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Original Article

Phytochemical Profiling and Evaluation of Cytotoxic Potential of *Cucumis prophetarum* L. Fruit Extract

Sadique Akhtar Javed[1,*], Asaad Khalid[2], Rym Hassani[3], Syam Mohan[4], Ashraf Nabiel Abdalla[5], Zia ur Rehman[1,2], Asim Najmi[1], Khalid Zoghebi[1], Hafiz Antar Makeen[6], Mohammed Albratty[1]

[1]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, Jazan University, Jazan, SAUDI ARABIA.
[2]Health Research Centre, Jazan University, Jazan, SAUDI ARABIA.
[3]Environment and Nature Research Centre, Jazan University, Jazan, SAUDI ARABIA.
[4]Center for Global Health Research, Saveetha Medical College, and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Kuthambakkam, Tamil Nadu, INDIA.
[5]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Umm Al-Qura University, Makkah, SAUDI ARABIA.
[6]Department of Clinical Pharmacy, Pharmacy Practice Research Unit, Faculty of Pharmacy, Jazan University, Jazan, SAUDI ARABIA.

ABSTRACT

Objectives: The present study examined phytochemical composition of ethanolic extract and evaluated cytotoxic properties of *Cucumis prophetarum* L. **Materials and Methods:** Phytochemical composition of extract was evaluated by GC-MS and screened for cytotoxic activity by MTT assay. The human cancer cell-lines including MCF7, A2780, HT29, and Normal Fetal Lung Fibroblasts (MRC5), used to determine cytotoxicity and selectivity index of the extract. **Results and Discussion:** Fatty acid esters (43.74%), phthalic acid diesters (11.38%), fatty acid silyl esters (4.15%), and siloxane derivatives (3.88%), aromatic monoterpenes (2.75%) and pentacyclic triterpenoids (1.21%) were the main constituents detected in the extract. Results indicated that MCF7 cells exhibited highest sensitivity (IC_{50}: 3.30 µg/mL) and the extract was selective and caused early apoptosis against the same cell-lines. **Conclusion:** The outcomes of this investigation indicated that the bioactive components in the extract might be employed for cytotoxic purposes. The study will be useful to establish the scientific basis for ethnopharmacological usage of the tested plant.

Keywords: *Cucumis prophetarum* L., GC-MS, Phytochemical, Cytotoxic, Selectivity index, Apoptosis.

Correspondence:
Dr. Sadique Akhtar Javed
Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, Jazan University, Jazan-45142, SAUDI ARABIA.
Email: sajaved@jazanu.edu.sa

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INTRODUCTION

Cancer is one of the leading causes of deaths worldwide. According to WCRF international, there were around 19.97 million cancer cases diagnosed in 2022.[1] WHO estimated that cancer accounted for nearly 10 million deaths in 2020, which is one-sixth of all deaths occur globally.[2] The cancer is usually treated by one or combination of more than one of the different methods including radiotherapy, surgery, chemotherapy, immunotherapy, hormonal treatment and targeted biological therapies.[3-5] Apart from their effectiveness in cancer treatment, the modern methods are associated with serious harmful effects. Moreover, resistance to chemotherapeutic agents is also a major problem associated with the anti-cancer therapy, which is directly or indirectly linked to cancer-related deaths.[6] Medicines from natural sources are used from time immemorial for treating a variety of serious diseases and are considered to be safe and effective. Even today, natural products are being considered as alternative therapy for a number of diseases and various anticancer natural products are currently in clinical practice. However, the reference of traditional and ethnopharmacological use of natural medicines cannot be a basis for the substitution for scientifically developed and well-regulated modern system of medication. Therefore, studies on natural products to establish their chemical and pharmacological profiling, safety and efficacy using modern techniques is of utmost importance.

Cucumis prophetarum L. is an ancient perennial climber herb with broad and simple leaves, commonly known as cucumis, wild cucumber, globe cucumber or wild gourd and belongs to the family Cucurbitaceae.[7] Its fruit has longitudinal coloured stripes and resembling a gooseberry. The plant is mainly native to the Asian and African semi-arid regions and found in different parts of the world including Saudi Arabia, where it is locally known as Shari-al-deeb.[8,9] *C. prophetarum* L. along with other species from the same family is historically and traditionally quite popular and used for treating a wide range of ailments

