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Metabolite Profiling Foamy Substance of Cucumis sativus L. white local cultivar studied by HR-LCMS

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Abstract
Cucumis sativus L. belong to Cucurbitaceous. The Phytochemical profiling of white foamy substance was performed using HR-LCMS, details of projecting compounds with MS spectra, peak list, and compound structure were studied. The prominent constituent analysis (PCA) of the investigative data displayed the existence of Triterpenes: Gitoxigenin (7.76ppm), Strophanthidin(13.79ppm), Cucurbita in-C (-0.8ppm), Cucurbita in-E(5.53ppm), and Cardiac glycosides: and Digitoxigenin(-2.13ppm) in 10µl of the loaded sample.100 other composites were identified by accurate mass of Q-TOF/MS and confirmed by database compounds (IRM calibration) which comprises, abscisic acid amino acids, fatty acids pyridylactic acid etc. Strophanthidin in the premier attention in the white foamy extract is a noteworthy finding where it has interaction with various proteins in biochemical reactions. This study reports the customary implication of removing white foamy substances before slicing for ingestion. It is used as both vegetables as well as fruit in Indian traditional medicine. It is a source of bioactive compounds.

Keywords: *Cucumis sativus, L. Cucurbita in Cardiac glycosides, Gitoxigenin, High- Mass spectrometry, Resolution Liquid Chromatography,*

1. Introduction
Cucumis sativus L. white cultivar is a member of the Cucurbitaceous family observed as local ‘nati southe’. It is consumed as healthy food. It is a rich source of prophylactic and therapeutic ingredients of day-to-day life [1]. Since ancient times C. sativus is eaten as traditional food and used to treat constipation, headaches for soothing effects and for reducing swelling in skin [2]. Cucumbers are available in different sizes and shapes in various parts of the world [3]. It has also refreshing effects [4]. These properties were well documented in Indian Ayurveda [5]. It contains phytochemical such as, flavonoids, reducing compounds alkaloids, glycosides polyphenols, saponins, tannins highest concentrations of vitamin C and vitamin A [6]. C. sativus is a universally nurtured helpful plant seed studied for antioxidant, antimicrobial activity [7]. It is consumed as vigorous nourishing constituents to inhibit infections and to raise the immune system of living organisms [8]. Mass spectroscopy is the holistic approach to study metabolite profiling of natural product research. The present analysis was done to recognize the bioactive compounds existing in the soapy white foamy substance of Cucumis sativus L. local white cultivar grown in Hassan district Karnataka. This is generally removed by the local people to avoid bitterness before slicing for eating. The acetone extract of white foamy substance was analyzed by an analytical approach. HR LCMS QTOF-MS. (High-Resolution Liquid Chromatography Quadrupole Time of Flight Mass spectrometry).

2. Materials and methods
2.1 Chemicals
Chemicals prerequisite were procured from Himedia and Sigma Aldrich. The HR-LCMS of the sample was done in SAIF, IIT Bombay. Pawai. Mumbai.

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2.2 Plant Collection

The local cultivar of C. sativus white (local white nati southe) was collected from the farm in the village called Kattebelaguli near Holenarasipura taluk, Hassan district, Karnataka, India. Fresh fruits were used for the extraction of white foamy substances. The white foamy substance was obtained by cutting a thin slice and placing it back rubbing on the flat surface on both blossom and stem ends, against cucumber in a circular motion. 100gm of fresh foamy substance was collected dried and dissolved in 100ml acetone. Soxhlet extraction was done, the residue dissolved in 10ml of acetone was stored in a tight container and used for analysis shown in Figure 2 Cucumber climber. Initial Phytochemical screening was done according to Begum *et al.* 2019 [9]. Extract showed the presence of sugar, cardiac glycoside, phenol, and terpenoids.

Figure 1 Cucumis sativus L. white cultivar

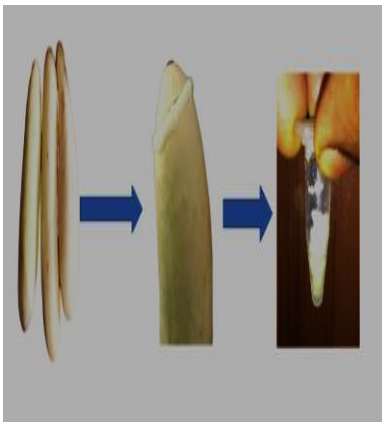

Figure 2 Foamy extract of Cucumis sativus L. white cultivar and its powdery form.

