev. The total running time for a sample was 45 min. The chemical components from the ethanolic extracts of fruit and seeds were identified by comparing the retention time of chromatographic peaks using Quadra pole detector with NISI Library to relative, retention indices. Quantitative determinations were made by

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relating respective peak areas to TIC areas from the GIC-MS.

III. RESULTS

Chemical components and medicinal properties of *Citrus medica* L. in fruit and seeds are shown in Table- 1 and Table- 2. Mass spectra analysis of bioactive volatile compounds of *Citrus medica* fruit and seeds are shown in Table -3 and Table-4. While the mass fragments are presented in Figure 1 and Figure 2. It is clear from the tables and figures that fruit and seeds of *Citrus medica* L. are rich in bioactive volatile compounds.

Table 1 Name of the Bioactive Volatile Compounds and their uses in Fruit of *Citrus medica*.

Sr. No.	Name of the Bioactive Volatile Compounds	Uses of the Bioactive Volatile Compounds
1	beta.-Myrcene	It is Used as a painkiller,used as antioxidant,anti inflammatory,anti-aging,anxiolytic,analgesic
2	Hexanoic acid, ethyl ester	It has Antioxidants,hypo-cholesterotemic,nematicide
3	2,4-Heptadienal, (E,E	It is a Derivative of ascorbic acid which naturally acts as natural flavor for antifungal properties
4	D- Limonene	It is Used for obesity,cancer and bronchitis.It is used as anti-inflammatory, antioxidant,anti-stress .
5	Citronellal	Has antimicrobial, antihelmintic, antioxidant, anticonvulsant, antitrypanosomal and wound healing
6	Octanoic acid, ethyl ester	It is used as a fragrance ingredient in personal care product.
7	Octanoic acid	It is an antibacterial agent,a human metabolite**.**
8	2,6-Octadienal, 3,7-dimethyl-, (Z)	It is Used as a natural antimicrobial agent.Essential oils in citral shows antifungal, antimicrobial, antiparasitic features
9	2-Decenal, (E)-	It is used as a nematicide
10	Citral	It shows antibacterial, antioxidant, anticancer, anti-diabetic and anti-inflammatory properties.
11	Nonanoic acid, ethyl ester	It has a role as a metabolite
12	Geranyl acetate	It is used in the fragrance formulation of perfumes, cosmetics, and soaps, treatments of many different health concerns such as fungal, viral, bacterial infections, inflammation, and even cancer treatment.
13	n-Tridecan-1-ol	It has a role as a bacterial metabolite, a plant metabolite and a human metabolite
14	Decanoic acid, ethyl ester	It is Used in the fragrance industry. Has a role as an antibacterial agent, an anti-inflammatory agent, a human metabolite, a volatile oil component, a plant metabolite and an algal metabolite.
15	cis-.alpha.-Bergamotene	It is Used as an antioxidant,anti-inflammatory, immunosuppressive, cytotoxic, antimicrobial ,anti-diabetic
16	Caryophyllene	It is stress, anxiety and pain-relieving terpene and shows analgesic properties, antibacterial gastroprotective, anxiolytic, anti-inflammatory properties.
17	Cyclohexane, octyl	It can serve as *probable alternatives to antibiotics with potential antimicrobial properties*
18	cis-.beta.-Farnesene	Has Anti-carcinogenic, antibacterial and antifungal activity. Also has anti-inflammatory and anti-allergy properties.
19	Bicyclo[2.2.1]heptane, 2-methyl-3-methylene-2-(4-methyl-3-pentenyl)-, (1S-exo)-	It is used in Cosmetic *as a* perfuming agent.
20	cis-.alpha.-Bisabolene	It is used as a flavoring and fragrance agents
21	Beta Bisabolene	It is a Major component of essential oil
22	1-Hexadecanol	*It has antimicrobial activity*
23	9-Tetradecen-1-ol, acetate, (E)-	*It is used* in dermatological products such as skin creams
24	1-Eicosene	It has anti-microbial and anti-inflammatory properties.
25	Heptadecane	It has Antibacterial, antioxidant
26	alpha.-Bisabolol	It shows anti-inflammatory properties**.**
27	Ethyl 14-methyl-hexadecanoate	It has Antibacterial activity
28	7-Hexadecanone	7-HEXADECANONE is widely used in cosmetics, personal care products and fragrances to impart a specific fragrance to products.
29	Lidocaine	It is Used as an anesthetic. Also used to treat minor burns, scrapes and insect bites.
30	Hexadecanoic acid, methyl ester	It is a Plant metabolite
31	n-Hexadecanoic acid	It is an Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Anti-androgenic Flavor Hemolytic 5-Alpha reductase inhibitor

