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Research Article

Cucumis Sativus L. Foamy Extract Induces Apoptosis In MDA-MB 231 Cells

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Abstract

Cucumis sativus L. is considered as an important salad fruit as well as vegetable, fruits. It is consumed as herbal medicines. We have investigated the effects of foamy extract on the viability of the MDA-MB-231 human cells. Results revealed that extract significantly ($P < 0:05$) inhibited the proliferation of cells in comparatively lower toxic effects. The IC50 was found to be 116.11 µg/mL against MDA-MB-231 cells, Annexin V/PI staining assays indicated that it stimulates apoptosis in MDA-MB-231. Treated cells were observed for greater incidence of apoptotic cells, when compared to the untreated cells. Thus, it is efficient enough to induce apoptosis, in MDA-MB-231 cells. Cucumber foamy extract may prove to be an important source of natural anticancer agents.

Keywords: Apoptosis, *Cucumis sativus* L, Foamy extract, HR-LCMS, MDA-MB-231cells.

Introduction

Cucumber originated in India, where it appears to have been cultivated for more than 3,000 years ago. *C. sativus* L.is noticeable inexpensive crop cultivated globally. It endures fruit of a cylindrical shape with a light aroma and a crisp texture with high water content and an essential component in salad and raita. They are widely grown and consumed in either raw or preserved pickled form all over the world. China produces 61.9 million tonnes, followed by European Union, Russia, Turkey. India is the leading exporter of cucumber and gherkins in 2023-2024 with the production of 54,015.97 metric ton. It has earned 58.71 million US $ by export. India's major export destinations are USA, Germany, France, Spain and Russia as per APEDA statistics (Agricultural and Processed Food Products Export Development Authority). Cucumber is divided into pickling and slicing varieties (Grumet et al., 2022).

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Different types of Cucurbitacin are identified in cucumbers, they are cytotoxic in nature which inhibit proliferation of tumours. The Indian thali is incomplete without a slice of Cucumber. It is consumed all over the world majorly as salad fruit as well as vegetable. It is listed as naturally low caloric and ancient food. It is an important income earning crop. Literature survey indicates that India possesses heterogeneous germplasm collection. It is a site of large genetic diversity, only small part of it exists in different parts of the world (Pal and Saha 2019).

The study of Globocon, 2020 every four minutes woman is diagnosed with breast cancer, about 1,78,000 new cases being diagnosed every year and mortality 12.7 per 100,000 women and 685000 deaths globally (Kharat et al., 2022). Numerous studies have demonstrated that the chance of developing breast cancer rises with age cancer dealing still has many unmet requests and needs new beneficial representatives' drugs. New and competent bioactive compounds against cancer should be developed for public health, for this purpose identification of new substances with anticancer activity is critical and crucial in the development of anticancer drugs (Inthi et al.,2023). Anticancer chemical research has advanced during the past few years. Many scholars have scrutinized plant-obtained bioactive complexes' possible as preventive agents to decrease cancer. *C. sativus* is considered as an important medicinal plant (Latha, 2022). It is one of the supreme members of the *Cucurbitaceae* family. It is believed to originate in Indian subcontinent and is recognized as a hub of diversity Sharma et al. (2022). Phytochemicals present in this family are often used as diuretic and cardiotonic managers. They also demonstrate good anti-inflammatory, antitussive, cytotoxic and expectorant effects. Apart from the biological profile, the *Cucurbitaceae* family has several therapeutically important chemical compounds Since ancient time (Habib et al., 2023). It is consumed as traditional food and used to treat constipation, headache for soothing effects and for reducing swelling in the skin. Its fruit juice has refreshing effective and fruits were used as cooling vegetable. Many chemo preventive chemicals have been discovered based on their ability to regulate one or more specific chemical processes (Bakam et al., 2023). In the current study showed that foamy extract of *C. sativus* L. is rich in tetracyclic compounds among them gitoxigenin is abounded in foamy extract of local green cultivar of *Cucumis sativus* L. of Hassan District, Karnataka, is examined on MDA-MB-231 cells human breast cancer cell lines. This study suggests that bioactive compounds found in foamy extract is the potential candidate to hit the growth of MDA-MB-231 cells.

