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ORIGINAL ARTICLE

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Cytotoxic Activity of *Capparis cartilaginea* Leaf Extract against Human Hepatocellular Carcinoma Cell Line

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ABSTRACT

Background: *Capparis cartilaginea* is a medicinal plant traditionally used in Yemen for cancer treatment. Its bioactive compounds exhibit antioxidant, anti-inflammatory, antidiabetic, antibacterial, and cytotoxic activities. Liver cancer is the third cause of cancer-related mortality globally, with hepatocellular carcinoma (HCC) accounting for about 75–90% of cases. However, scientific evidence regarding the cytotoxic activity of *C. cartilaginea* leaf extract against HCC remains limited. This study aimed to evaluate the in vitro cytotoxic activity of a hydromethanolic extract of *C. cartilaginea* leaves against the human HCC cell line (HepG2).

Methods: *C. cartilaginea* leaves were collected, air-dried in shade at room temperature, and extracted using 70% hydromethanolic solvent. The obtained extract was then evaporated and freeze-dried, and its cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on HepG2 cells. Cells were exposed to different concentrations of the extract for 24 hrs. Cell viability was then determined spectrophotometrically. Vinblastine sulfate was used as a reference cytotoxic drug. Dose-response curves were used to determine the half-maximal inhibitory concentration (IC_{50}) of the extract.

Results: The hydromethanolic leaf extract of *C. cartilaginea* exhibited a weak cytotoxic activity against HepG2 cells, with an IC_{50} value of 167.81±6.23 µg/mL compared with the standard cytotoxic drug vinblastine sulfate that showed an IC_{50} value of 3.04±0.22 µg/mL.

Conclusion: *C. cartilaginea* leaf extract exhibits a weak cytotoxic activity against the human HCC cell line (HepG2).

Keywords: *Capparis cartilaginea* ▪ Hepatocellular carcinoma ▪ HepG2 ▪ Cytotoxic activity ▪ Anticancer agent

1. Introduction

Capparis (caper), a genus of the family Capparaceae, has been widely studied phytochemically and pharmacologically. It comprises 140 species largely distributed in the Mediterranean basin, the Middle East, Southwest Asia, Northern Africa, and tropical and subtropical zones.[1,2] It is found as shrubs and lianas that can withstand natural adversity. About 13 *Capparis* species have been identified for their therapeutical and nutritional properties, including *C. spinosa*, *C. brevispina*, *C. decidua*, *C. grandiflora*, *C. tomentosa*, *C. zeylanica*, *C. sepiaria*, and *C. cartilaginea*. Four species have been reported in Yemen: *C. cartilaginea*, *C. spinosa*, *C. tomentosa*, and *C. decidua*.[3, 4]

C. cartilaginea grows in stony and rocky soils.[5] In Yemen, it is called lusaf, rusaf, khasruf, or shaflah. Various parts of *C. cartilaginea* have been used as a traditional medicine in conditions like rheumatism,

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gout, paralysis, earache, headache, snakebites, intestinal worms, diabetes, asthma, allergies, and tumors.[6] Its leaf extract contains alkaloids, saponins, diterpenes, flavonoids, phenols, and tannins. These bioactive constituents have antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, antipeptic, and antibacterial activities.[4, 6–9]

C. cartilaginea has been traditionally used as an anticancer, and several studies have investigated this activity using different plant parts, solvents, and methods. Table 1 summarizes some published experiments that used the MTT assay to evaluate *C. cartilaginea* cytotoxic activity. Most of these investigations employed the MTT assay to evaluate cytotoxic activity. However, findings across studies have been inconsistent, with reported half-maximal inhibitory concentration (IC_{50}) values ranging from significant cytotoxic activity to complete inactivity, depending on the plant part, extraction method, and tested cell line.

Table 1: Published studies evaluating the cytotoxic activity of *C. cartilaginea* using the MTT assay