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32	Hexadecanoic acid, ethyl ester	It shows Antioxidant, antimicrobial and anti-inflammatory activities.
33	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	It has Anti-inflammatory, analgesic properties.
34	6-Octadecenoic acid, methyl ester, (Z)-	It is used in diarrhea, abdominal pain and bleeding.
35	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z	It has analgesic, anti-inflammatory, anti- diarrheal, antmicrobial, anti-plasmodial, anti-ulcer, contraceptive and estrogenic properties.
36	Methyl stearate	*role as a metabolite*
37	Ethyl Oleate	*Antibacterial property*
38	Ethyl 9,12,15-octadecatrienoate	Decrease the risk of heart disease by helping to maintain normal heart rhythm and pumping**.**
39	Octadecanoic acid, ethyl ester	Anti-inflammatory, hypocholesterolemic, cancer preventive, nematicide**.**
40	Acetyl betulinaldehyde	Anticancer activity

Table 2 Name of the Bioactive Volatile Compounds and their uses in Seeds of *Citrus medica*.

Sr. No	Name of the Bioactive Volatile Compounds	Uses of the Bioactive Volatile Compounds
1	2,4-Heptadienal, (E,E)-	It can be used as Natural flavor for antifungal properties.
2	Octanoic acid, ethyl ester	It can be used as a fragrance ingredient in personal care products
3	2-Decenal, (E)-	It can be used as a nematicide
4	2-Dodecenal, (E)-	It can be Used as an anthelminthic drug,a plant metabolite and an antibacterial agent
5	Hexadecanoic acid, methyl ester	It has antimicrobial activity
6	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	It can be used in personal care products
7	n-Hexadecanoic acid	It Has Anti-inflammatory agent Antioxidant Hypocholesterolemic Nematicide ,Anti-androgenic
8	Hexadecanoic acid, ethyl ester	It has Antioxidant Hypocholesterolemic Nematicide ,Anti-androgenic Flavor Hemolytic 5-Alpha reductase inhibitor
9	12,15-Octadecadienoic acid, methyl ester	It has Anti-inflammatory properties in addition to analgesic and ulcerogenic properties
10	6-Octadecenoic acid, methyl ester, (Z)-	It is used in Diarrhea, abdominal pain and bleeding
11	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z	It has Antibacterial and anticandidal Anti-inflammatory, hypocholesterolemic, cancer preventive
12	Heptadecanoic acid, 16-methyl-, methyl ester	Has potent effect on skin cancer protein
13	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	It has Anti-inflammatory and antioxidant property
14	Glycidyl palmitate	It Can promote smooth skin
15	Oleoyl chloride	It is Used as a chemical reagent in the preparation of Vitamin E analogs as well as antiviral nucleoside phosphoramidate prodrugs used in the treatment of HIV and HBV
16	Glycidyl oleate	It Can be used as an antioxidant and antimicrobial effects.