Materials and Methods

Collection of Cucumber Fruits

The native local variety of C. *sativus* L.*green (nati southe)* was harvested from a farm in the Karnataka, India. Katte Belaguli, village which is 5km from Holenarasipura taluk, Hassan district. White frothy substance was extracted from fresh fruits. Cutting a thin slice of cucumber at end, placing it back on the flat

surface, and rubbing it between the blossom and stem ends of the cucumber in a circular motion produced the white foamy substance. For the experiment,100 gm of fresh frothy stuff was collected and used for the experiment showed in Figure -1.

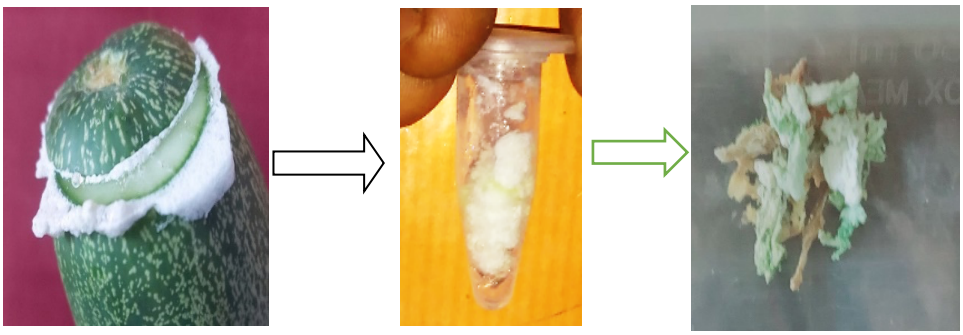

Fig. 1. C. *sativus* L. fruit foamy extract and dried powder

Extraction and Separation

Soxhlet extraction of foamy extract was done with 70 methanol. Distillation was performed to isolate the solvent; the concentrate was evaporated to desiccation and the remainder was dissolved in 5ml of 70% methanol. Preliminary Phytochemical Analysis was done according to Rai et al. (2023).

HR-LCMS Q-TOF/MS study of foamy extract

The 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 *C. sativus* L. ZORBAX Eclipse Plus-C18, 5micron (Agilent) Mode, 150x2.2 mm. 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 masses 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 250^0C. 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-minute acquisition period, the solvent composition flow A: Milli-Q water containing 0.1% formic acid B: Acetonitrile and TOF/6500 series. 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.

MTT Assay

Briefly, around 1×10^4 MDA-MB-231 cells human breast cancer cell lines purchased from NCCS Pune. Cells were seeded into each well of 96 well plate which were placed for incubation at 37 °C and 5% CO_2 in a CO_2 incubator. After 24 hr the cells were treated with foamy extract at various concentrations (0, 20, 40, 80, 100, 200 µg/mL). All the mentioned concentrations were tested in triplicates and the results were given as an average of each combination. The cells were again incubated in the CO_2 incubator for 24 hr under the same incubation condition. After the incubation 20µL of MTT(3-(4,5-Dimethylthiazol-2yl)-2,5-Diphenyltetrazolium Bromide reagent) 5mg/ml was added to each well and further incubated for another 4hr in Co_2 incubator, After the incubation time, the 96 well plates were removed and MTT media in each well are replaced with 100µl of DMSO (Dimethyl Sulfoxide), were placed for 30 min at RT dark. Followed by this the plates were subjected to spectrophotometric reading at the λ570nm using microplate reader.

Apoptosis Assay

Briefly, around 1×10^6 cells were seeded into each well of 6 well plate which were incubated at 37°C and 5% CO_2 in a CO_2 incubator. After 24hr, the cells were treated with the specific concentration of the compound (0% to 30%) to induce apoptosis in cells. The well plate was incubated at 37°C and 5% CO_2 in a CO_2incubator. After 24 hr, the cells were harvested and washed with 1mL cold phosphate-buffered saline (PBS). The washed cells were centrifuged, the supernatant was discarded, and the pellet was resuspended in 1mL of 1X annexin-binding buffer. A volume of 2.5 µL Annexin V stain and 2.5 µL 100 µg/mL Propidium iodide (PI) working solution was added to each 100µL of cell suspension. The cells were incubated at room temperature for 15 minutes. After the incubation period, 400 µL(1X) annexin-binding buffer was added, and mixed gently. As soon as possible, the stained cells were analysed by flow cytometry, measuring the fluorescence emission at 530nm and 575nm (or equivalent) using 488-nm excitation. Apoptotic activity by flow cytometry was studied by FITC V Kit (no. 51-65874X), Propidium Iodide (PI) (no. 51-66211E) Annexin V binding buffer (no. 51-66121E).