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due to its anticancer, immunomodulatory, hepatoprotective, cardiovascular anthelminthic and anti-inflammatory activities.[10] For example, in Saudi folk-medicine, fruit has been used for liver disorders[9] and in Ethiopia, it is used for treating diarrhea, skin infections, lung disorder, back pain, intestinal complications, cardiac failure and cancer.[11,12] In the modern time, *C. prophetarum* L. has been considerably investigated for a variety of biological potential and found to possess antibacterial, antioxidant, anticancer, antidiabetic and anti-inflammatory activities.[9,13-15] Literature indicated that the wide range of biological activities of *C. prophetarum* L. is mainly attributed to the presence of a group of phytoconstituents known as *cucurbitacins*, which are highly oxygenated tetracyclic triterpenes produced by the plants and used to defend themselves from external parasites.[10]

In a bioassay-guided fractionation study, Alsayari *et al*., (2018)[9] evaluated cytotoxic property of *C. prophetarum* L. fruit extract using cancer cell-lines. The ethylacetate fraction reported to show highest anticancer potential and purification of which led to the identification of few cucurbitacin compounds including B, D, E, F-25-acetate and hexanorcucurbitacin D. The compounds, B and E displayed highest cytotoxicity against the cell lines including MCF7, MDA-MB-231, HCT-116, A2780CP, A2780 especially against the breast cell lines. Other compounds previously isolated from *C. prophetarum* L. include spinasterol, cucurbitacin B, 2-O-β-D-glucopyranosylcucurbitacin E, cucurbitacin F 25-*O*-acetate, dihydroisocucurbitacin D, isocucurbitacin D, cucurbitacin E, isocucurbitacin E, dihydroisocucurbitacin E, dihydrocucurbitacin D, dihydrocucurbitacin E, cucurbitacin Q1, cucurbitacin O and cucurbitacin P. Several of these compounds showed cytotoxic activities and anticancer potential in different studies.[15-18]

C. prophetarum L. attracted the attention of the researchers involved in natural product research mainly due to its wide range of application in Ethiopian traditional medicine for treating a number of diseases. Although the reports regarding the extraction and a number of biological screenings of this plant is available, to the best of our apprehension, no study conducted on phytochemical profiling by using GC-MS analysis. Furthermore, it is well known, that the phyto-components and bioactivities varies among the same plant species, if collected from different climatic conditions and time of cultivation. Therefore, the aim of the current investigation was to perform extraction of fruit sample of *C. prophetarum* L. and study the phytochemical composition by automated GC-MS analysis, which show volatile and semi-volatile plant constituents and screen the extract for *in vitro* cytotoxic effects against Human Cancer Cell-Lines *including* MCF7, A2780, HT29 and Human Fetal Lung Normal Fibroblasts (MRC5). In addition to the determination of the IC_{50}, Selectivity Index (SI) and induction of apoptosis for the main plant extract were also evaluated.

MATERIALS AND METHODS

Sample Collection and Extraction

The *C. prophetarum* L. fruit sample was collected from southwestern region (Jazan province) of Saudi Arabia and authenticated. The sample was air-dried in shade and powdered. The extraction was performed by maceration according to the procedure described by Harborne (1984) with slight modification.[19] The powdered sample (approximately 500 g) was mixed in 80% ethanol (2.5 L) and left at room temperature for 72 hr with continuous shaking, allowed the undissolved components to settle down and the supernatant was collected after filtration. The process was repeated twice. The solvent was evaporated, and the residue was dried. The dried extract was stored in refrigerator at 2-8°C until further use.

GC-MS parameters

GC-MS instrument (Thermo Scientific, USA) was used for phytochemical composition analysis. Helium was set to flow at 1.2 mL/min utilizing a constant flow mode. The instrument was equipped with AS-3000 autosampler and ISQ type detector. The sample was injected into TR-5MS column as stationary phase to accomplish chromatographic separation. A GC-column of 30 m in length, an internal diameter of 0.25 mm and 0.25 mm film thickness coating was utilized. Split-less mode was used to inject 2 µL diluted plant extract into the column. Initially, the injector port was at 320°C for 5 min and then a temperature ramp was followed: to 205°C at 5°C/min - hold-time 5 min; 280°C at 5°C/min - hold-time 5 min and 300°C at 5°C/min-hold-time 5 min. Oven was fixed at 320°C as highest temperature. The MS instrument was run in EI mode with a 60=900 AMU (min) mass range. Transfer line and MS ion source temperatures were adjusted to 350°C and 320°C respectively using an electron-multiplier voltage of 1 Kv. The spectral data of the phytochemical constituents of the sample extract was acquired by using Xcaliber software.

