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Liquid Chromatography Mass Spectrometry method in profiling of bioactive compounds in various extracts of Benincasa hispida (Thunb.) Cogn. fresh fruit pulp

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Abstract: **Exceedingly sensitive liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) with positive ion detection method was elaborated and validated to resolute prime compounds present in the active fraction of five various extracts of Benincasa hispida (Thunb.) Cogn. fresh fruit pulp. Five different polarity extracts of Benincasa hispida fresh fruit pulp crude were compared and screened for their identities and concentration of compounds present in the samples with authentic chemical β-sitosterol. Benincasa hispida a potential source for valuable nutrition and traditional medicines.**

Keywords: **LCMS Analysis, β-Sitosterol, Benincasa hispida, Bioactive compound.**

1. INTRODUCTION

Mass spectrometry observation coupled to LC/ESI has become the dominating technique in compound analysis. In the LC-MS of atmospheric pressure ionization API) interfaces such as electrospray ionization(ESI) and Atmospheric pressure chemical ionization are almost exclusively applied. Mass spectrometry particularly LC-ESI-MS provides high sensivity and selectivity even for compound biological matrices. Benincasa hispida fresh fruit extracts. analysed different plant extracts without purification and chromatographic separation, by direct infusion into an ESI-MS apparatus with aim to obtain their finger prints [1]. LC coupled mass spectrometry is more desirable for the non-volatile polar compounds in their existing. Based on comparison of their rention times amd mass spectral data with those of available reference compound and those from the literature, similarly to the fresh fruit pulp.

Benincasa hispida (Thunb.) Cogn. of family Cucurbitaceae, a trailing gourd commonly known as ash gourd, winter melon, wax gourd cultivated in plains and hills. Ash gourd is a cylindrical fruit and completely covered with wax and can be stored for more than one year. BH fruit pulp is mainly applied in ayurvedic medicine for neurodegenerative disorders. BH antioxidant property revealed in the literature of [2]. ant-inflammation [3] analgesic uses. A potential source for valuable nutrients and functional foods [4]. Ash gourd used in the traditional system of medicine for Alzheimers disease [5]. Gastrprotective effect of BH fruit [6] have reported that beta- sitosterol present in Benincasa hispida as chemopreventive agent in Breast cancer. Fatariah [7] study explore the glycemic effect of BH in aqueous extract. Anti-convulsant activity by [8] and antidiabetic activity by [9].

2. MATERIALS AND METHODS

Benincasa hispida was cultivated in the green house of Pachaiyappa's college, Department of Botany. Fruit samples were collected and authencated in the same department

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Solvents and Chemicals

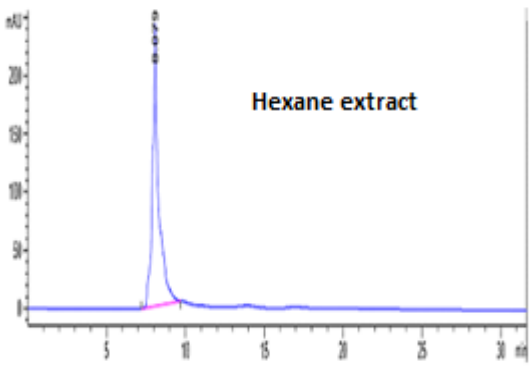
Hexane, Ethyl acetate, Chloroform, Methanol and Aqueous were used for the extraction . The fresh Benincasa hispida fruit was washed with distilled water and dried. The fruit rind was peeled off and seeds inside were removed. The pulp then meshed to fine juice in an air blender. The fresh juice was serially extracted with the solvents based on their polarity. For each extraction 500ml of fresh pulp was mixed with 1500ml of each solvents separately. Extracts were concentrated on a vacuum rotatory evaporator and fractions of samples from each concentrated extracts were used for further studies. β-sitosterol a plant phytosterol, procured from Sigma Aldrich is used as a standard biomarker.