Table 3 Mass Spectral Analysis of Bioactive Volatile Compounds of *Citrus medica* L. Fruit

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	8.395	8.250	8.560	1515001	0.08	.beta.-Myrcene
2	8.747	8.585	9.010	7625250	0.38	Hexanoic acid, ethyl ester
3	9.306	9.060	9.490	14178917	0.71	2,4-Heptadienal, (E,E)-
4	9.577	9.465	10.040	351173751	17.69	D-Limonene
5	13.409	13.355	13.545	2480290	0.12	Citronellal
6	14.662	14.550	14.765	4706169	0.24	Octanoic acid, ethyl ester
7	15.070	14.910	15.265	4640973	0.23	Octanoic acid
8	16.006	15.910	16.195	8797010	0.44	2,6-Octadienal, 3,7-dimethyl-, (Z)-
9	16.666	16.625	16.745	1961287	0.10	2-Decenal, (E)-
10	16.846	16.745	17.055	12367433	0.62	Citral
11	17.303	17.185	17.340	1065678	0.05	Neodecanoic acid
12	17.455	17.370	17.545	2063897	0.10	Nonanoic acid, ethyl ester
13	18.341	18.280	18.415	1755663	0.09	2,4-Decadienal, (E,E)-
14	19.173	19.110	19.210	3000120	0.15	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-
15	19.275	19.200	19.375	-741477	-0.04	Heptafluorobutyric acid, n-tetradecyl ester
16	19.429	19.375	19.500	2766329	0.14	2-Undecenal
17	19.696	19.610	19.825	6337539	0.32	Geranyl acetate
18	20.001	19.935	20.050	3504857	0.18	n-Tridecan-1-ol

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Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
19	20.109	20.060	20.140	1906706	0.10	Decanoic acid, ethyl ester
20	20.585	20.540	20.645	1554479	0.08	cis-.alpha.-Bergamotene
21	20.784	20.695	20.825	5572164	0.28	Caryophyllene
22	21.093	20.970	21.165	27832758	1.40	cis-.alpha.-Bergamotene
23	21.459	21.400	21.500	524562	0.03	Cyclohexane, octyl-
24	21.573	21.520	21.655	2471777	0.12	cis-.beta.-Farnesene
25	21.762	21.695	21.810	452316	0.02	Bicyclo[2.2.1]heptane, 2-methyl-3-methylene-
26	22.786	22.730	22.830	2033472	0.10	cis-.alpha.-Bisabolene
27	22.971	22.880	23.140	44448619	2.24	.beta.-Bisabolene
28	24.951	24.885	25.010	2009143	0.10	1-Hexadecanol
29	26.693	26.625	26.770	558844	0.03	9-Tetradecen-1-ol, acetate, (E)-
30	26.861	26.750	26.940	5923839	0.30	1-Eicosene
31	27.389	27.300	27.445	501719	0.03	Heptadecane
32	27.527	27.455	27.595	1357064	0.07	.alpha.-Bisabolol
33	27.966	27.915	28.025	764541	0.04	14-Methylpentadec-9-enoic acid methyl ester
34	29.582	29.520	29.650	6265563	0.32	Ethyl 14-methyl-hexadecanoate
35	31.733	31.685	31.830	1277981	0.06	7-Hexadecanone
36	32.477	32.380	32.730	11749829	0.59	Lidocaine
37	33.002	32.930	33.135	38921352	1.96	Hexadecanoic acid, methyl ester
38	33.792	33.680	34.025	270645034	13.64	1,4-Benzenedicarboxylic acid, bis(2-methylprc
39	34.258	34.130	34.495	79941620	4.03	n-Hexadecanoic acid
40	34.583	34.455	34.720	52064498	2.62	Hexadecanoic acid, ethyl ester
41	36.713	36.625	36.755	43238576	2.18	9,12-Octadecadienoic acid (Z,Z)-, methyl este
42	36.856	36.755	36.910	122062678	6.15	6-Octadecenoic acid, methyl ester, (Z)-
43	37.144	37.025	37.270	50270318	2.53	9,12,15-Octadecatrienoic acid, methyl ester, (Z
44	37.413	37.345	37.500	25490830	1.28	Methyl stearate
45	38.198	37.935	38.315	609655178	30.72	Ethyl Oleate
46	38.495	38.410	38.565	27661214	1.39	Ethyl 9,12,15-octadecatrienoate
47	38.731	38.640	38.870	24506734	1.23	Octadecanoic acid, ethyl ester
48	40.645	40.525	40.810	10362475	0.52	Glycidyl palmitate
49	43.670	43.530	43.795	32558582	1.64	Glycidyl oleate
50	44.345	44.270	44.460	6340342	0.32	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-am
51	48.210	48.000	48.475	44670881	2.25	Acetyl betulinaldehyde
				1984794375	100.00	