Evaluation of Phytochemical Composition

The fragmentation pattern of each chemical constituents detected in the extract was recorded and evaluated through comparison of the instrumental mass spectral data. The identification of each phytochemical ingredient was established through comparison of their chemical structures with those present in the built-in computer libraries such NIST, REPLIB and MAINLAB and the peak area of the components recorded in the mass spectrum was used to calculate their percent abundance in the extract sample. The Dr Duke's Phytochemical and Ethnobotanical Database was taken as a reference to report the biological profiles of the compounds identified in this investigation.[20]

Cell culture

In order to test cytotoxic property of the fruit extract on cell survival, human cancer cell lines including ovary (A2780), breast

(MCF7) and colon (HT29) adenocarcinomas were employed. Additionally, MRC5 normal cells were used for comparison. The cell cultures were procured from ATCC, Rockville, MD, USA. RPMI-1640 (10% FBS) media was used to maintain the cancer cell-lines and MRC5 cells cultured in Eagle minimal essential medium (EMEM, 10 % FBS). All the mixtures were kept at 37ºC temperature, 100% RH and 5% CO_2.

Cytotoxicity Assay

Cytotoxicity of 80% ethanolic extract of *C. prophetarum* L. fruit was estimated using the 3-(4, 5-Dimethylthiazol-2-Yl) - 2, 5 - Diphenyltetrazolium Bromide (MTT) assay, which estimated the conversion of tetrazolium salt to a blue formazan product by metabolically active cells. The procedure previously described in the literature was followed.[21,22] Cancer cell lines and human fibroblasts were grown in the appropriate media and seeded in 96-well (3×10^3/well) plates and left overnight at 37ºC to attach to the well. The fruit extract dissolved in DMSO (0.1%) to obtain solutions of different concentrations from 0 to 100 µg/mL and inoculated into the wells in triplicate. Just prior to incubation of plates for 72 hr, MTT (2 µL, 5 mg/mL) was incorporated into the wells and the supernatant was aspirated from each well. Absorbance of purple formazan was measured at A_{550}, that is proportional to the viable cell-counts. The viability results were expressed in IC_{50} i.e., extract concentration inhibiting cell growth by 50% in comparison to the control cell growth rate. Selectivity index (SI) was calculated as ratio of the IC_{50} values of the extract for normal cell line to that of selected cancer cell line. The extract was considered highly selective if the SI value is ˃2 and lower selectivity if the SI values is ˂2.

Morphological assessment of apoptotic cells

As MCF 7 cells showed most promising results in cytotoxic assay compared to other cells screened in this study, it was alone used to estimate the potential of *C. prophetarum* L. fruit extract to induce programmed cell death. These apoptosis assays were carried out using Acridine Orange (AO), a cell-permeable DNA binding dye in live cells and Propidium Iodide dye (PI), which is permeable to viable and dead cells. One million MCF7 cells were treated with plant extract after a 24 hr adherence to culture plates. The extract concentration used was 3.3 µg/mL (IC_{50} value), and treated for different time points such as 24, 48 and 72 hr. Upon completion of treatment, the cells were extracted from the flask using Trypsin and a cells scrapper. The cells were washed twice with 1X sterile PBS. The PBS was removed from the cell pellets using centrifugation. Then the cells were exposed to 1 µL of AO (10 mg/mL) and PI (10 mg/mL) with gentle shaking. Pellets were then set aside in the dark for 5 min and immediately transferred to a clean glass slide which was covered with coverslips. Each sample was separately checked under a fluorescent microscope for different morphological changes. One hundred cells were randomly counted from each sample (*n*=3) and cell number was used for quantification and statistical analysis. Cells were differentiated into four categories such as viable cells, early apoptosis, late apoptosis and dead cells. In principle, AO emits green fluorescence, and PI emits red fluorescence while binding to DNA. Cells with an intact nucleus and green colour were classified as viable cells. Early apoptosis cells were differentiated from viable cells using the difference in brightness of green fluorescence and condensation of chromatin inside the cytoplasm. Whereas the late apoptosis cells exhibited chromatin condensation as well but with a slight orange colour and the dead cells showed an intense orange nucleus.

RESULTS

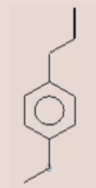
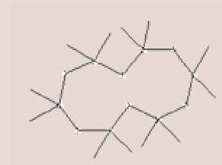
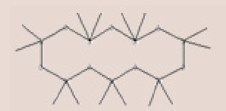
Phytochemical Composition of extract