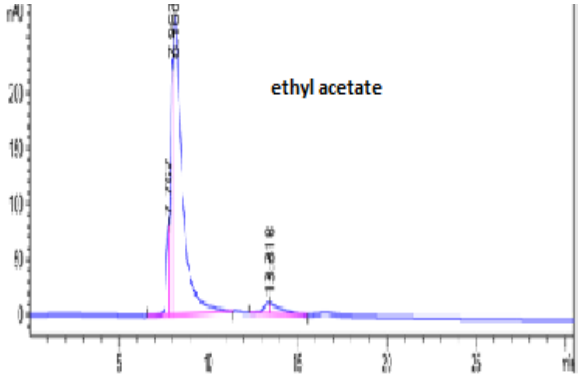
***LC-MS* Analysis**

The Hexane, Ethyl acetate, Chloroform, Methanol and Aqueous concentrated extract fraction of Benincasa hispida fresh fruit pulp were scrutinized by LC-MS technique. This Quantitative analysis was performed by Agilent 1260 infinity series coupled with Shimadzu LC-MS 20-20 quadrupole mass analyzer. With Agilent HPLC condition, column name Zorbax Eclipse C18 250 4.6 mm, 5µm particle sze flow rate 0.3 mL/min, mobile phaseA: HPLC grade water with 0.1% formic acid mobile phase . Phase B: HPLC grade Acetonitrile 95% and water isocratic flow throught the run , total run time 30 minutes. Wave length 196nm, 254nm, 210nm, 202nm, 230nm, 280, 320nm: samples were diluted in acetonitrile and filtered through 0.22µM syringe filter before injection. 2µl of sample was injected through auto sampler. The MS condition was Shimadzu LCMS -2020 in Electrospray ionization positive mode scan range 50-2000m/z. Drying gas flow 1.5L/min, DL temperature 250ºC, heat block temperature 200ºC. Detector voltage 0.8kv, spray flow 0.3/min.

3. RESULTS AND DISCUSSION

Preliminary information on bioactive compound present in different polarity extract of Benincasa hispida fresh fruit crude pulp was obtained by using LC-MS analysis. Maximum absorption of distinctive peak spectrum of beta-sitosterol compound was focused, which is the disticnt characteristic profile of BH fruit [10], [11]. LC-DAD profile of different Polarity extracts of crude is given in fig.1. Base peak chromatograms of fractions of hexane, ethyl acetate, chloroform, methanol and aqueous extracts of Benincasa hispida fresh fruit pulp is compared with the standard compound Beta-Sitosterol. For methanol, chloroform, ethyl acetate and hexane shows unambiguous identification retention time, spectral data and fragmentation pattern of compounds. In case of aqueous extracts, chromatograms shows ambiguous peak conditions when compared with those of available standard compound and those from the literature. [12] [13] In Fig.1 LC:DAD profile of concentrated fractions of various crude extracts from Benincasa hispida fresh fruit pulp chromatograms were detected at different wavelengths. (Absorbance vs. time (min). Fig. 2 illustrates LC-MS/MS data of identified and characterized compound profile in Benincasa hipida fresh fruit pulp. Peak number, retention time, width, area, height and area percentage refers to LC chromatograms of chromatographic conditions given in figure 1. LC-DAD chromatograms were acquired at 196nm, 254nm, 210nm, 202nm, 230nm and 320nm as the most selective wavelengths for the detection of plant sterol. [14] [15]. In fig. 3 products ion spectra of various extracts of Benincasa hipida and Beta-sitosterol is given. Fig. 4 illustrates relative abundance of product ion spectra of BH extracts and standard biomarker. Beta-sitosterol. Significant influence on the relative abundances of ion spectra was similar in almost all the extracts of BH.





