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Study of Metabolites of *Cucumis melo subsp. agrestis var conomon* by HR-LCMS Q-TOF/MS

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ABSTRACT

Cucurbitaceae includes *Cucumis melo subsp. agrestis var conomon* (Kani Vellari). Study of phytochemical profile of white foamy soapy substance was carried out, using HR-LCMS. The compound structure, peak list, and details of projecting components were examined by QTOF MS-Quadrupole Time of Flight Mass Spectrometer investigation. Prominent constituent analysis of the investigative data of 1 µL of the loaded sample included 14.96ppm of tyrosyl glutamate and 14.62ppm of hydrocortisone cypionate. Accurate mass Q-TOF/MS and IRM calibration identified 50 more compounds. It comprises phenyl butyryl glutamine, sorbose, norethynodrel, and pyrethrin. Methotrimeprazine, D-Pipecolic acid, 2-Octyl-4-propylthiazole, Methyprylon, Tranexamic acid, and Isopentenyladenine. In traditional Indian medicine, it is consumed as a vegetable. It has rich source of bioactive substances.

Keywords: *Cucumis melo subsp. agrestis var conomon* Tyrosyl Glutamate, Hydrocortisone cypionate, High-Mass spectrometry, Resolution Liquid Chromatography.

INTRODUCTION

The *Cucumis* genus includes ornamentals and several regularly grown crops, making it one of the most important genera of flowering plants economically. *Cucumis* species are distinguished by their bushy, ascending, trailing growth habits[1].It is widespread in South America and other tropical regions of Asia, yet it is endemic to arid regions of India[2]. *Cucumis melo* cultivars belong to the *Cucurbitaceae* family have bitter taste and maximum length of 10 cm before reaching maturity, *Cucumis melo subsp. agrestisis* conversely, specific cultivar locally called Mangalore southekaayi is sweet in taste and grows to a maximum size of 20 cm to 25 cm[3]. There are several variations in the pulp texture. In Coastal Karnataka, this fruit is known as mage-kaayi or moge-kaayi, a typical vegetable grown on a backyard plot[4]. It is eaten as wholesome food. It is a great source of preventive and healing bioactive components. Since ancient times, *C. melo* has been consumed as a traditional meal and used to soothe headaches for its calming properties, constipation, and skin swelling[5]. Additionally, it has revitalizing properties. *C melo subsp. agrestis var conomon*, also acknowledged as Dosakaya Kani Vellari, is a golden cucumber cultivar that is used to make sambar and pachadi. It has broad green patches

that turn dark brown when they mature and white flesh[6]. Local names for this cultivar include golden yellow and Malabar cucumber. This useful climber seed is widely cultivated and is being researched for its antibacterial and antioxidant properties. It is ingested as a powerful nutrient to prevent infections and strengthen immune systems[7].

The objective of this analysis was to detect the organic bioactive substances existing in the frothy, soapy substances of *Cucumis melo subsp. agrestis var conomon* fruit that is sold in the Karnataka market. The comprehensive analytical method for studying metabolite profile in natural product research is mass spectroscopy. The High-Resolution Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry or HR LCMS QTOF-MS was applied to examine acetone extract of the frothy white substance.

MATERIALS AND METHODS

Compounds prerequisite for study was procured from Himedia. The HR-LCMS of the sample was done in SAIF, IIT Bombay. Pawai. Mumbai.

Plant Collection

The *C. melo subsp. agrestis var conomon* fruit cultivar was gathered from a farm in the village of Kattebelaguli, which is close to Holenarasipura taluk in the Hassan district of Karnataka, India. The white, frothy compounds were extracted using fresh fruits. Cutting the tip into thin piece and rubbing it back against the cucumber in a circular motion the flat surface of the flower and stem ends produced the white frothy substance. Fresh foamy material weighing 100 g was gathered, dried, and dissolved in 10 mL of acetone. Following on Soxhlet extraction, the residue was dissolved in 2 mL of acetone, sealed, and used for analysis. Figure 1

Extraction and Separation

Soxhlet extraction of foamy extract was done. Distillation was performed to isolate the solvent; the concentrate was evaporated to desiccation and the remainder was dissolved in 5 mL of acetone Preliminary phytochemicals study was done according to Deepika *et al.,*[8].

