Original Article

GC-MS analysis of the bioactive phytochemical compounds with anticancer activity in the Capparis cartilaginea fruit extracts

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ABSTRACT

Capparis cartilaginea (*C. cartilaginea*) is a plant with various bioactivities found in the Kingdom of Saudi Arabia. It has the biological and chemical properties of medicinal plants, used traditionally for the treatment of many diseases. Bioactive phytochemicals that are present in medicinal plants are promising alternatives to improve the treatment efficacy of human diseases. In developed countries, prostate cancer (PCa) is the most common cancer in males. Various therapies have been proposed for cancer treatment, many of which use products derived from plants. The active compounds of *C. cartilaginea* were found in nearly all plants of the *Capparidaceae* family, such as alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, phlobatannins, phenolic constituents, glycosides. This study aimed to screen the bioactive phytochemicals compounds and anticancer activity of *C. cartilaginea* fruit extract. Gas chromatography-mass spectrometry (GC-MS) analysis was used to highlight the major components of the *C. cartilaginea* fruit extracts. The GC chromatogram profile determined the existence of six main peaks, and the related components were known to be 2-Furancarboxaldehyde, 5-methyl-; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 5-Hydroxymethylfurfural; Hexadecanoic acid, ethyl ester; 9-Octadecenamide, (Z)-; and 13-Docosenamide, (Z)-. Our research is novel and the results obtained suggested the possible use of the ethanol extract of *C. cartilaginea* fruit as a medicinal plant for preparing an anticancer treatment.

Keywords: *Capparis Cartilaginea,* Phytochemicals, GC-MS, Prostate cancer

Introduction

The history of traditional medicine dates back to the search for botanicals that could treat various illnesses, including cancer [1-3]. Many of the plants used in traditional medicine to treat illnesses contain bioactive compounds that can cure illnesses and can help to prevent disease as well. Additionally, plants have made a significant contribution to the global modern pharmaceutical industry [4]. Our research group has been studying the Capparis cartilaginea (*C. cartilaginea*) fruit extract and has published on the many beneficial aspects of this plant. It has been found to possibly reduce and treat osteoporosis and could alter the levels of the parathyroid hormone (PTH) and 1α,25-Dihydroxyvitamin D3 (25(OH)2D3) hormone [5, 6]. Moreover, it induced morphological alterations in 22RV1 human prostate cancer cells and had a notable impact on cell death and mortality. So, as a preliminary study, we speculated that it could be used as a medicinal plant to treat prostate cancer [7]. In light of the prior research, we learned about, examined, and checked for the presence of the phytochemical compounds that might contribute to the beneficial effects described earlier. As a follow-up to our earlier data, gas chromatography–mass spectrometry (GC-MS) was selected as the method of analysis to screen the phytochemical chemical composition of the *C. cartilaginea* fruit extract and determine which phytochemical components of the extract had a cytotoxic effect on 22RV1 human prostate cancer cells.

C. cartilaginea belongs to the Capparidaceae family [8]. Active compounds such as alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, phlobatannins, phenolic constituents and cardiac glycosides are found in almost all plants of the Capparidaceae family [9]. *C. cartilaginea* has been used

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successfully in traditional medicine to treat a wide range of human illnesses. Additionally, it has applications in medicine, cosmetics, food, and animal nutrition [10]. A Phytochemical screen of the chemical composition of the essential oil in crude extracts fractions of freshly flowered aerial parts of *C. cartilaginea* yielded 0.04 % pale yellowish oil. A complex mixture of compounds was obtained from the GC-MS analysis of the essential oils. The mixture included nitrogen, sulfur-containing compounds, oxygenated sesquiterpenes, and monoterpene hydrocarbons [11]. All extracts of C. cartilaginea contained alkaloids, carbohydrates, protein, coumarin, phytosterols, bitter principles, phenols, and tannins. The main constituents of the essential oil found in the leaves were isopropyl isothiocyanate (69.4%), butane-1-isothiocyanate (26.97%), and isobutyl isothiocyanate (3.26%) [12]. C. cartilaginea was found to contain a variety of secondary metabolites, such as alkaloids, flavonoids, tannins, phenolics, phenylpropanoids, coumarins, glucosinolates, lignans, quinones, terpenes, glycosides, and saponins [11, 13, 14]. Many plants, fruits, vegetables, and leaves contain flavonoids that have potential uses in medicinal chemistry. Flavonoids offer a range of health benefits, including but not limited to antiviral, anti-cancer, antioxidant, and anti-inflammatory effects [15]. *Capparis spinosa L* is also a plant belonging to the Capparidaceae family. The antioxidant compound analysis of Capparis spinosa L seed extracts revealed high levels of total phenolic, flavonoid rutin, and quercetin. The MTT assay showed that in comparison to the hacked cells, *Capparis spinosa L*. seed extracts at a concentration of 1000 g/mL reduced the viability of the SH-SY5Y cancer cell lines (P 0.001), which could be due to the high antioxidant content of the seed extract of *Capparis spinosa L* [16].