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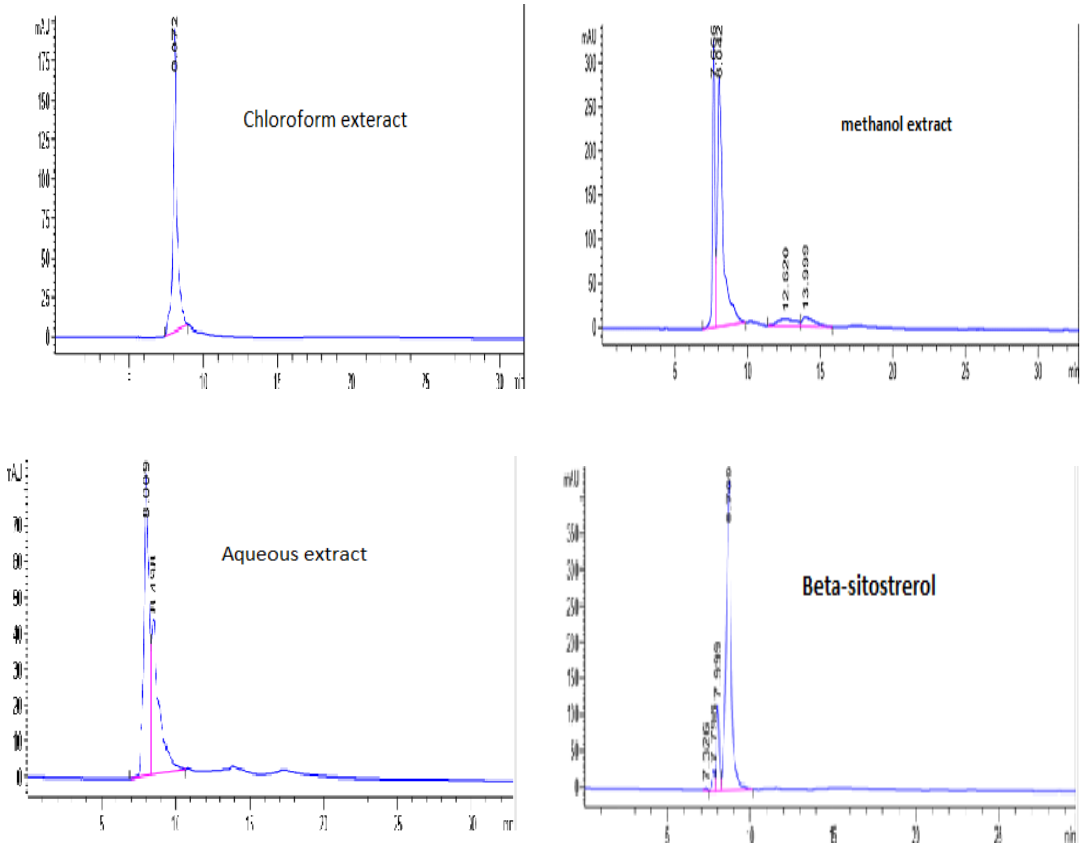

Fig 1: LCMS-DAD Profile of various extracts of Benincasa hispida fresh fruit pulp and standard biomarker.[Absorbance Vs. time (min)] chromatography detected.

Hexane extract

Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.079	0.3382	6445.42920	242.40579	100.0000

Ethyl Acetate extract

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.666	BV	0.1898	2.49351e4	2051.13232	21.4052
2	8.001	VB	0.4764	7.69417e4	1975.25269	66.0496
3	13.378	BV	0.8471	5228.62061	83.43505	4.4884

Chloroform extract

Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.072	0.2553	3560.28418	191.10544	100.0000

Methanol extract

Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.666	0.1679	3699.22217	327.20554	29.8052
2	8.042	0.3468	7457.42920	283.55713	60.0856
3	12.620	1.0657	667.29785	7.27794	5.2959
4	13.999	0.7267	597.39386	9.67030	4.8133

Aqueous extract

Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.009	0.3090	2045.40674	83.73665	56.5415
2	8.498	0.4706	1572.12207	43.39897	43.4585

Beta-Sitisterol

Peak #	RetTime [min]	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.799	0.1801	355.85199	27.29421	9.6972
2	7.999	0.1657	844.52643	79.62990	23.0138
3	8.248	0.1919	242.19226	21.10865	6.5999
4	8.717	0.6180	2227.07764	48.29341	60.6891

Fig 2: peak number, retention time, width, area, height and area percentage refers to LC-DAD chromatograms (chromatography conditions see in fig.1) of various extracts of BH fruit and standard biomarker.