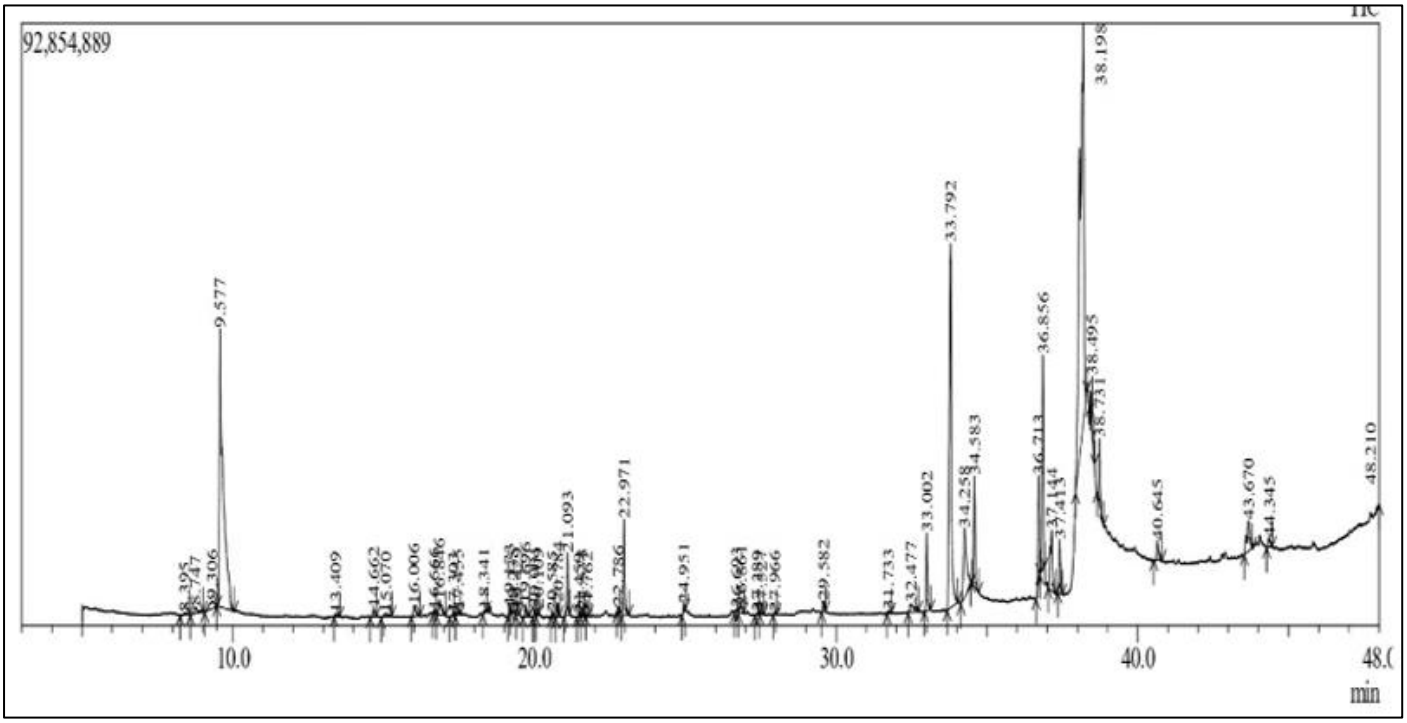

Fig 1 GC-MS Spectra of Fruit of *Citrus medica* L.