Materials and Methods

Plant material

In October 2018, the C. cartilaginea fresh fruit was collected from Wadi Molham, Wadi Abu Al-Haza, and Wadi Al-Quraine in the Tuwaiq Mountains, Kingdom of Saudi Arabia. The plant was authenticated by Dr. Amal Y Aldhebiani, Assistant Professor of Plant Taxonomy, Department of Biology, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

Plant material and preparation of ethanolic extract

The fresh fruit of C. cartilaginea was freeze-dried for 24h at −60 °C under 5 mTorr pressure using a freeze dryer (ilShin BioBase, Gyeonggi-do, South Korea). The dried fruit was then ground into powder. Using the Soxhlet apparatus (Sigma, St. Louis, Missouri, USA) and 70% ethanol for 5–6 h at 70 °C, the powdered plants were extracted. The ethanol extract was evaporated and concentrated at 60 °C under reduced pressure (100 Torr) using a rotary evaporator (Hahnvapor, San Rafael, California, USA). Then, 50 g of the dried fruit powder was used to create 11.5 g of dried extract. The final extract was stored at -80°C for later use [6].

Phytochemical screening

The GC-MS analysis was carried out at the Faculty of Science, Biochemistry Department, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia. GC-MS allowed us to highlight the main constituents of the C. cartilaginea fruit extracts. Initially, a 10 µL volume of C. cartilaginea fruit's ethanolic extract was loaded into a gas chromatograph (7890A) that was linked to an Agilent mass spectrometry detector (5975C) made by Agilent Technologies, located in Santa Clara, California, USA. A split mode injection of 1.0 µL was conducted, with a ratio of 10:1, while maintaining the injector temperature at 280 °C. The mass spectrum source temperature was kept constant at 250 °C. The mobile phase was N2 at a constant flow rate of 0.7 mL/min. Peak identification was accomplished through computer matching of the mass spectra using the National Institute of Standards and Technology (NIST) and the United States Army Criminal Investigation Laboratory (USACIL) library, as well as through direct comparison with published data [17].

Results and Discussion

Phytochemical screening by GC-MS analysis

To identify the bioactive chemical composition of C. cartilaginea fruit extract, the chloroform fraction of the fruit extract was subjected to GC-MS analysis. The chemical compounds are shown in **(Table 1)**, and the corresponding chemical shift peaks of the spectrum are shown in **Figure 1**. The GC chromatogram profile determined the existence of six main peaks, and the related components were identified as follows. The first peak recognized indicated 2-Furancarboxaldehyde, 5-methyl, an organic compound belonging to the aryl-aldehydes class **(Figure 1b)**.The second peak corresponded to 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, a compound belonging to the class of organic compounds known as dihydropyranones **(Figure 1c)**. The third peak indicated 5-Hydroxymethylfurfural. This organic compound belongs to the aryl-aldehydes class **(Figure 2)**.

Table 1. The main bioactive chemical composition of the C. cartilaginea fruit extract from the GC-MS analysis.

RT (min)	Compound Name	MF	MW	Exact Mass	Prob %
3.6953	2-Furancarboxaldehyde, 5-methyl-	C6H6O2	110	110.0367794	83.1
6.053	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	144	144.042258	95.9
7.135	5-ydroxymethylfurfural	C6H6O3	126	126.031694	4.6

15.814	Hexadecanoic acid, ethyl ester	C18H36O2	284	284.27153	75.6
19.151	9-Octadecenamide, (Z)-	C18H35NO	281	281.271864	97.2
22.346	13-Docosenamide, (Z)-	C22H43NO	337	337.334465	91.6

Data are expressed as NO: number, RT: retention time in minutes, MF: Molecular Formula, MW: molecular weight, and Prob %: the probable percentage of the compound present in the extract.