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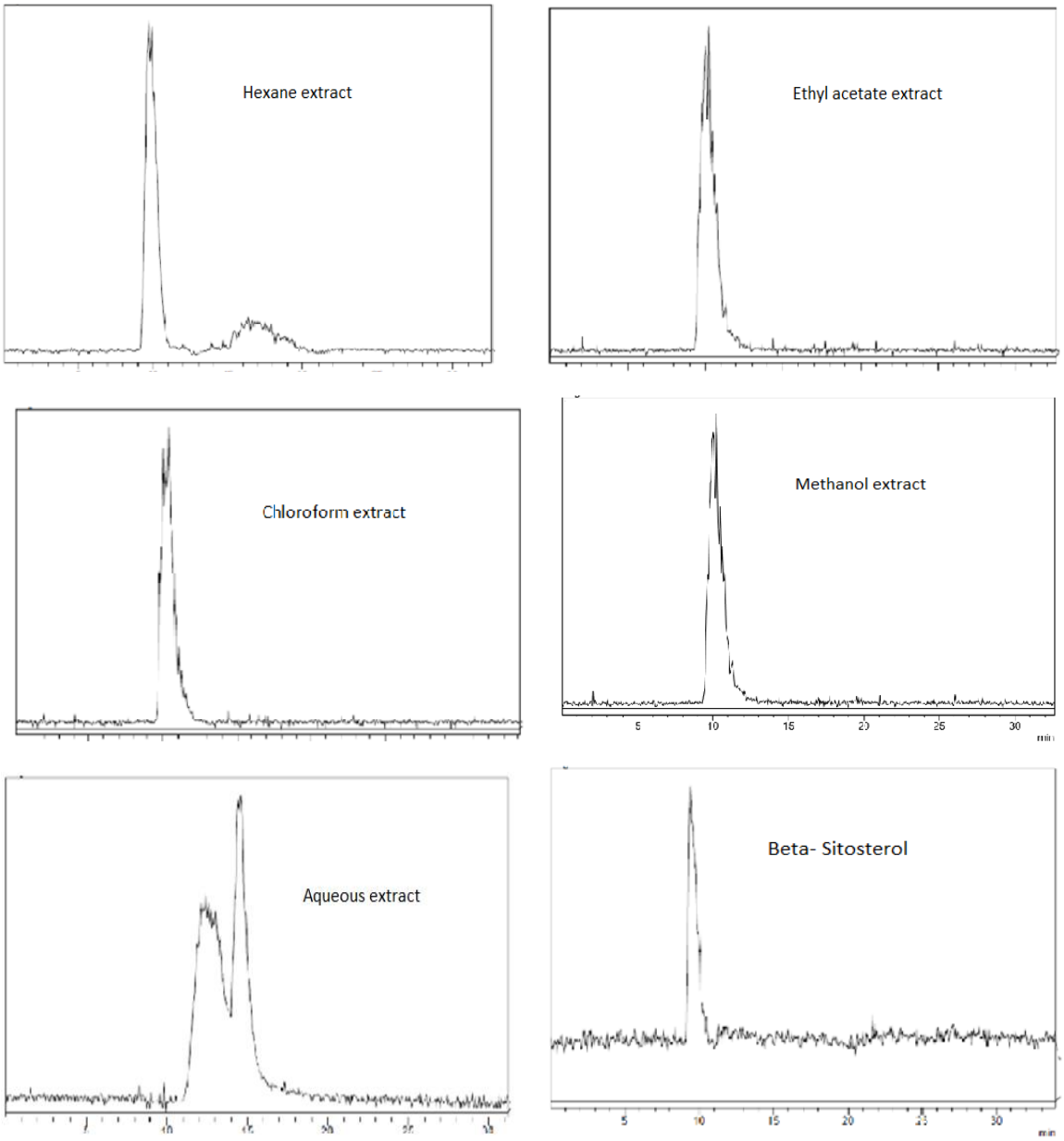
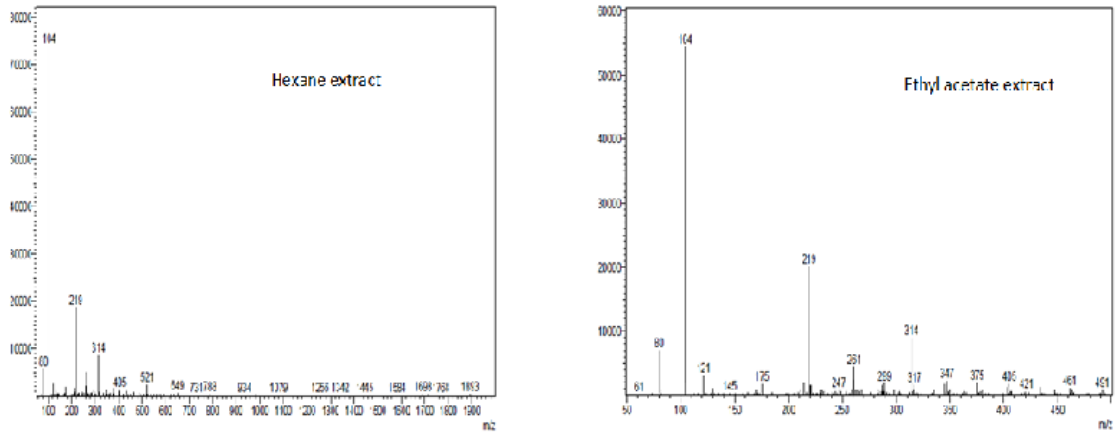