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Table 4 Mass Spectral Analysis of Bioactive Volatile Compounds of *Citrus medica* L. Seeds

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	9.255	9.125	9.350	5738714	0.14	2,4-Heptadienal, (E,E)-
2	14.666	14.580	14.880	4557333	0.11	Octanoic acid, ethyl ester
3	16.652	16.530	16.770	6288184	0.15	2-Decenal, (E)-
4	18.294	18.175	18.665	20127287	0.47	2,4-Decadienal, (E,E)-
5	19.403	19.265	19.565	8765676	0.21	2-Dodecenal, (E)-
6	24.940	24.685	25.115	8397991	0.20	1-Hexadecene, 16-bromo-
7	26.691	26.635	26.765	1106969	0.03	cis-7-Dodecen-1-yl acetate
8	29.574	29.425	29.840	2827722	0.07	Ethyl 14-methyl-hexadecanoate
9	32.989	32.745	33.120	63180203	1.49	Hexadecanoic acid, methyl ester
10	33.747	33.580	33.860	106531887	2.51	1,2-Benzenedicarboxylic acid, butyl 2-ethylhe:
11	34.358	34.185	34.460	183922844	4.33	n-Hexadecanoic acid
12	34.570	34.505	34.645	46868750	1.10	Hexadecanoic acid, ethyl ester
13	36.706	36.620	36.795	19381127	0.46	12,15-Octadecadienoic acid, methyl ester
14	36.853	36.760	36.985	237782820	5.60	6-Octadecenoic acid, methyl ester, (Z)-
15	37.135	36.970	37.215	66895819	1.57	9,12,15-Octadecatrienoic acid, methyl ester, (Z
16	37.404	37.300	37.565	42856627	1.01	Heptadecanoic acid, 16-methyl-, methyl ester
17	38.196	37.790	38.910	3386213494	79.68	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-
18	40.636	40.535	40.730	8065083	0.19	Glycidyl palmitate