2.3 HRLCMS Analysis

High-resolution liquid chromatography and mass spectrometry (HR-LCMS) analysis of the extract prepared in acetone and then subjected to HR-LCMS analysis. The HR-LCMS of the sample was performed in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Pawai, Mumbai. Metabolite fingerprint of the foamy substance of *C sativus* obtained by Agilent Technologies, USA Mode Hypersil gold 3micron 100 x 2.1 mm column. High-resolution liquid chromatography and mass spectrometry model- G6550A with 0.01% mass resolution was used. The acquisition method was set to be MS- minimum range 60 (m/z) and maximum 1000 Dalton (m/z) with scanning rate each spectrum per second. Gas chromatography has maintained at 250^0C with a gas flow of 13 psi/minute. Hip sampler with model-G4226A was used with auxiliary speed 100 µl/minute, ejection speed 100µl/minute, flush out factor 5µl and 8µl injection volume used for HR-LCMS. Within 30 minutes Acquisition time, initial 2 minutes the flow of solvent composition A: B was 95: 5. The solvent used for HR-LCMS. A. 100% Water B. 100% Acetonitrile. Metabolites were identified by matching retention time as well as mass spectra with those of the corresponding reference standards, and by comparison with an in-house mass spectral library (IRM calibration). A chromatogram was obtained with a composite pattern of major and minor peaks. Bioactive compounds were identified with TOF/Q-TOF mass spectrometer of Dual AJS ESI ion source (Dual Agilent Jet Stream Electrospray Ionization).

3. Results and Discussion

Phytochemical analysis: Preliminary phytochemical analysis indicated the presence of

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Steroids, Flavonoids, Terpenoids Reducing Sugar and Cardiac glycosides.

3.1 HRLCMS study

Phytochemical analysis of foamy extract Figure 1 indicated the presence of triterpenes and cardiac glycosides prominently**.** The specific confirmatory test for lactone ring and deoxysugars of cardiac glycosides was conducted found positive for triterpenes and cardiac glycosides. The previous study on phytochemical analysis methanol and acetone leave extract of C. *sativus* showed the presence of cardiac glycosides, tannins, carbohydrates, terpenoids, saponins resins phytosterols, and exhibited antibacterial and anticancer activity [10]. Plants are the rich source of bioactive compounds. High-Resolution Liquid Chromatography coupled with a Quadrupole ion trap Mass Spectrometer is the rapid analytical technique. Taking sensitivity and resolution into consideration the qualitative and quantitative phytochemical composition of acetone extract of the foamy substance of *C. sativus* was done. It is composed of multiple classes of metabolites. The chromatogram of foamy extract made up of a complex pattern of major and minor peaks shown in Figure 3 The chemical nature of each peak was identified. The metabolite profile highlighted the presence of hundred different organic compounds consisting of amino acids, fatty acids, pyridylactic acid, abscisic acid, etc. The qualitatively identified metabolites were compared with ms/ms spectra with standards C. sativus and fruit pulp extract was qualitatively identified with a skeleton of triterpenes and originally identified in cucurbitaceous plants by [11], [12], [13]. The principal component analysis (PCA) of the analytical data showed the presence of Triterpenes:Cucurbitacin-C(-0.8), Cucurbitacin-E(5.53ppm),and Cardiac glycosides: Gitoxigenin(7.76ppm), Strophanthidin(13.79ppm), and Digitoxigenin(-2.13ppm) in 10µl of the loaded sample. The recorded spectra were compared with the reference standard. All the compounds were detected with mass spectra (m/z)-Mass to charge ratio. The data displays the mass of prominent compounds as a plot of the ion signal as a function of the mass to charge ratio. These spectra are used to determine the elemental signature of the material, the density of the particles and molecules, and to elucidate the chemical identity and composition of the molecules. The MS spectrum peak list includes data on relative abundance, the most typical ion fragment produced, such as the relative strength of the compound. All ions produced are noticed in the detector. The ratio m/z, retention, time-molecular formula chemical formula of all prominent compounds is defined below.