Statistical Analysis

Entirely experiments were completed in triplicate at three independent times. The calculations were executed in Excel, and the statistical significance was analysed one-way or two-way ANOVA depending on the experiment. The results were reported as mean ± SD (the standard error of the mean). The statistical difference was set at * $p < 0.5$, ** $p < 0.05$, *** $p < 0.001$.

Results and Discursion

In this study we collected 100gm of fresh frothy substances, followed by Soxhlet extraction with 70% methanol. Approximately 2mg of residue was obtained. It was dissolved in 2ml 70% methanol and 1ml of this extract was analysed for preliminary phytochemical analysis it indicated the presence triterpenes (Latha and Raja Naika 2020). It was sent to Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Pawai, Mumbai, for HR-LCMS analysis. Result showed in Table-1: Gitoxigenin 8.33ppm in 10µL sample loaded is significant result. The local green cultivar of C. *sativus* L is the rich source of bioactive compounds *C. sativus* L. (Latha and Raja Naika, 2020).

Table 1. Prominent Compounds of Foamy Extract of *C. sativus* L data by SAIF IIT Bombay

Mass	m/z	Name	Formula	ppm
574.314	597.3039	Cucurbitacin A	C_{32} H_{46} O_9	0.35
390.2374	413.2267	Gitoxigenin	C_{23} H_{34} O_5	8.33
560.3346	583.3238	Cucurbitacin-C	C_{32} H_{48} O_8	0.65
558.3226	581.3109	Cucurbitacin B	C_{32} H_{46} O_8	5.93
404.2195	407.2338	Strophanthidin	C_{23} H_{32} O_6	0.96
556.2996	579.2885	Cucurbitacin E	C_{32} H_{44} O_8	7.17
374.2479	397.2368	Digitoxigenin	C_{23} H_{34} O_4	5.95

MTT Assay

The cytotoxic effect of methanolic foamy extract was subjected evaluation by means of 3-(4,5 dimethyl-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay. Cells have been exposed to different concentration The IC50 value of the given compound was found to be at 116.11 µg/mL concertation. Table-2 and Figure -2

Table 2. MTT Assay

Concentration(µg /mL)	OD	Cell inhibition (%)	Average	SD
Control	0.778	-	-	-
	0.7839	-	-	-
	0.7956	-	-	-
20	0.7737	1.543966721	3.76666119	1.99773135
	0.7517	4.34354373		
	0.7433	5.412473134		

Concentration(µg /mL)	OD	Cell inhibition (%)	Average	SD
40	0.6047	23.04980829	20.6701678	2.52388961
	0.6442	18.02329503		
	0.6213	20.93740019		
80	0.4864	38.10389739	34.3244684	3.77308238
	0.5162	34.31174308		
	0.5457	30.55776482		
100	0.4666	40.6235167	43.0625421	2.75915209
	0.4518	42.50686851		
	0.4239	46.05724117		
200	0.1226	84.39872085	83.4146271	1.99789347
	0.1484	81.11558054		
	0.12	84.72957995		