Fig 3: Base peak spectrum of chromatograms of different extracts of BH fruit pulp and standard biomarker.[response units vs.time (min)]



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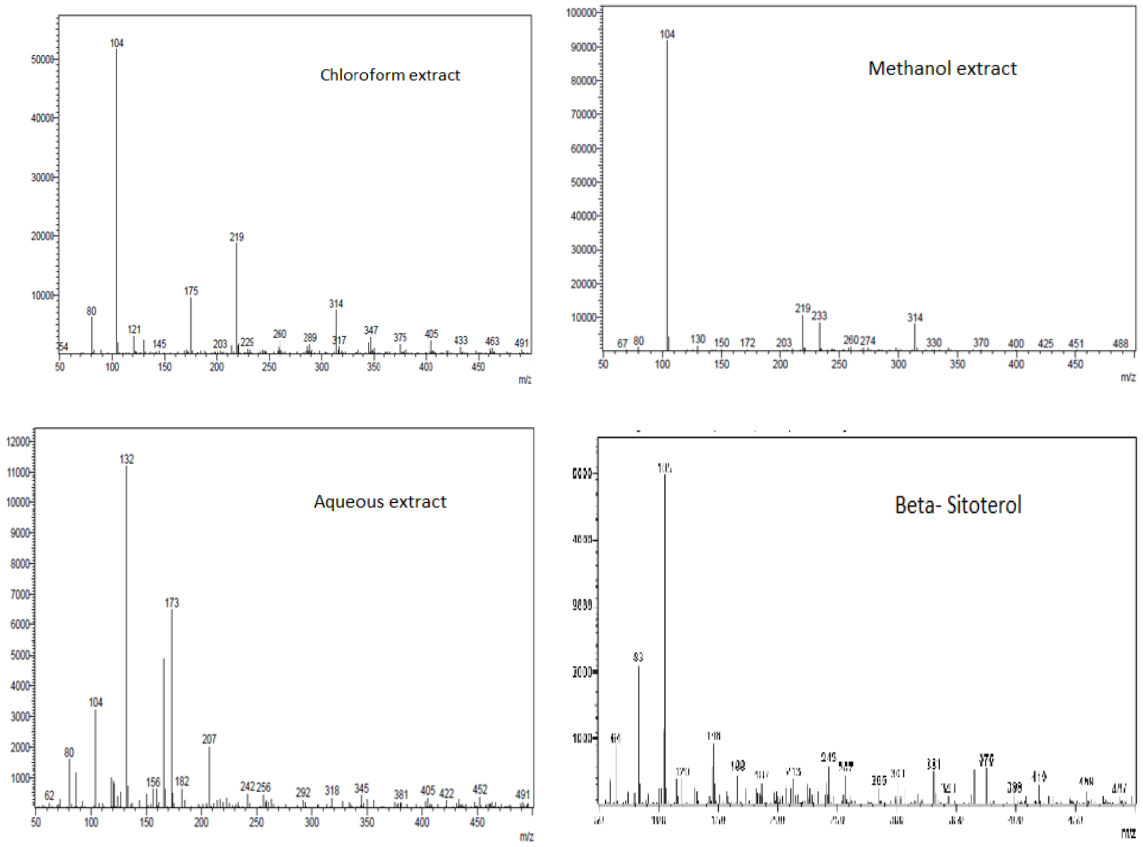

Fig 4: Product ion spectra of [M-H] ions of various extracts of BH fruit pulp and standard biomarker.

4. CONCLUSION

Aim of our study was comprehensive characterization of the bioactive compound profile of crude extract of Benincasa hispida fresh fruit pulp by Electrospray Ionization Tandem Mass Spectrometry coupled to LC-MS with particular attention to β-sitosterol. According to the results of LC-ESI/MS analysis bioactive compound investigation of various extracts from BH fruit pulp, that is used in traditional medicine and standard compound were compared and provided basic impression of distinct components profile of Benincasa hispida.LC-MS/MS and LC – DAD quantitative analysis for its selective bioacitive compound were developed , validated and compared, in order to pertain in quality control of Benincasa hispida.

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