HRLCMS Analysis

The acetone-prepared extract that was subsequently analysed by HR-LCMS. HR-LCMS analysis was carried out at the IIT Bombay, Pawai, Mumbai, Sophisticated Analytical Instrument Facility (SAIF). Agilent Technologies, USA was able to obtain the metabolite fingerprint of the foamy substance that is *C. melo subsp. agrestis var conomon*. ZORBAX Eclipse Plus-C18, 5 micron (Agilent) Mode, 150x2.2mm. Model G6550A, a high-resolution liquid chromatography and mass spectrometry system with a mass resolution of 0.010%, was employed. The MS acquisition method, with a scanning rate of one spectrum per second, was configured with a minimum range of 60 (m/z) and a maximum range of 1000 Dalton (m/z). By comparing the retention time (RT) and mass of the compounds, Agilent Mass Hunter Qualitative Analysis B.06 was able to identify the bioactive chemicals. With a gas flow rate of 13psi/minute, gas chromatography has been sustained at 250^{0}C. Using a hip sampler model G4226A, the auxiliary speed and ejection speed were both set at 100 µL/minute. The injection volume utilized for HR-LCMS was 8 µL, and the flush out factor was 5 µL. In the first minute of the 30 min acquisition period, the solvent composition flow A: Milli-Q water containing 0.1% formic acid B: Acetonitrile and TOF/6500 series Table 1 and Table 2. By comparing retention times and mass spectra with the respective reference standards, metabolites were found. A composite pattern of major and minor peaks were seen in the chromatogram that was produced. The analysis was completed at the advanced analytical instrument facility located in Mumbai, India, at the Indian Institute of Technology.

Fig. 1. *Cucumis melo subsp. agrestis var conomon* cultivar and collection foamy extract. (Image source:-Captured by the Author in the field, by Samsung Galaxy A70s 64mp camera)

Table 1: Solvent Arrangement of HRLCMS

	Channel	Ch 1 Solv	Name 1	Ch2 Sol	Name 2	Selected	Used	Percent
1	A	100.0% Water V.02	0.1% FA in water	100.0% V.02	0.1% FA in Water	Ch 2	yes	95.00%
2	B	100.0% Methanol V.03		100,0% Acetonitrile V. 02		Ch 2	yes	5.00%

Table 2: Time Table

	Time	A	B	Flow	Pressure
1	1.00 min	95.00%	5.00%	0.300 mL/min	1200.00 bar
2	25.00 min	0.00%	100.00%	0.300 mL/min	1200.00 bar
3	30.00 min	0.00%	100.0%	0.300 mL/min	1200.00 bar
4	31.00 min	95.00%	5.00%	0.300 mL/min	1200.00 bar
5	35.00 min	95.00%	5.00%	0.300 mL/min	1200.00 bar

RESULTS

Preliminary phytochemical analysis

The examination of phytochemicals Salkowski, Molisch, and Libermann-Buchard test findings were positive, indicating the presence of sugars and steroids in the *Cucumis melo subsp. agrestis var conomon* cultivar. It was determined that steroids were present after a specific confirmatory test for lactone rings and deoxysugars was carried out. Previous studies have identified the presence of tannins, polysaccharides, terpenoids, saponins, resins, phytosterols, and cardiac glycosides in the phytochemical analysis of *C. melo* leaves extract in methanol and acetone[9]. The presence of glycosides, tannins, alkaloids, flavonoids, phlobatanin, and anthraquinone saponin were observed in the epicarp, mesocarp, and seeds of mature fruits from *Cucumis melo* (L.), *Lagenaria reviflora* (Benth), and *Citrullus lanatus* (Thunb). The stems of *Cucumis melo* included cucurbitane-type triterpenoids, and spectroscopic investigation revealed the presence of cucurbitacin B and cucurbitacin. Gallic acid content was demonstrated by chloroform extract from the dried fruit *Cucumis melo* L., sometimes known as honeydew melon[10].

