

PHYTOCHEMICAL ANALYSIS IN FOAMY EXTRACT OF CUCUMBER (*CUCUMIS SATIVUS* L.)

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ABSTRACT

The metabolite profiling of white foamy substance local white cultivar of Cucumber (*Cucumis sativus* L.) was performed using HR-LCMS. Prominent compounds with MS spectra, peak list, and compound structure were studied. Which pertains to *Cucurbitaceae*. The cytotoxic activity was studied on onion root tip squash and microscopic evaluation was done. The principal component analysis (PCA) of the analytical data showed the presence of Triterpenes: Cucurbitacin-C (0.8ppm), Cucurbitacin-E (5.53ppm) and Cardiac glycosides: Gitoxigenin (7.76ppm), Strophanthidin (13.79ppm), and Digitoxigenin (2.13ppm) in 10 μ l of the loaded sample. 100 other compounds were identified by accurate mass of Q-TOF/MS and verified by database compounds (IRM calibration). Strophanthidin in the highest concentration in the foamy extract is a significant finding. This study reports the traditional significance of removing white foamy substances for bitterness in cucumber before slicing. The cytotoxic study on onion root tips by photomicrograph study indicates reduction in root growth and chromosomal aberrations, disturbed telophase, anaphase, abnormal cell shape, dissolved chromosomes, membrane damage and nuclear lesions.

Keywords: Cucumber, Cucurbitacin, Cardiac glycosides, Gitoxigenin, Strophanthidin, High-resolution liquid chromatography, mass spectrometry.

INTRODUCTION

Cucumis sativus L. is a member of the *Cucurbitaceae* family. It is consumed as healthy food. It is a rich source of prophylactic and therapeutic ingredients of day-to-day life as reported by Suma et al. (2019). It is studied that since ancient times *C. sativus* is eaten as traditional food and used to treat constipation (Pan et al., 2022), headaches for soothing effects and for reducing swelling in skin (Nwosisi et al., 2022). Cucumbers are available in different sizes and shapes in various parts of the world. It has also

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refreshed effects (Kumari et al., 2018). These properties were well documented in Indian Ayurveda (Olaniyan et al., 2019; Ebanu et al., 2019). It is reported that it contains phytochemicals such as saponins, tannins, flavonoids, reducing compounds, alkaloids, glycosides polyphenols, high concentration of vitamin C and vitamin A. *C. sativus* is a globally cultivated beneficial plant seed studied for antioxidant, antimicrobial activity by Huang et al. (2019). It is consumed as active nutritional ingredients to prevent microbial infections and to increase the immune system of living organisms (Krishna et al., 2019). Mass-spectroscopy is the holistic approach to study metabolite profiling of natural product research. The present analysis was done to identify the bioactive compounds present in the white foamy substance of *Cucumis sativus*, White local cultivar grown in Hassan district Karnataka; the substance was removed for bitterness before 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).

MATERIALS AND METHODS

Chemicals

Chemicals required were purchased from *Himedia* and Sigma Aldrich. The HR-LCMS of the sample was carried out in SAIF, IIT Bombay. Pawai, Mumbai.

Plant Collection

The local cultivar of *C. sativus* white (local *bili 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.

Phytochemical Analysis

Preliminary Phytochemical screening was done according to Begum et al. (2019).

Preparation of plant extract

In the present investigation the aqueous extract of white foamy extract was studied. The white foamy substance was obtained by cutting thin slices and placing it back rubbing on the flat surface on both blossom and stem ends, against cucumber in circular motion. 200g of fresh foamy substance collected shade dried made into powder. Soxhlet extraction was done in 50% acetone. residue dissolved in 10ml of acetone was stored in a tight container and used for HR LCMS mass spectrometry analysis.

Study of Cytotoxic activity

The residue of foamy substances was dissolved in 100ml of distilled water. The different stock solutions were prepared (1mg/ml, 2mg/ml and 3mg/ml). The aqueous white foamy extract was evaluated by exposing onion bulbs to concentrations of 1 to 5mg the extract for 48-96h. The macroscopic and microscopic analysis of onion root tips was done after 48-96h.

Allium Cepa test: The medium sized onion bulbs were purchased from local market

near to Government Science College, Hassan. Outer dry scales dry bottom plates were removed, they were stored in dry and well aerated condition before use to encourage bulb viability, moldy and sported bulbs were discarded. Exposure of onion bulbs to 1 to 5mg/ml concentrations of the extract for 48-96h.