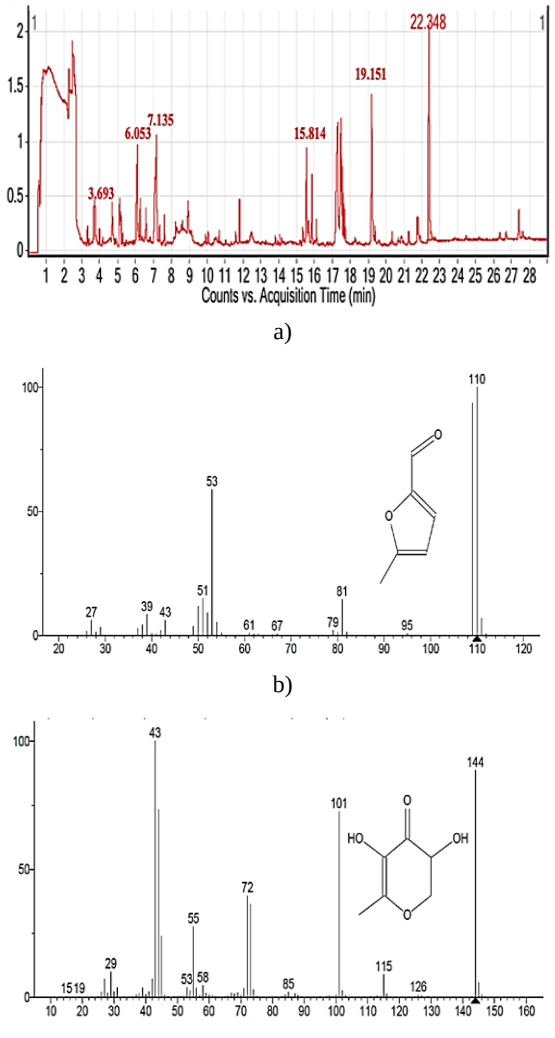

Figure 1. a) GC-MS analysis of the chemical composition of the C. cartilaginea fruit extract. The GC-MS chromatogram and the peak area of the separation of the components are shown; b) The black arrow indicates 2-Furancarboxaldehyde, 5- methyl- in the C. cartilaginea fruit extract; c) The black arrow indicates 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- in the C. cartilaginea fruit extract.

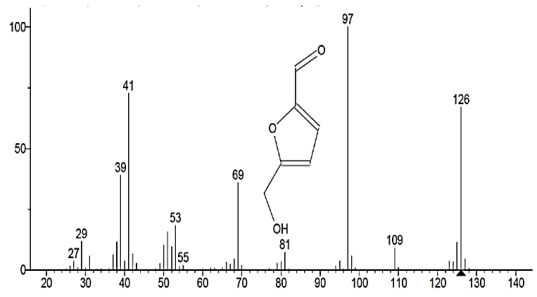

Figure 2. The black arrow indicates 5-Hydroxymethylfurfural in the C. cartilaginea fruit extract.

The fourth peak indicated Hexadecanoic acid, ethyl ester. This compound belongs to the class of organic compounds known as fatty acid esters **(Figure 3)**. The fifth peak is indicated 9-Octadecenamide, (Z)-, which belongs to the class of organic compounds known as fatty amides **(Figure 4)**. The sixth peak indicated 13-Docosenamide, (Z). This compound also belongs to the fatty amides class (HMDB version 4.0; [18] **(Figure 5)**. The six distinctive peaks were identified in the MS fingerprinting analysis.

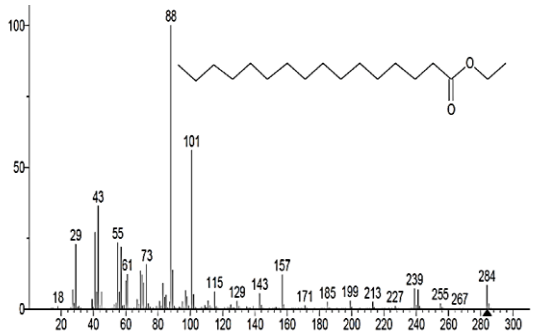

Figure 3. The black arrow indicates Hexadecanoic acid, ethyl ester in the C. cartilaginea fruit extract.

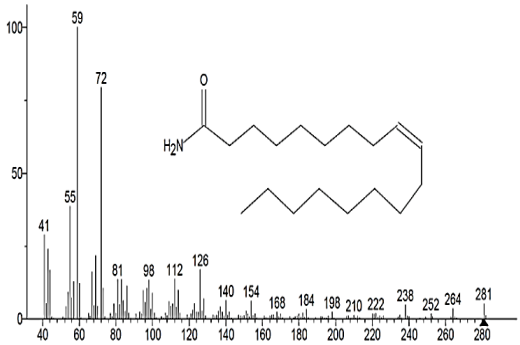

Figure 4. The black arrow indicates 9-Octadecenamide, (Z)- in the C. cartilaginea fruit extract.