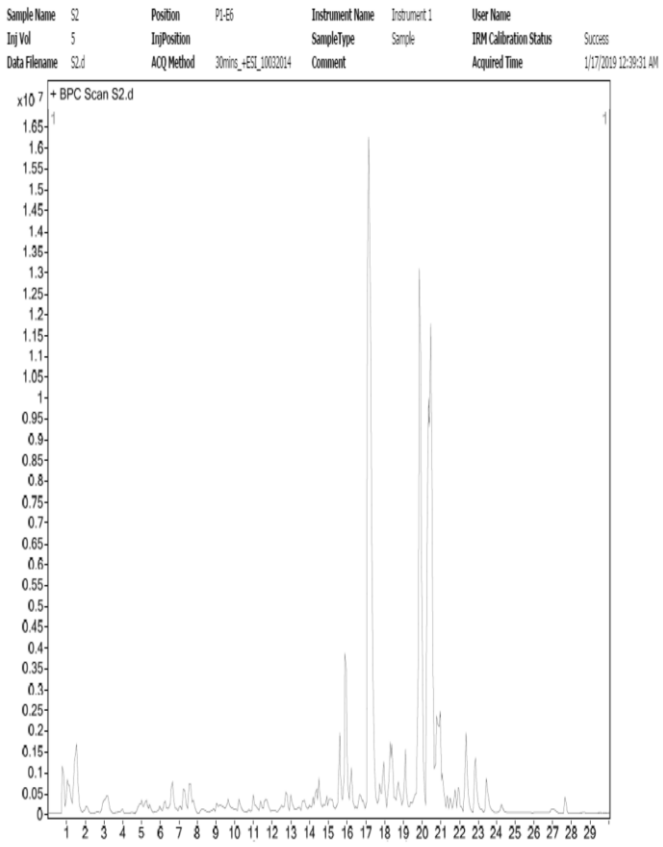

Figure 3 Chromatogram of Foamy extracts of Cucumis sativus

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3.2 Details of Prominent Phytochemicals of foamy Substance Along with MS spectrum peak and compound structure

Table 1 Cpd 1 : Digitoxigenin

Compound label	Name	m/z	RT	Algorithm	Mass
Cpd 1 : Digitoxigenin	Digitoxigenin	397.2357	19.92	Find by formula	374.2465

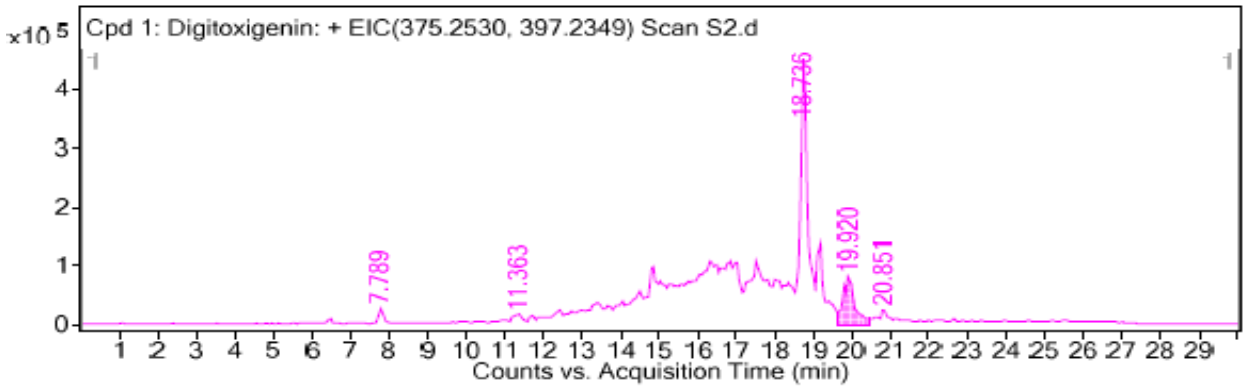

Figure 4 Digitoxigenin

Table 2: Digitoxienin MS spectrum peak list

m/z	Z	Abund	Formula	Ion
375.2477	1	4559.96	$C_{23}H_{34}O_4$	(M+H)+
397.2357	1	5369.53	$C_{23}H_{34}O_4$	(M+H)+

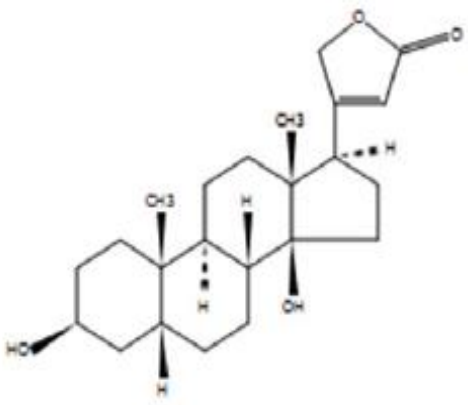

Figure 5 Digitoxigenin Structure

The Table1, Figure 4, Table 2 & Figure 5 shows the mass of Digitoxigenin as a plot of the ion signal as a function of the mass to charge ratio. The ratio m/z 397.2357 retention time 19.92 molecular formula chemical formula of Digitoxigenin is defined above. It was observed in the range of -2.13 pp.