Macroscopic evaluation

The onions were exposed to 48h to test samples of concentration of 1mg/ml, 2mg/ml and 3mg/ml. Onion Bulbs were first kept in tap water in dark for 48h then bulbs are shifted test tube containing different concentration of sample. The test samples were changed every 24h with fresh test samples. The growth inhibition was observed, each sample was kept in 5 replicates. The best growth bulbs of root length (2.5-3cm) were selected for study from each concentration. Twenty best root lengths were measured as metric rule and used as index of general toxicity. Visible morphological changes such as change in color, hooks, and twists in roots were observed.

Preparation of onion root tip squash for microscopic evaluation

About 2-3cm the onion roots tips were fixed in Conroy's fluid (3ml alcohol and 1ml acetic acid) for 15 minutes. Transferred into 70% alcohol for storage. The squash was prepared after washing the root tips in water for 2 minutes. Tips were hydrolyzed in 1N HCl (2-3 drops) for 2min on watch glass and 9 drops of acetoorcein stain was added, warmed gently for 5 minutes and allowed it to cool for 10-15 minutes. The root tips were kept on slide, 45% acetic acid was added, covered with cover slip and finally squash was done gently by keeping the slide in between the blotting paper to spread the cells uniformly with help of thumb. Excess stain was removed by blotting paper and observed under the microscope.

A total of about 1500 dividing cells from five scorable slides per sample i.e., 100 dividing cell per slide made from onion root tips excised from each concentration of extract in which they are placed. (1mg/ml, 2mg/ml and 3mg/ml). Onion bulbs kept in tap water pH 7.3 was used as positive control and Ethyl Methane Sulfonate EMS (2.10^{-2} M) as negative control. The root tips were cut from each bulb examined for detectable morphological abnormalities. The bulb of satisfactory root length (2.5-3cm) was used in the study. The cells were observed at 100x magnification for different mitotic stages and chromosomal aberrations. Photomicrographs were taken using 100x light microscope with Nikon photographic camera. The mitotic index and frequency of chromosomal aberrant cells per total cells each sample at each concentration of was calculated by using the formula.

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\% \text{Aberrant cells} = \frac{\text{Number of Aberrant cells}}{\text{Total number of cells}} \times 100$$

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 carried out in Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. Metabolite fingerprint of the foamy substance of *C sativus* obtained by Agilent Technologies, USA Mode Hypersil gold 3micron 100 x 2.1mm 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 1000Dalton (m/z) with scanning rate each spectrum per second. Gas chromatography has maintained at 250°C with a gas flow of 13psi/minute. Hip sampler with model- G4226A was used with auxiliary speed 100µl/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 complex 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).

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical analysis indicated the presence of alkaloids, reducing sugar, steroids, terpenoides, cardiac glycosides, tannins, phenols and flavonoids (Table 1).

Table 1. Phytochemical analysis

| S. No | Phytochemical compounds | <i>Cucumis sativus</i> L white peel |
|-------|-------------------------|-------------------------------------|
| 1 | Alkaloids | + |
| 2 | Reducing Sugar | +++ |
| 3 | Steroids | ++ |
| 4 | Terpenoids | ++ |
| 5 | Cardiac glycosides | ++ |
| 6 | Tannins | + |
| 7 | Phenols | ++ |
| 8 | Saponins | + |
| 9 | Amino acids | - |
| 11 | Proteins | + |
| 12 | Starch | - |

| S. No | Phytochemical compounds | <i>Cucumis sativus</i> L white peel |
|-------|-------------------------|-------------------------------------|
| 13 | Flavonoids | ++ |

+marginally present. ++ Discreetly present. +++extremely present – absent

HRLCMS study

Phytochemical analysis of foamy extract indicated the presence of triterpenes and cardiac glycosides. 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 leaves extract of *C. sativus* showed the presence of cardiac glycosides, tannins, carbohydrates, terpenoids, saponins resins phytosterols, and exhibited antibacterial and anticancer activity by Feng et al. (2018). Plants are the gem house 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 is made up of a complex pattern of major and minor peaks (Fig. 1).