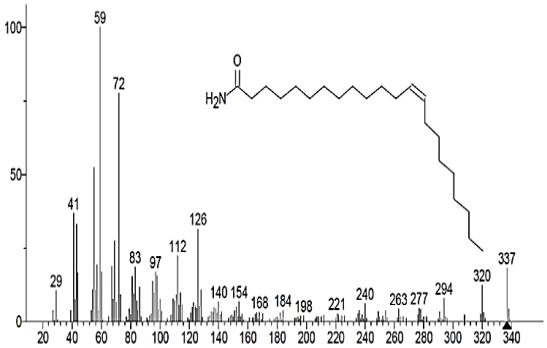

Figure 5. The black arrow indicates 13-Docosenamide, (Z)- in the C. cartilaginea fruit extract.

Natural bioactive substances can both inhibit and prevent all types of cancer. Globally, there is a greater demand for products made from plants for therapeutic and nutraceutical uses as a result of research being conducted to determine which chemical compounds are present in each plant and their various pharmacological activities [19]. The methanolic extract of C. Cartilaginea fruit contains a steroid, carbohydrate, alkaloid, saponins, and flavonoids [9, 11]. The fruit of the C. cartilaginea extract was examined to characterize the phytochemical profiles of the plant to gain a better insight into the optimal consumption levels, which could provide pharmacologically significant concentrations in body fluids and tissues. Our group studied the anticancer activity of the ethanolic extract of C. Cartilaginea fruit along with the apoptotic machinery and evaluated its effects against the 22RV1 human prostate cancer cell line. The C. Cartilaginea fruit extract possessed an effect against human prostate cancer affecting the cell migratory capacity, as the total scratch area widely opened and the number of dead cells increased by ~70% [7].

In this study, the GC-MS screening revealed that the ethanolic extract of C. cartilaginea fruit may contain novel phytochemical compounds that could be isolated and may be used for therapeutic purposes. Each of the compounds had significant therapeutic potential. The GC chromatogram profile determined the existence of six main peaks, and the related components were determined to be 2-Furancarboxaldehyde, 5-methyl-; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; 5-Hydroxymethylfurfural; Hexa-decanoic acid, ethyl ester; 9-Octadecenamide, (Z); and 13-Docosenamide, (Z). **Table 2** shows the primary bioactive chemical composition of the C. cartilaginea fruit extract as determined by GC-MS analysis and its potential relevance to cancer.

Table 2. The main bioactive compounds of interest from the GC-MS analysis are relevant to cancer.

NO	Compound Name	Anticancer Activity	Organism/Cell Line
1	2-Furancarboxaldehyde, 5-methyl-	Yes	Human colon adenocarcinoma-derived (CaCo-2) cell line, male Swiss mice, and male Sprague–Dawley rats [20].
2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Yes	liver cancer (HepG2), breast cancer (MCF7), and normal human embryonic kidney (HEK 293) cell lines [21].
3	5-ydroxymethylfurfural	Yes	(Min/+) mice, prostate cancer (PC-3), breast cancer (MCF-7), and human colon cancer (HT-29) cell lines [22-24].
4	Hexadecanoic acid, ethyl ester	Yes	HeLa and human neuroblastoma (SH-SY5Y) cell lines [25, 26].
5	9-Octadecenamide, (Z)-	Yes	Mammary epithelial carcinoma cell [27].
6	13-Docosenamide, (Z)-	Yes	Mammary epithelial carcinoma cell [28].