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Table 2 Cpd 2: Gitoxigenin

Compound label	Name	m/z	RT	Algorithm	Mass
Cpd 2: Gitoxigenin	Gitoxigenin	413.2266	9.893	Find by formula	390.2376

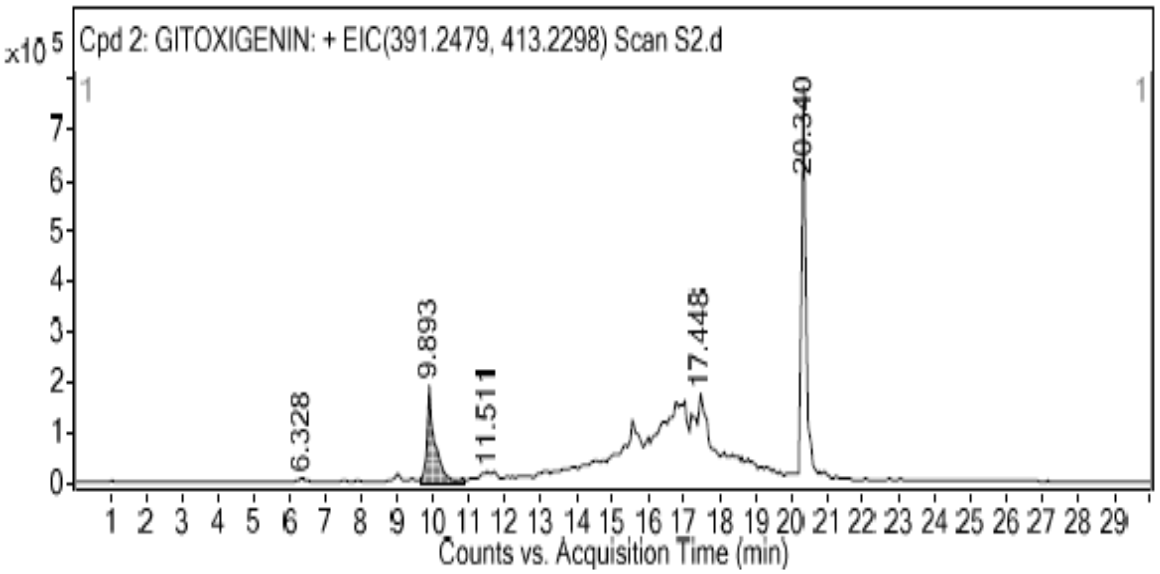

Figure 6 Gitoxigenin

Table 3 Gitoxigenin MS spectrum peak list

m/z	Z	Abound	Formula	Ion
413.2266	1	12981.34	$C_{23}H_{34}O_5$	(M+Na)+
414.2305	1	4854.74	$C_{23}H_{34}O_5$	(M+Na)+

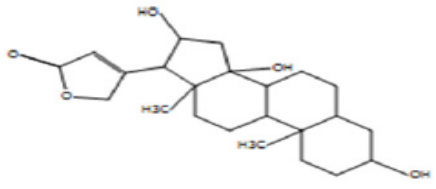

Figure 7 Gitoxigenin structure

The Figure 6, Table 3 & Figure 7 above shows the mass of Gitoxigenin as a plot of the ion signal as a function of the mass to charge ratio. The ratio m/z 413.2266, retention time 9,893 molecular formula chemical formula of Gitoxigenin is defined above. It was observed in the range of 7.76 ppm.