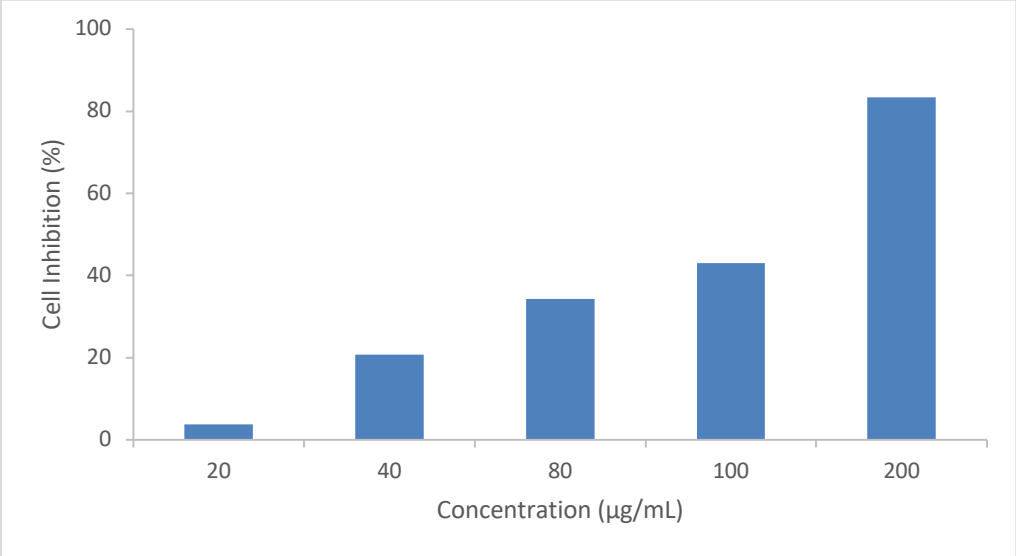

Fig. 2. Cell Inhibition (%)

Apoptosis Assay

After being exposed to foamy extract for 24 hours, MDA-MB-231 cells were driven to undergo apoptosis. The study in the graphical representation of apoptosis assay, the conjugation of 10^3-10^4field on both X-axis and Y-axis represents the appearance of

apoptotic cells. The field beyond 10^4 range represents cells in late apoptotic phase and death phase, whereas field below 10^3 range represents live cells. FIGURE -3 and FIGURE 4. results indicate that gitoxigenin induces cell death via the apoptotic pathway in Human MDA-MB-231 cancer cells, so that this compound can be developed as an anticancer agent in the future from C. *sativus* foamy extract.

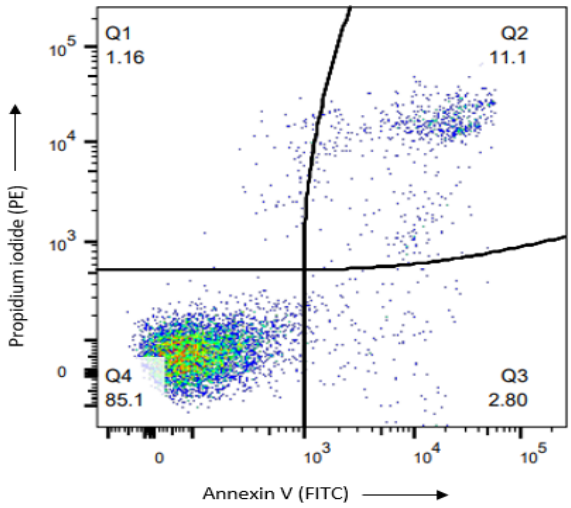

Fig. 3. Appearance of Apoptotic Cells

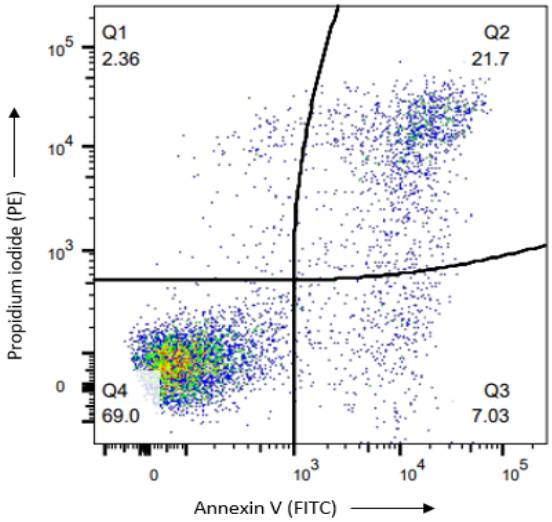

Fig. 4. FACS graph representing cells in the 30% concentration sample

Phosphatidyl (PS) can be bound by annexin V an anticoagulant, when calcium is present. PS will go to the surface of the cell during the early phases of apoptosis. Due to its conjugation with fluorescein isothiocyanate (FITC), annexin V can be identified via flow cytometry.