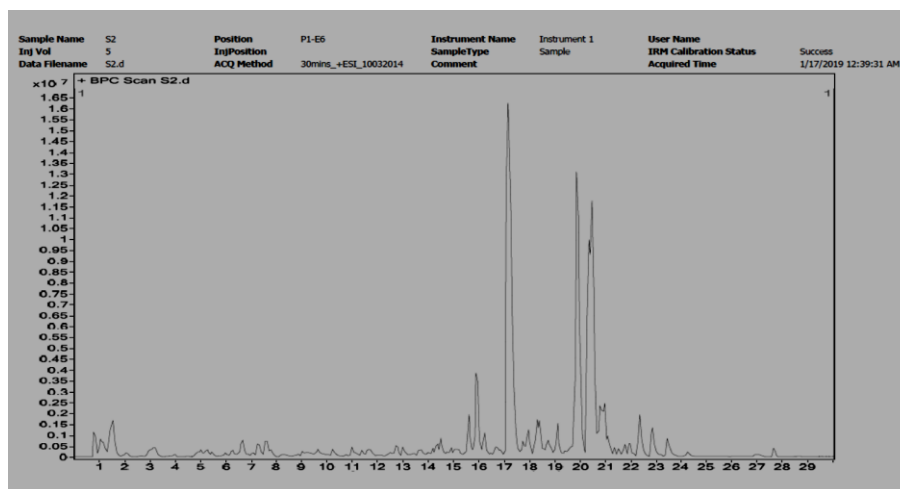


Figure 1. Chromatogram of Foamy extract of *Cucumis sativus* L.

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

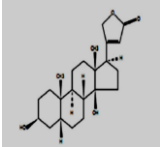
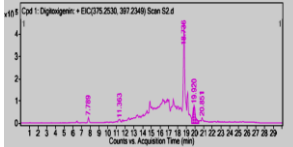
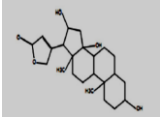
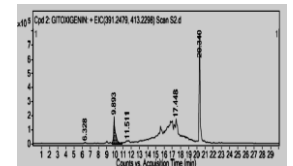
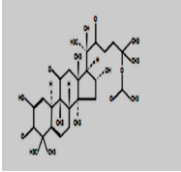
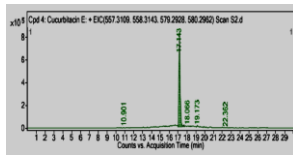
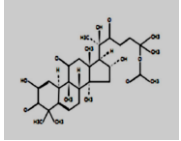
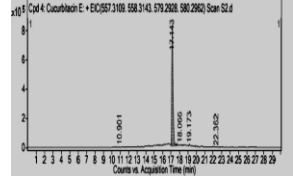
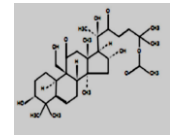
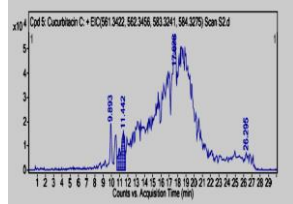
with ms/ms spectra with standards *C. sativus* and fruit pulp extract was qualitatively identified with a skeleton of triterpenes and originally identified in cucurbitaceous

plants by Rajasudha and Manikandan, 2019; (Liang et al., 2019; Che Zhang., 2019).

Principal component analysis (PCA)

The analytical data presented in Table 2 of showed the presence of Triterpenes: Cucurbitacin-C (0.8ppm), Cucurbitacin-E (5.53ppm), and Cardiac glycosides: Gitoxigenin (7.76ppm), Strophanthidin (13.79ppm), and Digitoxigenin(2.13ppm) in 10 μ l 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. Triterpenes were 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 is 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 low concentration 0.05-0.128 μ M were operative as anticancer drugs by Siti and Nurhanan (2018).

Table 2. HRLCMS details of prominent compounds with MS spectra, peak list and compound structure of white foamy substances of *Cucumis sativus* L. local white cultivar

| Compound label | Compound Structure | m/z | RT | Formula | Mass / MS spectrum peak list |
|--------------------------|---|-----------|--------|--|---|
| Cpd 1 : Digitoxigenin |  | 397.23577 | 19.92 | C ₂₃ H ₃₄ O ₄ | 374.2465  |
| Cpd 2: Gitoxigenin |  | 413.2266 | 9.893 | C ₂₃ H ₃₄ O ₅ | 390.2376  |
| Cpd 3: Strophanthidin |  | 407.2258 | 14.614 | C ₂₃ H ₄₄ O ₈ | 404.2143  |
| Cpd 4: Cucurbitacin E |  | 579.2895 | 17.143 | C ₃₂ H ₄₄ O ₈ | 404.2143  |
| Cpd 5: Cucurbitacin C |  | 561.3426 | 11.442 | C ₂₃ H ₄₈ O ₈ | 404.2143  |