HRLCMS study

To analyse the bioactive compounds found in the frothy, soapy substances HRLCMS tied with a Quadrupole ion trap Mass Spectrometer was done. It is the quick investigative technique captivating sensitivity and resolution into consideration the qualitative and quantitative phytochemical composition of acetone extract of the foamy substance of *C melo subsp. agrestis var conomon* was done. It exhibited 10 peaks,it is composed of manifold classes of metabolites. The first emerged compound wasTyrosyl-glutamate (14.59ppm) of mass 310.112.The second emerged compound was Tyrosyl-glutamate (14.96ppm) of mass 310.1119, fourth was Phaeophorbide (2.5ppm) of mass 606.2493, sixth compound was Hydrocortisone cypionate, (13.9ppm) of mass 486.2914., eighth compound emerged was Hydrocortisone cypionate(14.62ppm) of mass 485.2839, tenth was UDP-N-acetyl-2-amino-2-deoxy-D-glucuronate(2.62ppm) of 666. 0614. The third, fifth, seventh and ninth emerged peaks were not so conspicuous, chromatogram of foamy extract made up of 9 major and 8 minor peaks Fig. 2 and Fig. 3 totally 10 prominent bioactive compounds were identified along with MS spectrum peak, compound structure, ratio m/z, retaining, time, ppm, molecular formula and organic formula of altogether projecting compounds were identified showed in Table 3. This is the first report on analysis of foamy extract.

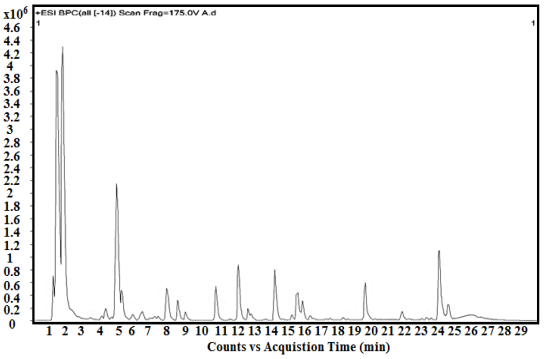


Fig. 2. High Resolution Liquid Chromatogram study of Foamy extracts of *Cucumis melo subsp. agrestis var. conomon.* (By SAIF Bombay)