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Table 4 Cpd 3: Strophanthidin

Compound label	Name	m/z	RT	Algorithm	Mass
Cpd 3: Strophanthidin	Strophanthidin	407.2258	14.614	Find by formula	404.2143

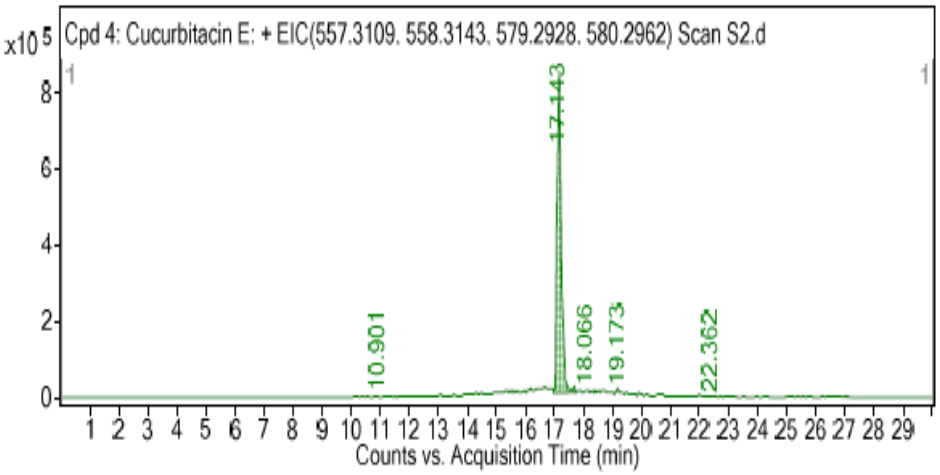

Figure 8 Strophanthidin

Table 5 Strophanthidin MS spectrum peak list

m/z	Z	Abound	Formula	Ion
579.2895	1	100272.21	$C_{23}H_{44}O_8$	(M+Na)+
580.2995	1	37338.36	$C_{23}H_{44}O_8$	(M+Na)+
581.2995	1	9001.24	$C_{23}H_{44}O_8$	(M+Na)+
582.309	1	1754.59	$C_{23}H_{44}O_8$	(M+Na)+

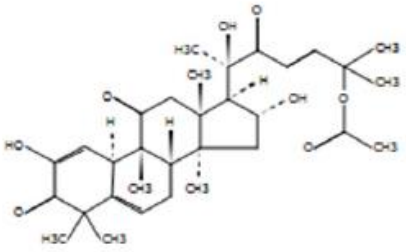

Figure 9 Strophanthidin structure

The Table 4, Figure 8, Table 5 & Figure 9 above shows the mass of Strophanthidin as a plot of the ion signal as a function of the mass to charge ratio. The ratio m/z 407.2258 retention time 14.614 molecular formula chemical formula of is Strophanthidin defined above. It was observed in the range of 13.79ppm in the highest concentration.

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Table 6 Cpd 4: Cucurbitacin E

Compound label	Name	m/z	RT	Algorithm	Mass
Cpd 4: Cucurbitacin E	Cucurbitacin E	579.2895	17.143	Find by formula	556.3005