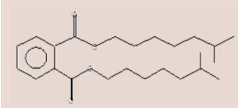
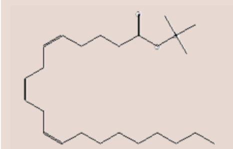
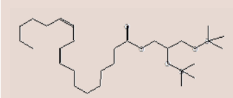
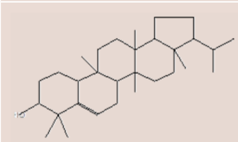
The 80% ethanolic fruit extract of *C. prophetarum* L. was estimated using GC-MS and the identified compounds have been listed along with their reported bioactivities in Table 1. Compounds detected in the extract were mainly fatty acid esters (43.74%); the derivatives including 9,12-octadecadienoic acid (Z,Z)-methylester (13.84%), 1,2-benzenedicarboxylic acid, diisooctyl ester (11.38 %), 9,12,15-Octadecatrienoic acid-2,3-dihydroxypropyl ester (Z, Z, Z) - (10.56 %) and 9, 12 - Octadecadienoic acid, ethyl ester (linoleic acid ethyl ester) (10.17%) identified in highest levels and considered to be the major components detected in the current investigation. The mass spectra along with their chemical structure are presented in Figure 1. Moreover, hexadecanoic acid methylester (2.19%), octadecanoic acid methylester (2.08%), hexadecanoic acid ethyl ester (1.1%), ethyl oleate (1.13%), and 13-docosenoic acid methyl ester (Z)- (1.58%) were the other fatty acid esters derivatives present in minor proportions. Additionally, *cis*-5,8,11-eicosatrienoic acid trimethylsilylester (3.14%) and 1-monolinoleoylglycerol trimethylsilyl ether (1.01%) were identified as fatty acid silyl esters; while cyclohexasiloxane dodecamethyl (1.99%), and cycloheptasiloxane tetradecamethyl (1.89%) were two siloxane derivatives present in the plant extract. Estragole (2.75%), 1,2-benzenedicarboxylic acid diisooctyl ester (11.38%) and D: B-Friedo-B': A'-neogammacer-5-en-3-ol (1.21%) were three phytocompounds present individually in different proportions. The compositions of plant extract recorded in this investigation is further highlighted in chart diagram (Figure 2).

Cytotoxic activity

The MTT assay analysis was conducted to investigate the cytotoxicity potential of the extract of *C. prophetarum* L. The extract was found to exhibit highest action against MCF7 cell line (IC_{50}: 3.30 µg/mL), followed by A2780 and HT29 cell lines, while the IC_{50} against MRC5 normal cell line was 20.49 g/mL. The extract exhibited excellent selectivity to MCF7 cells as compared to the MRC5 normal cells with a SI value of 6.20 (Table 2).

Table 1: GC-MS analysis and biological significance of phytocompounds from *C. prophetarum* L.

RT	Compounds	Molecular formula	MW	Area%	Nature of compound	Structure	Biological significance
13.76	Estragole (methyl chavicol)	$C_{10}H_{12}O$	148	2.75	Aromatic monoterpene		Cytotoxic, antimicrobial, anti-inflammatory, anesthetic, anticonvulsant, antioxidant.[23-27]
15.84	Cyclohexasiloxane, dodecamethyl- (D6)	$C_{12}H_{36}O_6Si_6$	444	1.99	Cyclic siloxane deriv.		Cosmetics, antifungal and personal care products, antiperspirants, deodorants, antibacterial, emollient, lubricant, de-foaming agent.[28-31]
20.29	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	1.89	Cyclic siloxane deriv.		Anticaking and skin conditioning agent, antioxidant, flavoring, hypocholesterolemic activities.[28,32]
31.08	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	2.19	Fatty acid ester		Hypocholesterolemic, nematicide, pesticide, antioxidant, antiandrogenic flavor, alphareductase inhibitor, hemolytic, anti-microbial agent.[31-33]
32.39	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	1.1	Fatty acid ester		Hypocholesterolemic, nematicide, antioxidant, flavor, 5-alpha reductase inhibitor, hemolytic and anti-andrigenic.[31,33,34]
34.80	9,12-Octadecadienoic acid (Z, Z)- methylester	$C_{19}H_{34}O_2$	294	13.84	Fatty acid ester		Analgesic, ulcerogenic, anti-inflammatory.[35]
35.70	Octadecanoic acid methylester	$C_{19}H_{38}O_2$	298	2.08	Fatty acid ester		Antiviral, cytotoxic antimicrobial.[36]
36.76	Linoleic acid ester (9,12-Octadecadienoic acid ethylester	$C_{20}H_{36}O_2$	308	10.17	Fatty acid ester		Anti-eczemic, antiacne, antimicrobial, anti- coronary, antihistaminic, 5-alpha reductase inhibitor, anti- androgenic, hepatoprotective, anti-arthritic, nematicide, hypocholesterolemic.[34]
36.95	Oleic acid ester (Ethyl Oleate)	$C_{20}H_{38}O_2$	310	1.13	Fatty acid ester		Antimicrobial.[34]