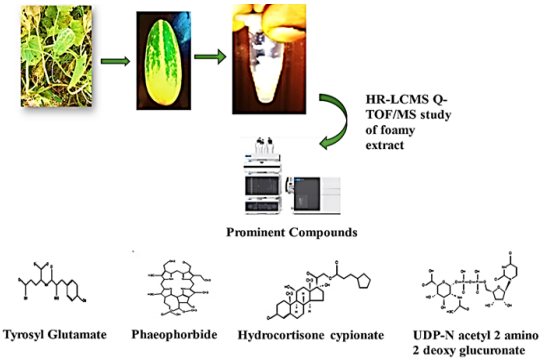
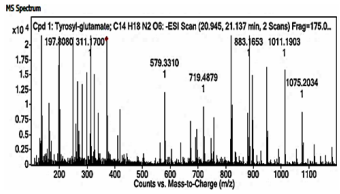
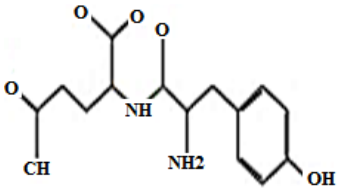
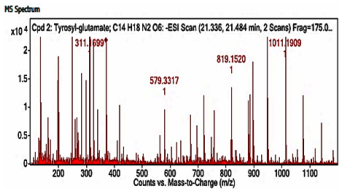
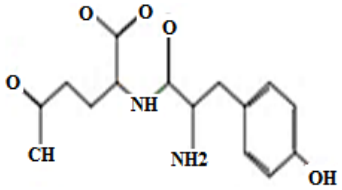
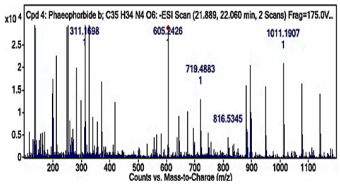
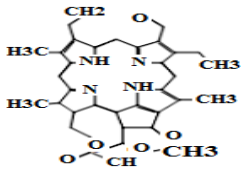
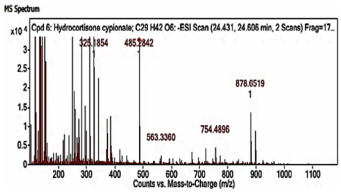
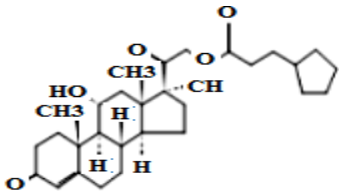
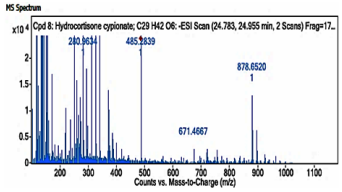
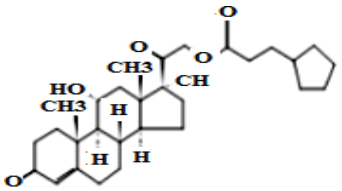
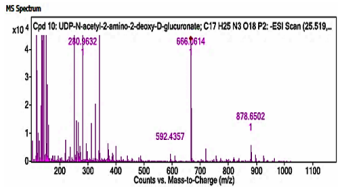
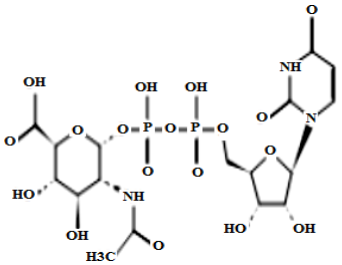


Fig. 3. Prominent Bioactive Compounds identified in Foamy Extract of *Cucumis melo subsp. agrestis var. conomon* (Image source:- Captured by the Author in the field, by Samsung Galaxy A70s 64mp back camera compound and formula by SAIF Bombay)

Table 3: Details of Prominent Phytochemicals of foamy Substance with Mass, MS spectrum peak, M/Z, PPM and compound structure of *Cucumis melo subsp. agrestis var. conomon*

SL	Name of the Compound and Formula	Mass	MS spectra	M/Z	PPM	Structure
1	Tyrosyl Glutamate $C_{14}H_{18}N_2O_6$	310.112		369.128	14.59	
2	Tyrosyl Glutamate $C_{14}H_{18}N_2O_6$	310.1119		369.1282	14.96	
3	Phaeophorbide $C_{35}H_{34}N_4O_6$	606.2493		605.2426	-2.45	
4	Hydrocortisone cypionate $C_{29}H_{42}O_6$	486.2914		485.2842	13.9	
5	Hydrocortisone cypionate $C_{29}H_{42}O_6$	485.2839		485.2839	14.62	
6	UDP-N acetyl 2 amino 2 deoxy glucuronate $C_{17}H_{25}N_3O_{18}P_2$	666.0614		621.0625	-2.62	