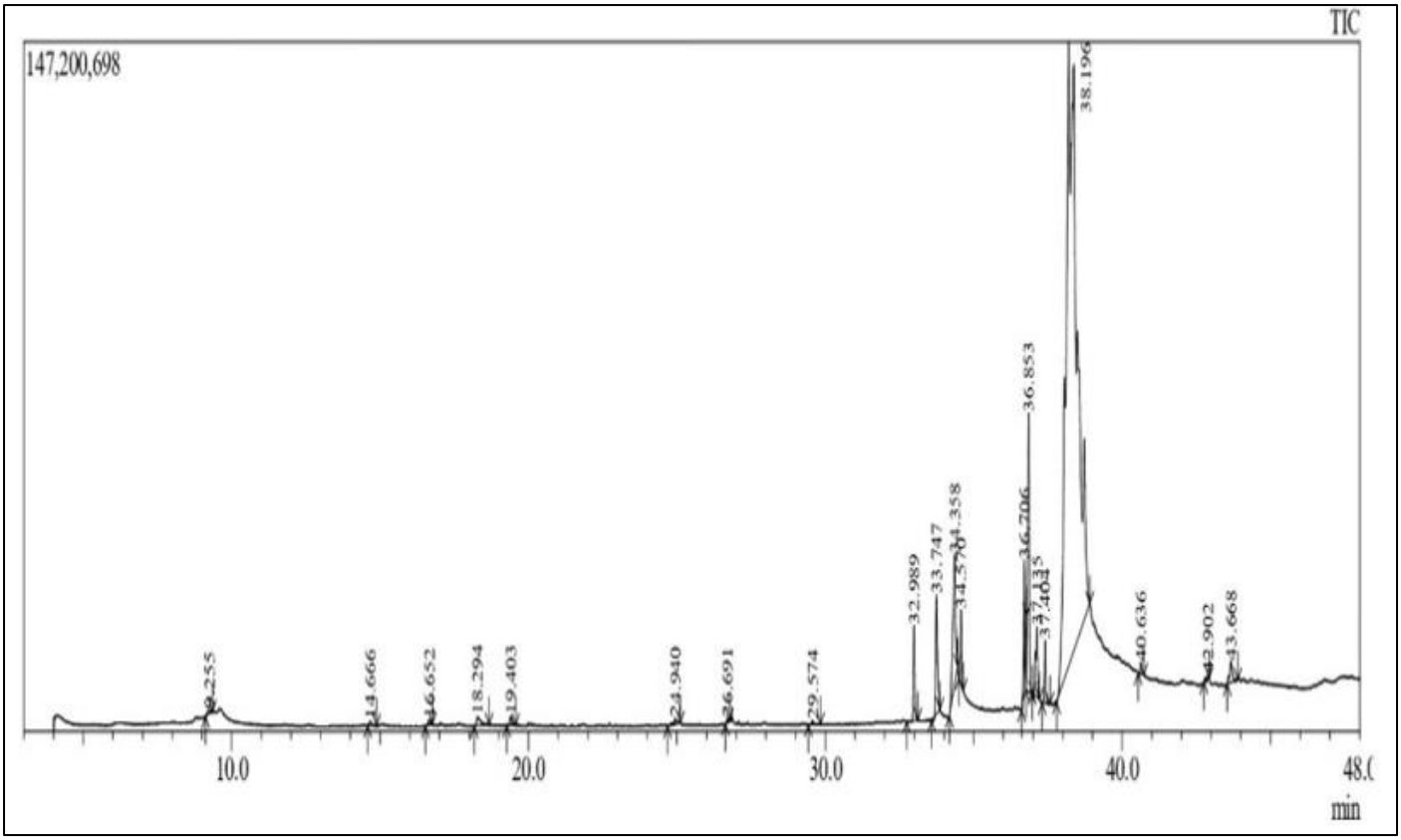

Fig 2 GC-MS Spectra of Seeds of *Citrus medica* L.

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Fig 3 Fruit

Fig 4 Seeds

IV. DISCUSSION

Citrus medica L. is an underutilized plant having various bioactive volatile components in fruit and seeds of the plant. The compounds possess anti-cancer and anti-tumour properties, anti-inflammatory, anti-oxidant, anti-stress, anti-diabetic and possible disease preventing properties, antibacterial, antioxidant, gastroprotective, anxiolytic, anti-inflammatory properties. It has an odour that can be used by perfumers to create floral scents. The compounds found are likely to be responsible for the special odour and taste. This comparision will lead to identification of novel flavour and odour compounds and be a step towards understanding the chemistry behind the *Citrus medica* fruit and seeds.

V. CONCLUSION

Citrus medica L. is a rich source of bioactive compounds capable of preventing and treating various diseases. The species is widely used in Ayurvedic medicine for antioxidant, carminative, antibacterial, anticancer and antiviral purpose among others. Due to the crucial role of *Citrus medica* L. drug industry, this systematic review presents a careful analysis of the studies regarding *Citrus medica* L with a particular focus on its bioactive volatile compounds. The results of this attempt have proved the resource vale of *Citrus medica* as cosmetics and pharmaceuticals. Major phytochemicals in the fruit like β-Myrene can is used as a painkiller, anti-inflammatory, anti-aging and analgesic medicine. D-Limonene can be used for obesity, cancer and bronchitis. β- Bisabolene forms the major component of essential oil. Citronellal, Citral can be used as antimicrobial , anti-inflammatory compound in drug industry. Major phytochemicals in the seeds that are promising in the drug industries are 2-Decenal (E), n-Hexadecanoic acid which can be used as a nematicide. Thus to summarise more clinical trials should be conducted to support the therapeutic use of *Citrus medica* L. In addition, aspiring new researchers must be made aware about the conservation and preservation of such ethnomedicinal properties of *Citrus medica* L. GC-MS analysis has proved the potential of *Citrus medica* L. as a 'Biosreource'.

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