Propidium iodide was utilised to distinguish between surviving, early apoptotic cells and necrotic, late apoptotic cells. Necrotic cells would bind to Annexin V-FITC and exhibit membrane permeabilization. Both early apoptotic and living cells released propidium iodide. Because of the delayed necrotic-like cell disintegration, late apoptotic cells will be stained with propidium iodide and FITC together. When Human MDA-MB-231 cells were exposed to gitoxigenin for 24 hours, the therapy induced apoptosis. Figure-5 and Figure- 6.

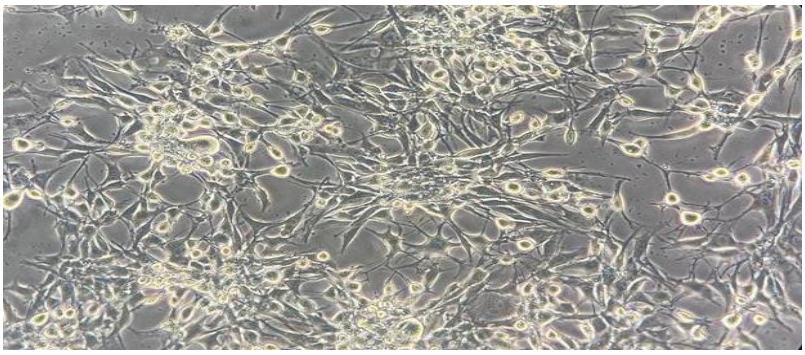

Fig. 5. Morphology of Human MDA-MB-231 cancer cells control and Human MDA-MB-231 cell lines

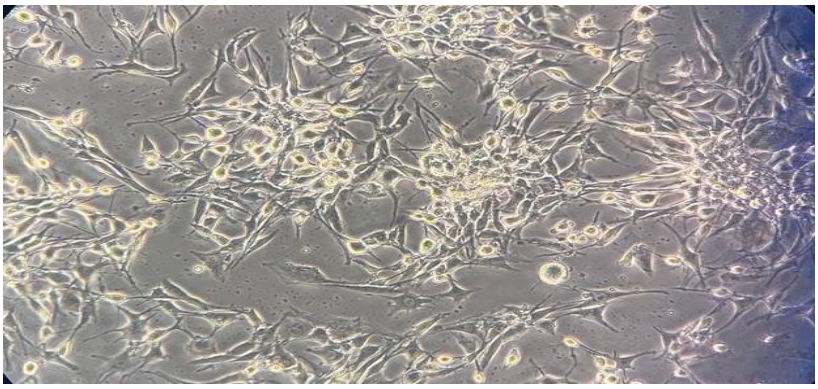

Fig.6.34.8 µg/mL treated cells Human MDA-MB-231

In the present study we recorded the therapeutic application, of *C. sativus*. We have explored the phytochemical and pharmacological profile foamy substance *C. sativus*. The HRLCMS study of substance revealed different bioactive compounds. Among the prominent compounds in the foamy extract Gitoxigenin (8.33 ppm) was found in uppermost concentration. The cells treated with the foamy extract are observed to show better incidence of apoptotic cells, when compared to the untreated cells (control). Thus, the bioactive compounds in foamy extract is efficient enough to induce apoptosis, in MDA-MB-231 cells.