5-Hydroxymethylfurfural has been shown to have a wide range of beneficial effects including antioxidant, antiallergic, anti-inflammatory, anti-hypoxic, antisickling, and antihyperuricemic effects [29]. It is a component of natural medicinal honey, known as an aquaporin-1 ion channel blocker. In aquaporin-1-enriched cancer cell lines, aquaporin-1-blocking furan derivatives prevented cancer wound closure and invasion. Therapeutic inhibition is dose-dependent, and the differences among compounds point to a relationship between chemical structure and activity. In vitro, these substances may have an impact on cell invasion, migration, and cytoskeletal dynamics. They were established in high-aquaporin-1-expressing cancer cell lines, colon cancer (HT29) and Aquaporin-1-expressing breast cancer (MDA), and the low-aquaporin-1-expressing SW480 cell line (colorectal cancer cells). The compound known as 5-hydroxymethylfurfural, along with two other molecules that have a similar chemical structure - 5-nitro-2-furoic acid and 5-acetoxymethyl-2-furaldehyde, were shown to have a specific effect of reducing the ability of cells to move in cell lines that have high levels of aquaporin-1 [30]. There was an assumption that 5-hydroxymethylfurfural was cancerogenic; on the other hand, the National Institute of Environmental Health Sciences found no evidence of any oncogenic activity when giving 5-hydroxymethylfurfural at a concentration of 750 mg/kg over 2 years in rats and in mice. Antiproliferative and antioxidative activities were found in 5-hydroxymethylfurfural, suggesting its potential chemoprevention in cancer [31], such as in melanoma cells [32]. Likewise, its antiproliferative and cytotoxic effects against the oral adenosquamous carcinoma cell line (CAL-27) were noticed. Accordingly, it was suggested as a case study by the pharmaceutical industry for the treatment of oral cancer [33-35].

Another bioactive compound present in the C. Cartilaginea fruit extract in the current study was 9-octadecenamide. It is also known to have cancer prevention activity [36]. Similarly, 13-Docosenamide was shown to have crucial neuro-signaling molecules because they act as endogenous bioregulators to control the growth of tumors, circulatory disorders, inflammation, nociception, nervousness, and depression [37].

2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one is found in foods and natural extracts and was noted to have strong antioxidant properties [38]. Similarly, the 2,3-dihydro-3,5 dihydroxy-6-methyl-4H-pyranone in P. Africana stembark extract was shown to have antiproliferative and proapoptotic effects on human prostate cancer cells, due to its capacity to inactivate NF-B (transcription protein) [39]. Flavonoid fractions have also attracted much attention for their antimicrobial activity [40]. Phenolics and flavonoids are known to have anticancer effects and induce apoptosis on various cancer cell lines [41].

Fagbemi *et al*. studied Amarindus indica, a tropical medicinal plant that has been said to have the ability to treat a variety of diseases. There were 37 compounds identified by the GC-MS analysis. Two of the bioactive substances were comparable to our research, including 5-Hydroxymethylfurfural (31.06%) and n-Hexadecanoic acid (1.38%), which have been reported to have anticancer activity, as well as a variety of prophylactic, antibacterial, antifungal, and antitubercular activities [42].

Similar compounds were detected in the methanolic extract fruit of Capparis spinosa L. (in the family Capparidaceae) by GC–MS, such as β-Sitosterol (28.70%), n-Hexadecanoic acid (26.25%), and 9,12,15-Octadecatrienoic acid, (Z, Z, Z). Other low-content molecules were obtained such as Octadecanoic acid (4.63%), 9,12-Octadecadienoic acid (Z, Z) (3.49%), 9 Octadecenamide, (Z) (2.86%), and Eicosane (2.39 %). The results obtained indicate that the fruit of the caper represents a rich source of bioactive compounds [43]. Moreover, Hexadecanoic acid and ethyl ester was attained in the C. spinosa L. essential oils that were analyzed using the GC-MS technique [44]. Similarly, phytoconstituent compounds were notable in the methanolic extract of Capparis spinosa L. roots, leaves, and fruits. GC-MS analysis in the extract of root revealed the existence of a similar compound to that in our study, including 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl [45, 46]. Subsequently, this kind of GC-MS investigation is an initial move towards recognizing the dynamic standards in this medicinal plant, and our research will be useful for further point-by-point study. The results obtained indicate that Capparis is a rich source of bioactive compounds. However, the diversity in the chromatogram profile may be due to different species of the Capparidaceae family and different geographical regions of the plant, as well as the part used from the plant, the extraction method, and the nature of the solvent used, in addition to the GC-MS conditions.

Conclusion

Our previous findings concluded that the C. cartilaginea fruit extract may have medicinal properties against prostate cancer 22RV1 cell line affecting cell migration. According to the results of the current study, C. Cartilaginea's fruit extract contains a variety of phytochemical compounds from different chemical classes. Six primary bioactive compounds were discovered through GC-MS analysis; each one has a significant pharmaceutical and anticancer effect, according to published data. It is worth noting that while some compounds have been found to have anti-cancer properties, this does not necessarily mean that they are effective treatments for prostate cancer in humans. Further research, including clinical trials, is needed to fully understand the potential effects of these phytochemicals on prostate cancer. This study provides a sound theoretical and experimental basis for further research on the development of new prostate-cancer drugs from *C. Cartilaginea* fruit extract.

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Conflict of interest: None

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Ethics statement: None

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