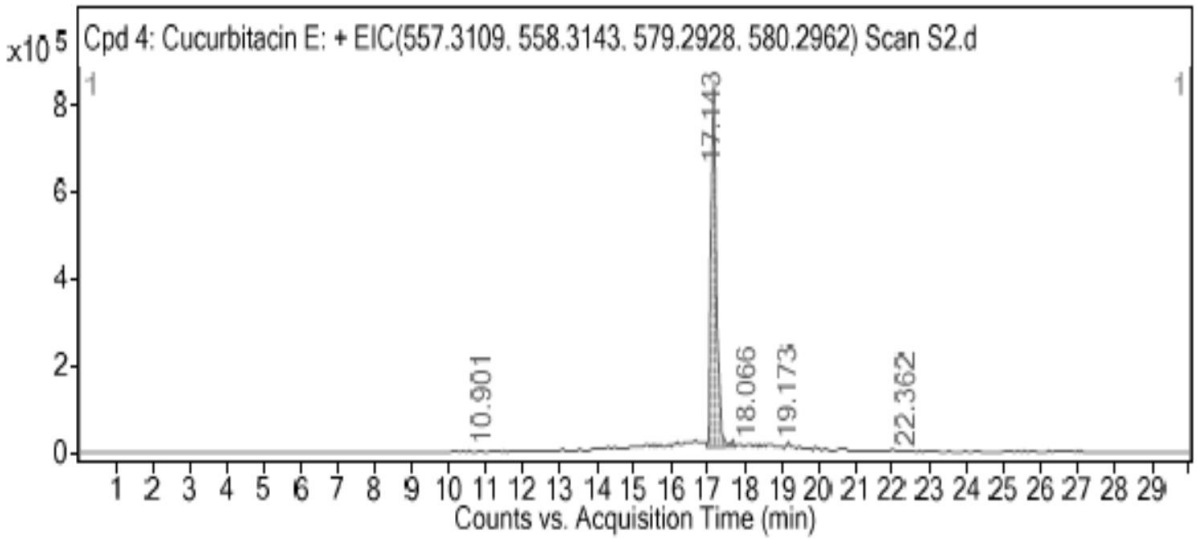

Figure 10 Cucurbitacin E

Table 7 Cucurbitacin E MS spectrum peak list

m/z	Z	Abound	Formula	Ion
579.2895	1	100272.21	$C_{32}H_{44}O_8$	(M+Na)+
580.2925	1	37338.36	$C_{32}H_{44}O_8$	(M+Na)+
581.2995	1	9001.24	$C_{32}H_{44}O_8$	(M+Na)+
582.309	1	1754.59	$C_{32}H_{44}O_8$	(M+Na)+

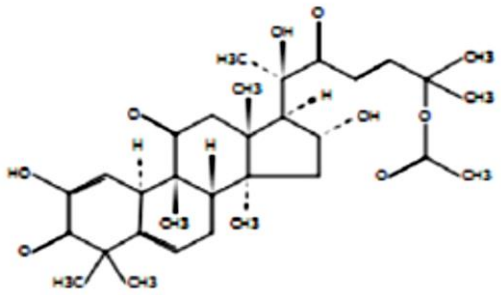

Figure 11 Cucurbitacin E structure

The Table 6, Figure 10, Table 7 & Figure 11 shows the mass of Cucurbitacin-E as a plot of the ion signal as a function of the mass to charge ratio. The ratio m/z 579.2895, retention time 17.143 molecular formula chemical formula of Cucurbitacin-E is defined above. It was observed in the range of 5.53.

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Table 8 Cpd 5: Cucurbitacin C

Compound label	Name	m/z	RT	Algorithm	Mass
Cpd 5: Cucurbitacin C	Cucurbitacin C	561.3426	11.442	Find by formula	560.3354