37.80	Octadecanoic acid ethylester	$C_{20}H_{40}O_2$	312	1.09	Fatty acid ester		antimicrobial, anticancer.[37,38]
44.58	(Z,Z,Z)-9, 12, 15 - Octadecatrienoic acid, 2,3-dihydroxy propylester	$C_{21}H_{36}O_4$	352	10.56	Fatty acid ester		Antioxidant, anti-inflammatory, nematicide, insectifuge, antimicrobial, antihistaminic, antieczemic.[39]
45.15	13-Docosenoic acid methyl ester, (Z)-	$C_{23}H_{44}O_2$	352	1.58	Fatty acid ester		Anti-microbial.[40]
45.73	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	11.38	Phthalic acid diester		Cosmetic, perfumes, softener, plasticizer, dyestuffs, textile.[41]
48.14	Cis-5,8,11-Eicosatrienoic acid trimethylsilylester	$C_{23}H_{42}O_2Si$	378	3.14	Fatty acid silyl ester		No reported activity.
59.48	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	1.01	Fatty acid silyl ester		Antiarthritic, antiinflammatory, antioxidant, antiasthma, diuretic, antimicrobial.[30,31]
61.81	D:B-Friedo-B':A'-neogammacer-5-en-3-ol	$C_{30}H_{50}O$	426	1.21	Pentacyclic triterpenol		Unknown

Morphological assessment of induction of apoptosis

Owing to highest cytotoxic potential, the MCF7 cell line was selected for morphological assessment using fluorescence microscopic method to estimate the viable cells, secondary necrosis and early and late apoptosis. The cells (*n*=200, 24 hr, 48 hr and 72 hr) were arbitrarily and differentially counted, and compared with the untreated ones. Early apoptotic events seen by intervened-AO within fragmented-DNA and bright-green fluorescence. On the other hand, the untreated cells were green and observed as intact-nuclear structure. At 24 hr point, nuclear chromatin condensation and blebbing observed, indicating moderate apoptosis caused by extract. Moreover, in the late apoptotic process, variations including presence of reddish-orange colour because of binding of AO to the denatured-DNA were perceived after 48 hr and 72 hr times. The results of the morphological assessment depicted in Figure 3.

DISCUSSION

It is well known that the biological potential of a plant extract is due to the bioactive constituents present in them. The components identified from the *C. prophetarum* L. extract in GC-MS analysis were also reported to display a variety of biological properties including cytotoxic effect, in several studies. It is noteworthy that the GC-MS analysis conducted in the present investigation primarily identifies volatile and semi-volatile compounds and therefore a range of potentially significant non-volatile biologically active components may not be detected. Although, the present study provides useful insights into the volatile and semi-volatile profiles of *C. prophetarum* L., it does not represent a complete phytochemical profile. Future studies using other advanced chromatographic and spectroscopic techniques such as LC-MS and NMR respectively are planned to explore a more comprehensive phytochemical profile of plant and that may better correlate specific constituents with observed biological activities.

Estragole is one of the constituents detected in this investigation, which is a volatile aromatic monoterpene present in the essential oils of many other plants. Many studies reported that estragole possessed several biological properties including cytotoxic, antimicrobial, anesthetic, anticonvulsant and antioxidant; it is also known for inhibition of Nitrous Oxide (NO) production, COX-2 enzyme inhibition and reduction of iNOS protein expression, thus preventing inflammatory diseases.[23-27] Lashkari *et al.*, (2020)[23] reported dose-dependent cytotoxic effect of estragole against MCF7 cell line in MTT assay. The compounds also considerably

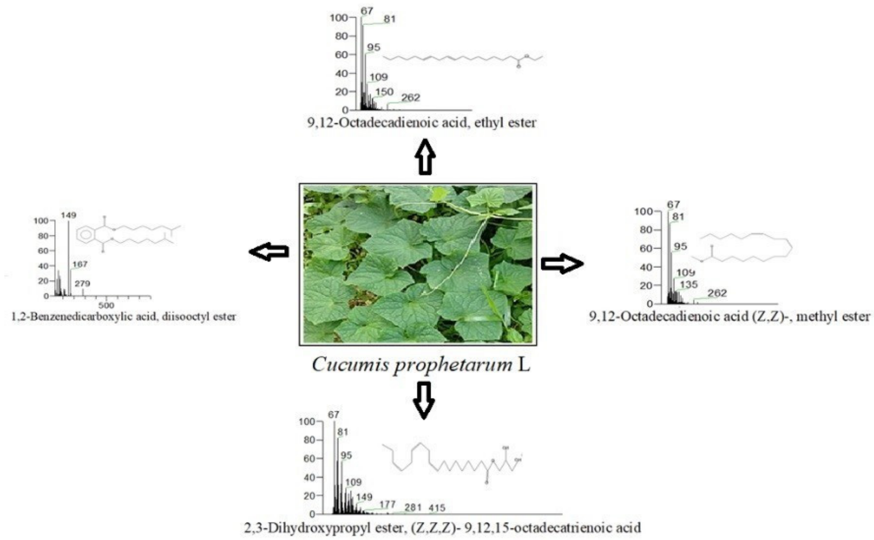


Figure 1: Main biologically active constituents detected in *Cucumis prophetarum* L. fruits extract using GC-MS.