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. The mass of compound-1 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 (Table 2). It was observed in the range of -2.13ppm. Compound-2 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 (Table 2). It was observed in the range of 7.76 ppm. The compound-3 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 Strophanthidin defined above (Table 2). It was observed in the range of 13.79ppm in the highest concentration. The compound-4 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 (Table 2). 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 (Table 2). It was observed in the range of -0.8 pp. 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 5.53. are used in arrow missile poisons as abortifacients, as heart stimulants, as diuretics Mallick et al. (2022). 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 NF/ κ B pathway it also revealed to upsurge the expression of death receptors (DR4, DR5) upsurgence the calcium concentrations DR4 (Trail receptor 1) and DR5 (Trail receptor 2) are transmembrane receptors that have an intracellular death domain by Kabouche et al. (2017) and Reddy et al. (2020). 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. (Patel et al., 2019). Glycosides related to puerarin, gymnemic acid I rutin, and sativoside have been stated for noteworthy antidiabetic activity. Aglycones like christinin, strictinin, and securigenin have been conveyed for their antidiabetic activity by Adki et al. (2021). 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. Cardiac Glycosides (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 defense. They can also be treated as preliminary assemblies for combinatorial chemistry to produce novel compounds comprising remedies with the anticipated properties (Karas et al., 2020, Putri et al., 2022). Strophanthidin the highest concentration in the foamy extract is a significant finding where it interacts with various proteins in biochemical reactions. It has a role in the down regulation of proto-oncogenes. It plays a significant role in cell cycle arrest at the G2/M phase. Inhibits the membrane protein $Na^+/K^+ ATPase$ in muscle tissue. *C sativus* is used as both vegetables as well as fruit in Indian traditional medicine. It is a source of nutritional benefits and bioactive compounds.

Cytotoxic activity

The morphological observation indicated brownish colour change in the roots and average root length was decreased depending upon increasing concentration. Photomicrograph study was done under 100x light microscope with Nikon photographic camera. The photomicrograph study indicates reduction in root growth and chromosomal aberrations, disturbed telophase, anaphase, abnormal cell shape, dissolved chromosomes, membrane damage and nuclear lesions (Fig. 2) The mitotic index in negative and positive control was 7.25% and 3.29% respectively.

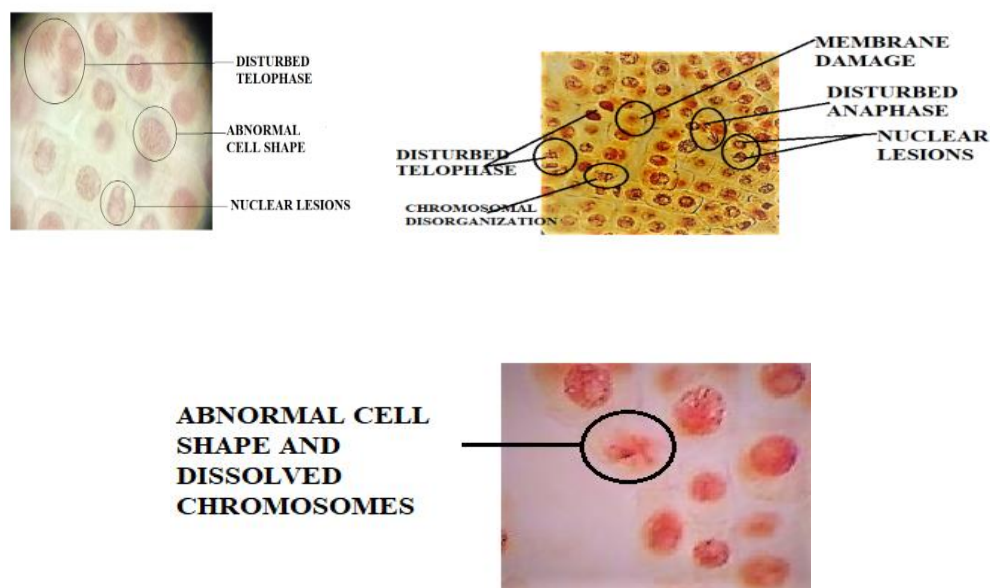


Figure 2. Photomicrograph study was done under 100x light microscope with Nikon photographic camera

CONCLUSION

The present study suggests that *Cucumis sativus* L. local white cultivar of Hassan district Karnataka is a good source of drugs to improve human health and enlightened us on the science behind the removal of white foamy substances before slicing the cucumber. In this regard, we have to expand our knowledge on phytochemical constituents of *Cucumbers* grown in different parts of the world. Animal model study is further recommended to evaluate cytotoxic study white foamy extract of *Cucumis sativus* L. for human welfare. The foamy extract could be the source of cheap medicine for cancer and cardiac patients.

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