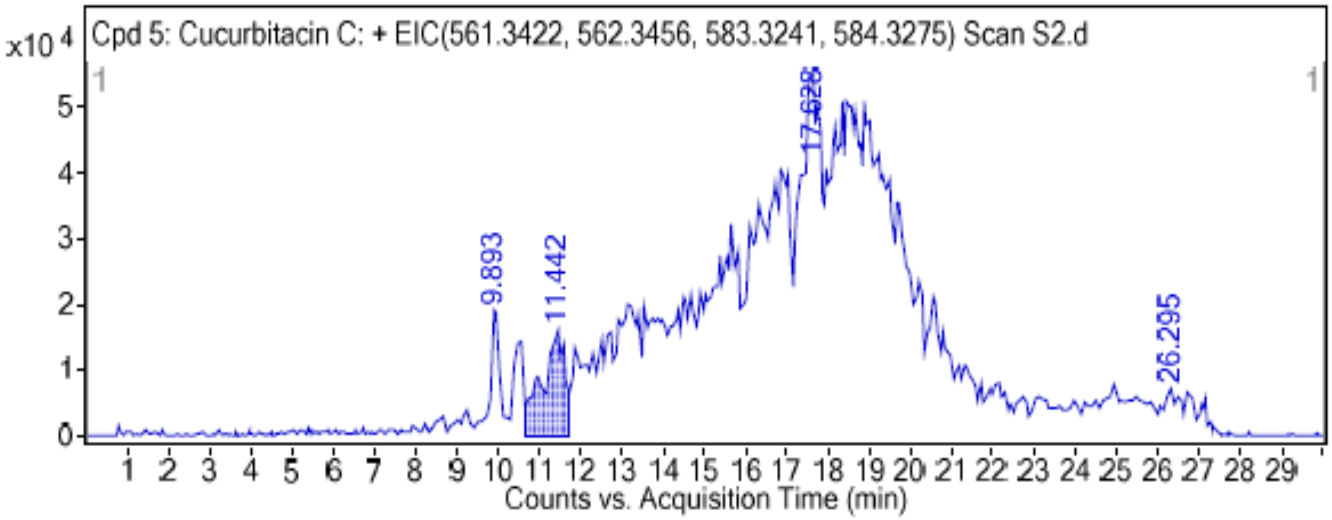

Figure 12 Cucurbitacin C

Table 9 Cucurbitacin C MS spectrum peak list

m/z	Z	Abound	Formula	Ion
561.3426	1	393.41	$C_{23}H_{48}O_8$	(M+H)+

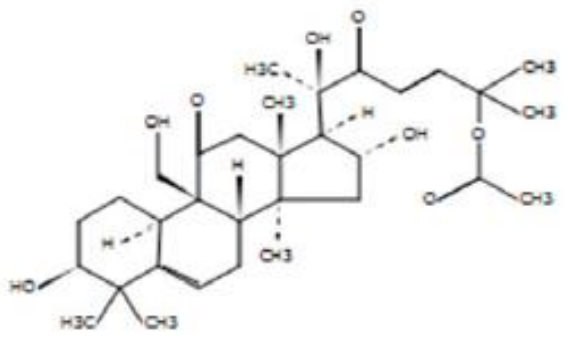

Figure 13 Cucurbitacin C structure

The Table 8, Figure 12, Table 9 & Figure 13 shows the mass of Cucurbitacin-C as a plot of the ion signal as a function of the mass to charge ratio. The ratio m/z 561.3426, retention time 11.442 molecular formula chemical formula of Cucurbitacin-C is defined above. It was observed in the range of -0.8 ppm. Triterpenes were indeed naturally occurring tetracyclic compounds. A study till today says that the main natural sources for cardenolides were extracted from Digitalis lanata and Digitalis purpurea species. Both species are cultivated for this purpose. Strophanthidin is a cardenolide found in species of the genus Strophanthus. Our study reported the Strophanthidin 13.77ppm in white foamy substances of C. sativus local white variety this is considered as potent pharmacologically cytotoxic compounds has immense pharmacological properties. To our knowledge this the first report of phytochemical analysis of a foamy extract of Cucumis sativus L. In existing exercise, Digoxin is the merely Cardiac Glycoside (CG) compound that has been accepted for clinical use in considering cardiac patients and had been permitted for clinical use in handling cardiac patients and had been revealed to constrain cancer cell viability at meditations of 10-100nM. The study exposed that

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low concentration 0.05-0.128µm were operative as anticancer drugs [14]. The precise usage and principal effects of CG in inhibiting Na+/K+ ATPase pumps are not yet fully understood. The application of CG reported more than 1550 years ago in early texts they are used in arrow missile toxins as aborticides, as heart stimulants, as diuretics [15]. All principle phytochemicals are depicted below.