Table 2: Cytotoxiciy (IC$_{50}$±SD µg/mL, 72 hr) and Selectivity Index (SI) results of *C. prophetarum* L. extract against human cancer cell-lines and normal fibroblast.

Celline	IC$_{50}$ (g/mL)	Selectivity Index
MCF7	3.30±0.54	6.20
A2780	16.44±2.55	1.24
HT29	45.83±1.51	0.45
MRC5	20.49±1.22	-

triggered apoptosis in MCF7 cell line and enhanced caspase-3 activity in comparison to the control untreated cells.

Cyclic siloxane derivatives have an important role in cosmetics, and personal care products.[28,29] These compounds were reported to be effective as antiperspirant, deodorant, antifungal, antibacterial, emollient, lubricant, de-foaming agent, anticaking and skin conditioning agents[28,30,31] Cyclohexasiloxane, dodecamethyl- and Cycloheptasiloxane, tetradecamethyl- are the two cyclic siloxane derivatives detected in this investigation. In earlier investigation extracts containing these two siloxane compounds among other constituents were reported to possess anticancer activity[42-44] Hexadecanoic acid methyl ester is frequently identified component in the plant extracts and reported to exhibit hypocholesterolemic, pesticide, nematicide, antioxidant, alpha reductase inhibitor, antiandrogenic, hemolytic and anti-microbial activities.[31,33,34] The compound was also detected in various plant extracts possessing anticancer potential.[43,45,46] Octadecanoic acid methyl ester was reported as antiviral and cytotoxic agent, antimicrobial.[36] Octadecanoic acid ethylester, oleic acid ethylester and 13-docosenoic acid, methyl ester, (Z)- were potent antimicrobial and antifungal compounds.[34]

9, 12-Octadecadienoic acid (Z, Z) - methyl ester exhibited analgesic, ulcerogenic, anti-inflammatory and antifungal properties.[35,47,48] Moreover, the extract containing the compound was found to possess anticancer activity as well.[49] Similarly, the extracts containing octadecanoic acid as one of the fatty acid ester constituents exhibited anticancer and antimicrobial activities.[37,38] 9, 12 - Octadecadienoic acid, ethylester (linoleic acid ethylester) is one of the major fatty acid ester components detected in this analysis and as reported by previous studies, it showed a number of biological properties including antieczemic, antiacne, antimicrobial, anti- coronary, antihistaminic, 5-alpha reductase inhibitor, anti-androgenic, hepatoprotective, anti-arthritic and nematicide, hypocholesterolemic activities.[34] Furthermore, (Z,Z,Z)-9, 12, 15 - octadecatrienoic acid, 2, 3-dihydroxypropylester was reported to display antioxidant, anti-inflammatory, nematicide, insecticide, antimicrobial, antihistaminic and antieczemic activities.[44] Manilal *et al.*, (2009) reported anticancer potential of Red Alga with fatty acid derivatives, mainly 9, 12-octadecadienoic acid (Z,Z) as the main component.[50] 1,2-Benzenedicarboxylic acid diisooctylester present in the extract was used in cosmetic, perfumes, dyestuffs and as a softener and plasticizer, and in textile industries.[41] Fatty acid silyl ester have anntiarthritic, antiinflammatory, antioxidant, antiasthma, diuretic, antimicrobial properties.[30,31]

Although the study of literature indicated that the crude extracts exhibited cytotoxic activity, a direct relationship between the bioactivity displayed by the extracts and the individually identified components could not be established. In the current investigation, the presence of estragole, Cyclohexasiloxane dodecamethyl-, Cycloheptasiloxane tetradecamethyl-, hexadecanoic acid methyl ester, Octadecanoic acid methyl ester, 9, 12-Octadecadienoic acid (Z, Z) - methyl ester and octadecanoic acid ester has been established through GC-MS analysis. The plant extracts containing the above compounds reported to exhibit cytotoxic activities in different previously reported investigations. Since