In the preliminary study, we carried out quantitative estimation of cardiac glycosides. Total glycosides of the sample quantitatively determined according to Raymond reaction (Latha. 2023). The IC50 value of the given compound was found to be at 116.11 µg/mL concentration. In the presence of calcium, annexin V functions as an anticoagulant and has the ability to bind phosphatidyl (PS) The translocation of PS to the cell surface occurs early in apoptosis. Because annexin V is coupled with fluorescein isothiocyanate (FITC), it can be identified using flow cytometry. While necrotic cells exhibited membrane permeabilization and would bind to Annexin V-FITC, surviving, early apoptotic cells could be distinguished from necrotic, late apoptotic cells using propidium iodide (Mazzio et al., 2021). Excreta of propidium iodide was observed in both early apoptotic and living cells. Because late necrotic-like cell disintegration occurs, late apoptotic cells will be stained with propidium iodide and FITC (Akhil et al., 2023). In the apoptosis assay we found field beyond 10^4 range represents cells in late apoptotic phase and death phase, whereas field below 10^3 range represents live cells. The cells treated with the compound are observed to show greater incidence of apoptotic cells. Phosphatidyl serine (PS) is a key biomarker of early apoptosis. So, it is necessary to perform further investigation to elucidate pathway which induce apoptosis MDA-MB-231 cell lines.

The recent data available on the study of cytotoxic activity using the MTT assay, the cytotoxic effect of A. indicum-mediated synthesized CuO NPs was assessed against human lung A549 and breast MDA-MB-231 cancer cell lines. The results of the investigation showed that ZnO-PB-NPs might, in a dose-dependent way, cause considerable cytotoxicity in human breast cancer cells. Treatment with ZnO-PB-NPs reduced the breast cancer cells capacity to proliferate (Nagarajaiah et al., 2023). The research demonstrated the green synthesized CuO Nanoparticles has potential biological activity, including good dye degradation in malachite green, effective anticancer activity against the MDA-MB-231 cancer cell line, and antibacterial action against gram positive bacteria. (Nafeh et al., 2023).

The MDA-MB-231 cells treated with MLPD underwent morphological examination, which revealed cytotoxic alterations such as vacuole formation, changed shape, and a decrease in the number of cells forming apoptotic bodies. The study was done fraction of the A. muricata leaf extract might stop the proliferation of the human MDA-MB-231 cell line, which is used as a model for triple negative breast cancer in

the culture media used RPMI 1640 (Sathiyavimal et al., 2022): After 72 hours of incubation, RNAs were extracted using TriPure isolation reagent from both treated and untreated MDA-MB-231 cell lines using A. muricata DMSO extract. The expression of P53 and Bcl2 was measured by qRT-PCR, with the β-actin gene serving as an internal control. ELISA was used to analyse the protein expression of the BRCA1, BRCA2, EGFR, p53, Bcl2, Cytochrome C, and Caspase 3 genes (Pal et al., 2023). The A. muricata DMSO extract-treated cell line exhibited overexpressed levels of pro-apoptotic P53 gene expression, suggesting that the extract may be effective in driving cancer cells towards programmed death (Grumet et al., 2022) The crude extract from the Southeast Asian medicinal herb Houttuynia cordata Thunb (HCT) significantly reduced the growth of MDA-MB-231 and MCF-7 cells (Chekuri et al., 2023). The purpose of the study was to determine how cytotoxic this plant extract and its fractions were to MDA-MB-231 and MCF-7 human breast cancer cells (Rai et al., 2023) The phytoactive components of HCT are identified. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess the vitality of the cells. Using a flow cytometry approach, the manner of cell death was determined by labelling with annexin V-FITC and propidium iodide (PI). Using fluorescence microplate readers and fluorescent probes labelled 2',7'-dihydrodichlorofluorescein diacetate (DCFH-DA) and dihydroethidium (DHE+), the oxidative stress was quantified. High performance liquid chromatography was used to characterise HCT phytochemicals (HPLC) (Ella et al., 2021) and (Menchikov et al., 2023). Plants are the prime source of folk medicine, homoeopathy and aromatherapy. Cucumber is a popular vegetable and also fruit, which has been widely used in folk medicine to reduce heat and inflammation. It is a rich source of vitamin C, and can be used externally to cool, cleanse and healthy. It is climber of several promising aspect (Sauter and Edward, 2020). This implies that ethnobotanical information of plant resources can be studied by its phytochemical analysis.

Conclusion

Very popular vegetable as well as fruit *Cucumis sativus* L. has many bioactive compounds found in foamy extract expressed the of activity of inhibiting proliferation and inducing apoptosis in MDA-MB-231 cells of breast cancer. Further investigation to be done to elucidate pathway which induce apoptosis MDA-MB-231 cell lines.

Acknowledgement

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