A validated RP-HPLC method was used to study and quantify cucurbitacin E in five distinct kinds of melon fruit. A 70:30 (v/v) ratio of acetonitrile to water (1% glacial acetic acid) was used. The results showed that the cucurbitacin E concentration varied amongst five different melon fruit cultivars by 0.0129%w/w to 0.231%w/w The fruit extract with the highest concentration was discovered to be *C melo var. flexuosus* (L.) Naudin fruit[11]. We discovered in our investigation that the fruit froth extract had no cucurbitacins for *C. melo subsp. agrestis var. conomon*. Using flow-injection analysis and detection based on atmospheric pressure chemical ionization-Fourier transform

mass spectrometry, the first proof of the presence of Cucurbitacins D, B, R, and C and their isomeric forms were found in a bitter-tasting fruit of the "Scopatizzo" melon, a landrace of *C. melo* typical of the Apulia region in Southern Italy[12]. The fruit extract from *C. melo* was shown to have a high level of Superoxide Dismutase Activity, which was thought to be caused by the fruit's cucurbitacin, phenolic compounds, vitamins, minerals, fatty acids, and essential oils. *C. melo* was widely recognized for its anti-inflammatory properties. The pharmacological characteristics of plants of the *Cucumis genus* include anti-inflammatory, anticancer, antidiabetic[13], antiwrinkle, antibacterial, antioxidant, and analgesic effects[14].

Our study showed that foamy extract is rich source of both Tyrosyl Glutamate, a dipeptide of mass 310.112 (14.59ppm), Tyrosyl Glutamate mass 310.1119(14.96ppm), Hydrocortisone cypionate a glucocorticoid of mass 486.2914(13.9ppm) and Hydrocortisone cypionate of mass 485.2839 (14.62ppm) Figure 3.

The mechanism of action of hydrocortisone cypionate for the treatment of numerous illnesses was investigated in this context due to its importance in pharmacology. Hydrocortisone is one of the most important glucocorticoids in humans. The homeostatic mechanisms of the body must be regulated. Hydrocortisone has anti-inflammatory properties and can be used to treat inflammation caused by corticosteroid-responsive dermatoses[15]. It is distinguished by its ability to attach to the cortisol receptor and start a variety of important effects on the immune system, metabolism, cardiovascular system, and homeostasis. They differ from sex steroids and mineralocorticoids in their receptors, target cells, and modes of action. It suppresses cell-mediated immunity. They function by inhibiting the genes that generate TNF-alpha Tumor Necrosis Factor, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, and IL-8, with IL-2 IL-Interleukin being the most important. Reduced cytokine synthesis limits T cell proliferation[16]. It affects humoral immunity, which results in decreased IL-2 and IL-2 receptor expression in B cells. This reduces the production of antibodies as well as the growth of B cell clones. Reduced levels of IL-2 also result in a decrease in the activation of T lymphocyte cells[17,21].

DISCUSSION

Information on the action mechanism of hydro cortisone is available. The cytosolic glucocorticoid receptor is the site of hydrocortisone binding. Following its attachment to the receptor, the newly created receptor-ligand complex moved into the nucleus of the cell, where it bound to many glucocorticoid response elements (GRE) in the target genes' promoter regions[18,19]. Following its interaction with fundamental transcription factors, the DNA-bound receptor causes the expression of particular target genes to rise. The anti-inflammatory properties of corticosteroids from lipocortins, phospholipase A2 inhibitory proteins that regulate the formation of prostaglandins and leukotrienes by inhibiting arachidonic acid[10]. In particular, lipocortin-1 (annexin-1) is synthesised in response to glucocorticoids, and it attaches to cell membranes to block phospholipase A2 from getting into contact with its substrate, arachidonic acid[16].

The two main products in inflammation Prostaglandins and Leukotrienes are inhibited by the action of Glucocorticoids[20]. Our study revealed that foamy extract of *C, melo subsp. agrestis var. conomon* could be the source of inexpensive bioactive compounds for the treatment of inflammation. Apart from prominent compounds other 50 compounds are also listed.

CONCLUSION

The presence of large number of phytoconstituents have been revealed *C. melo var. agrestis* commonly known sambar southe it has been used for its several properties in conventional usage . However, not much work is being performed in foamy extract which has been carried out to show its valuable effects. Hence, this study will provide base for further research in this field. The soapy foamy extract of might be the resource of inexpensive bioactive compounds for the treatment of inflammation in medical field.

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Conflict of Interest

Author has no conflict of interest.

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