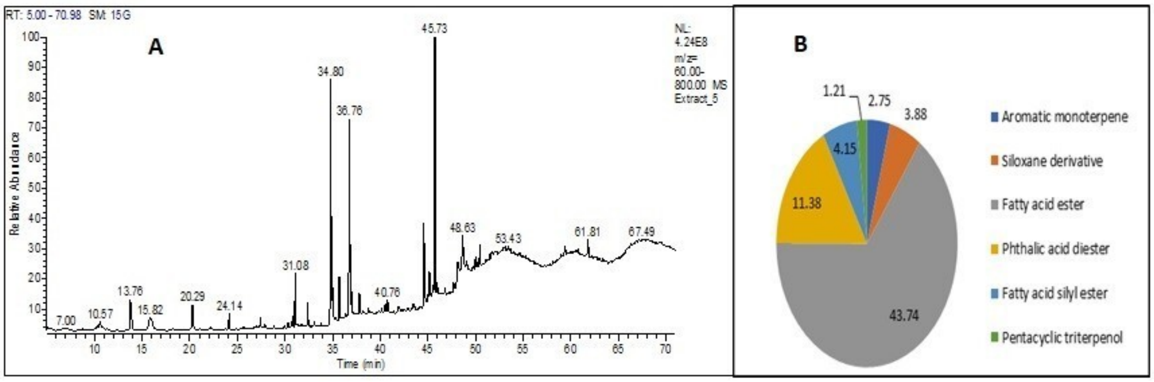


Figure 2: A: GC-MS Chromatogram and B: phytocompounds present in *Cucumis prophetarum* L.

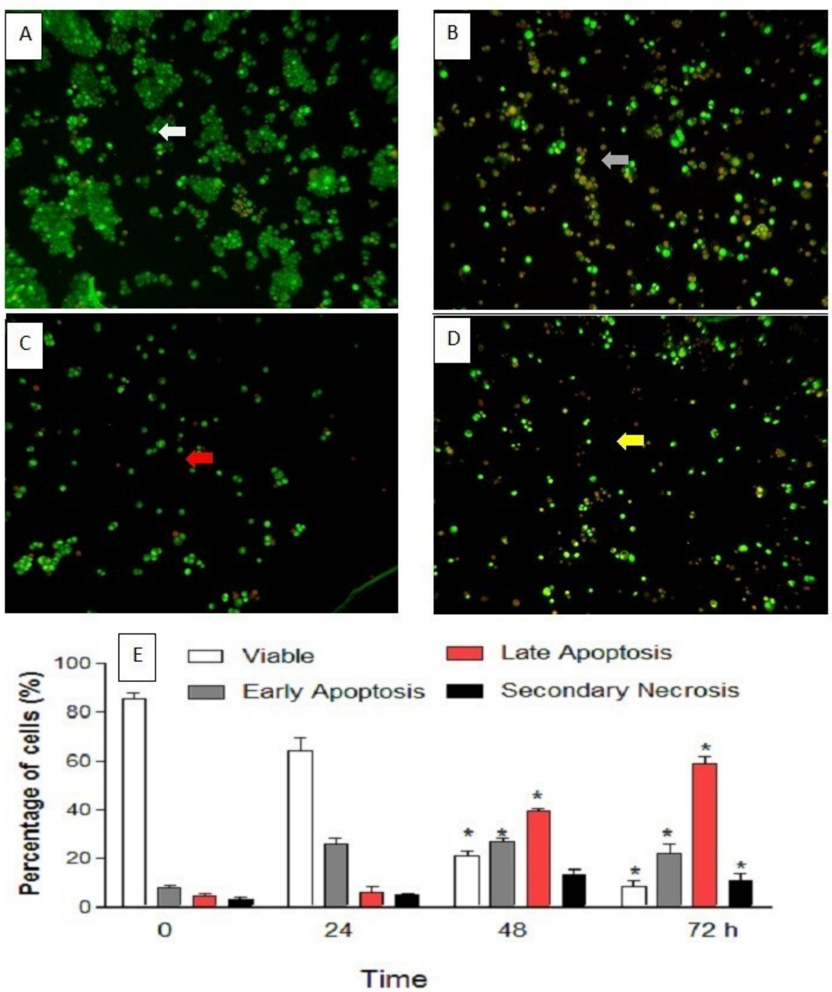


Figure 3: Apoptotic effect of *Cucumis prophetarum* extract against MCF7 cells. A: 0 hr; B: 24 hr; C: 48 hr; D: 72 hr and E: quantitative barchart. White, grey, red and yellow arrows indicate viable cells, early apoptosis, late apoptosis and secondary necrosis respectively.

the activity was evaluated on a complex mixture, synergistic or antagonistic interactions among different components of the extract cannot be ruled out. However, to establish the role of specific compounds, future investigation will involve isolation and purification of individual components followed by evaluating the cytotoxic properties and target-specific assays to explore their mechanism of action. Such investigations will be important to validate the identified compounds as lead molecule for developing anticancer agents.