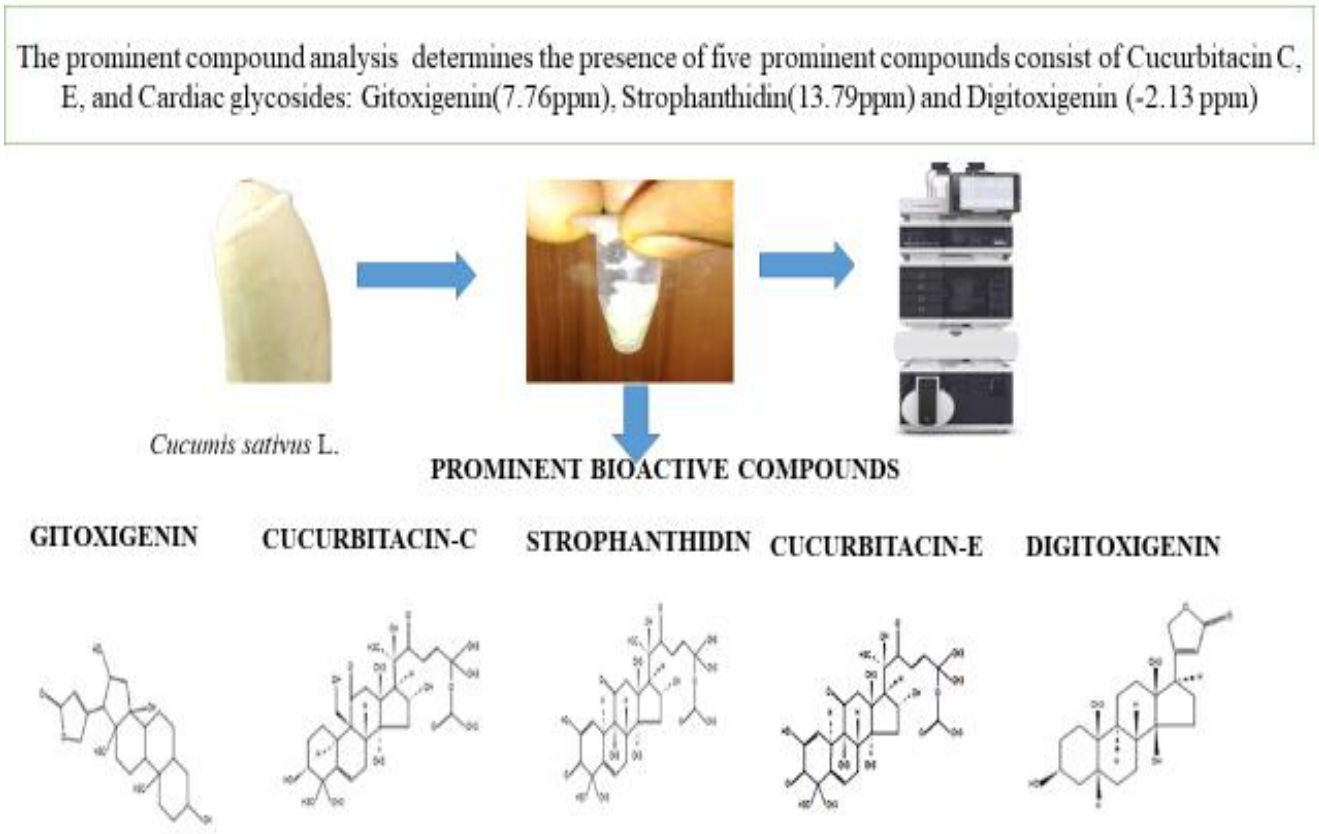

Figure 14 Prominent Compounds of Foamy extract of Cucumis sativus L

Cardenolides are also exhibited cytotoxic activity the possible mechanism depicted in literature are various signal transduction cascades that ultimately prevent cancer cell growth and persuade apoptosis through inhibition of TNF/NF-kβ (nuclear factor kappa-light-chain-enhancer of activated B cells.)pathway it also revealed to upsurge the expression of death receptors (DR4, DR5) upsurges the calcium concentrations DR4 (Trail receptor 1) and DR5 (Trail receptor 2) are transmembrane receptors that have an intracellular death domain DD [16],[17]. Cardiotonic drugs are used to upsurge the competence on the contraction of the heart muscle, which clues to improved blood flow to all tissues of the body and increases the force of the contraction of the myocardium of the heart [18]. Glycosides related to puerarin, gymnemic acid 1rutin, and stevioside have been stated for noteworthy antidiabetic activity. Aglycones like christinin, strictinin, and securigenin have been conveyed for their antidiabetic activity [19]. The study on relations of cardiac glycosides with the nuclear receptor superfamily of transcription factors triggered by fewer molecular ligands such as hormones that control several purposes of cells and organisms. CG of endogenous and exogenous sources by interacting with nuclear receptors can have impacted the processes regulated by these transcription factors, including carcinogenesis, immune system, hormonal management, body

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defense. They can also be treated as preliminary assemblies for combinatorial chemistry to produce novel compounds comprising remedies with the anticipated properties[20].Cucumis flexouses and Cucumis reticulatus was examined for the potential antimicrobial, antioxidant activities of ethanol extract of Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.[21], [22].The complete detail of all 100 compounds label, Name, m/z, RT, algorithm, mass, MS spectrum, MS zoomed spectrum, MS spectrum peak list, MS/MS spectrum, MS/MS spectrum peak list, and compound structure found in local white Cucumis sativus L. cultivar was given supplementary data.

Conclusion

The current study put forward that Cucumis sativus L. local white cultivars of Hassan district Karnataka is a worthy source of remedies to expand human health and educated us on the knowledge behind the elimination of soapy white foamy substances formerly cutting the cucumber for consumption. In this regard, we have to expand our knowledge on phytochemical constituents of different cultivars of Cucumbers grown in different parts of the world. The foamy extract could be the source of inexpensive bioactive compounds applied for cancer and cardiac patients.

Conflict of Interest

Author has no conflict of interest.

Acknowledgement

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