Cytotoxic activity and morphological assessment of induction of apoptosis

In cancer research Selectivity Index (SI) is an effective tool that help to identify the sample that show promising targeted cytotoxicity. SI is helpful in evaluating that how selectively a test sample kill the target cells (cancer cells) relative to the healthy cells. A greater SI value indicates that the sample has higher selectivity to the target cells than to the healthy cells and hence exhibit greater therapeutic potential. It also helps to estimate the potential side effects, as the sample with low SI may be harmful to the normal cells in addition to its effect against the cancer cells. Apart from its significance in the initial cytotoxicity testing, the SI suffers from certain limitations because the SI is usually determined based on *in vitro* data, which may not fully reflect the complexity of *in vivo* settings including drug distribution, immune related interference and metabolic processes. The SI results may significantly vary across different cancer and normal cell lines, while it does not provide an insight into the mechanism of action of the tested sample.[51,52]

The outcome of the cytotoxic activity screening in this investigation indicated that, the extract displayed excellent selectivity to MCF7 cells compared with MRC5 normal cells with SI value of 6.20, followed by 1.24 for the A2780 cells, while there was no selectivity against HT29 cells. Higher SI value indicates the safety of the extract and low toxicity towards the normal cell lines, which is opposite to its toxicity toward the cancer cell line (MCF7). The results of the morphological assessment indicated that the extract caused morphological changes which were related to time-dependent apoptosis. While doing differential-scoring of the treated cells, significant (p<0.05) difference in cell population was observed.

Overall, the extract selected in this study exhibited significant cytotoxic activity and demonstrated apoptosis as a primary mode of cell death. Mainly we focused on basic apoptosis assessment through morphological changes. Deeper mechanistic approaches including specific apoptotic pathways, mitochondrial potential disruption, ROS generation and cell cycle modulation may provide a more comprehensive understanding of cytotoxic mechanism of the extract.

CONCLUSION

Fruit sample *C. prophetarum* L. was extracted through maceration process in 80% ethanol and phytochemical components of the extract were determined by GC-MS screening. The extract screened for *in vitro* cytotoxic property against MCF7, A2780 and HT29 human cell lines. The outcome of the study indicated that the extract displayed highest cytotoxicity against MCF7 cell line. The extract showed selectivity to cancer cell lines in comparison to normal human cell line MRC5. The extract caused morphological changes which were related to time-dependent apoptosis. The cytotoxic activity of the plant might be due to the presence of various types of constituents detected in the extract, which include fatty acid esters, aromatic monoterpene, cyclic siloxane derivative and fatty acid silyl ester. Several phytocompounds detected in this investigation were previously reported to have anticancer potential along with a number of other properties including antioxidant, antiinflammatory, antimicrobial, 5-alpha reductase inhibitory and antiandrogenic properties. The study will be helpful to rationalize the use of the plant through scientific evidence.

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ABBREVIATIONS

MTT: 3-(4, 5 - Dimethylthiazol - 2 - yl) - 2, 5 - Diphenyltetrazolium Bromide; **GC-MS:** Gas Chromatography Mass Spectrometry; **MCF7:** Human Breast Adenocarcinoma; **A2780:** Human Ovary Adenocarcinoma; **HT29:** Human Colon Adenocarcinoma; **MRC5:** Normal Fetal Lung Fibroblasts, **IC_{50}:** Half-Maximal Inhibitory Concentration; **WCRF:** World Cancer Research Fund; **WHO:** World Health Organization; ***C. prophetarum:*** *Cucumis prophetarum*; **AMU:** Atomic Mass Unit; **EMEM:** Eagle Minimal Essential Medium; **RH:** Relative Humidity; **FBS:** Fetal Bovine Serum; **ATCC:** American Type Culture Collection; **DMSO:** Dimethyl Sulfoxide; **AO:** Acridine Orange; **PI:** Propidium Iodide; **SD:** Standard Deviation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUMMARY

The objective of the present study is to evaluate the phytochemical composition of ethanolic extract of *Cucumis prophetarum* L. and determine its cytotoxic properties. GC-MS analysis was performed to evaluate the phytochemical composition of the extract. The components were identified with the help of built-in libraries such as NIST, MAINLIB, and REPLIB in the instrument database. The extract was evaluated for cytotoxic potential by using MTT assay method. The primary chemical components detected in the plant extract were fatty acid and their esters, aromatic monoterpenes, cyclic siloxane derivatives and pentacyclic triterpenoids. The human cancer cell line cultures including MCF7, A2780, HT29, and Normal Fetal Lung Fibroblasts (MRC5), were used to determine the cytotoxicity and selectivity of the ethanolic extract. The extract displayed appreciable cytotoxic property on the tested cell-lines with a highest action against MCF7 cell line, followed by A2780 and HT29. The results indicated that the plant extract was selective and caused early apoptosis against MCF7 cells compared with MRC5 normal cells with good SI.

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