Part	Solvent	Cell line(s)	Finding	Ref.
Leaves	Methanol	Lung (A-427), urinary bladder (5637), and human breast adenocarcinoma (MCF-7)	No cytotoxic activity, with IC_{50} >50 µg/mL	[10]
Flowering parts	Methanol	Human breast adenocarcinoma (MCF-7), human hepatocellular carcinoma (HepG2), mouse fibrosarcoma (WEHI-164), non-small cell lung carcinoma (A-549), and Madin-Darby bovine kidney (MDBK)	No cytotoxic activity, with IC_{50} >50 µg/mL	[11]
Twigs, leaves, stem	Methanol	Human lung cancer cell line (A-549), human breast adenocarcinoma (MCF-7), and human colon adenocarcinoma (HCT-116)	Twigs showed the highest cytotoxic activity against A-549 cells (IC_{50} = 57.5 µg/mL), followed by stem extract (IC_{50} = 240 µg/mL)	[4]
Leaves	Methanol	African green monkey kidney cells (Vero), laryngeal cancer (HEp-2)	Moderate cytotoxic activity: IC_{50} ≈ 182.15 µg/mL (Vero) and 72.16 µg/mL (HEp-2)	[12]
Fruits	Ethanol	Human prostate cancer (22RV1)	Significant cytotoxic activity with IC_{50} <20 µg/mL after 72 h treatment	[13]
Leaves	Ethanol	Breast carcinoma cells (MCF-7), colon carcinoma cells (HCT-116), and human muscle rhabdomyosarcoma (RD)	Significant cytotoxic activity against HCT-116, MCF-7, and RD cell lines with IC_{50} values of 59.58, 39.14, and 102 µg/mL, respectively	[6]

Globally, liver cancer represents the third most common cause of cancer-related mortality overall and the second cause among men. Its incidence is increasing globally and is expected to reach over one million cases by 2025. The highest number of cases occur in Asia and Africa, and 75–90% of liver cancer cases are hepatocellular carcinoma (HCC).[14–17] According to the Global Cancer Observatory (GLOBOCAN) estimates, there were 905,677 new cases and 830,180 deaths in 2020 attributed to HCC, with incidence and mortality in males being two to three times higher than in females.[18]

Chronic infections with hepatitis B and C viruses are the primary and secondary causes of HCC in the Middle East and North Africa (MENA) region.[15] Alcohol consumption, tobacco smoking, aflatoxin exposure, lifestyle-related factors, and metabolic disorders, such as non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), also contribute to the pathophysiology of HCC.[15]

In Yemen, a mild but non-statistically significant increase in liver cancer incidence and mortality rates has been reported.[16] Because chronic hepatitis B virus infection is prevalent among approximately 5% of the general population, liver cancer represents a major public health concern in the country.[16] Although *C. cartilaginea* is used traditionally in Yemen to treat many types of cancer, no prior study investigated the cytotoxic activity of its leaf extract against HepG2 cell line of HCC. Therefore, this study aimed to evaluate the potential in vitro cytotoxic activity of the hydromethanolic extract of *C. cartilaginea* leaves against the HepG2 cell line.

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2. Methods

2.1. Plant identification and preparation of leaf extract

Fresh leaves of *C. cartilaginea* were collected in August 2021 from the Al-Qabbaitah Mountains, Lahj Governorate, Yemen. A voucher specimen (BHSS 709) of the taxonomically identified plant was deposited in the Herbarium of the Faculty of Biological Sciences, Sana'a University.

The leaves were ground into a coarse powder after being washed with tap water and allowed to air dry at room temperature in the shade. One hundred grams of the powder were macerated in one liter of 70% methanol solvent (v/v) for 72 hrs with frequent agitation.[19] Whatman® No. 1 paper was used to filter the extract. Then, a rotary evaporator (Rotavapor®, BÜCHI Labortechnik AG, Switzerland) was used to evaporate the filtrate at 45°C, and the filtrate was finally dried using a freeze dryer (Labconco®, Labconco Corporation, Kansas City, Missouri, USA) under reduced pressure and a temperature below -80°C.

2.2. Cell line propagation

RPMI-1640 medium (Capricorn Scientific GmbH, Hessen, Germany) was used to cultivate HepG2 cells. Inactivated fetal calf serum (10%) and gentamycin (50 µg/mL) were added to supplement the medium. The cells were kept at 37°C in a humidified atmosphere with 5% CO_2.

2.3. Cytotoxic activity assessment

Cytotoxic activity of *C. cartilaginea* leaf extract was assessed at the Regional Center for Mycology and Biotechnology (RCMB) in Al-Azhar University, Egypt. The 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay was used to determine the IC_{50} of the extract on the intact HepG2 cells.[20] Vinblastine sulfate was used as a standard drug against the HepG2 cell line.

In 96-well tissue culture plates (Corning Incorporated, New York, USA), HepG2 cells were suspended in RPMI-1640 medium and dispensed at a concentration of 5 x 10^4 cells per well. Plates were then incubated for 24 hrs. *C. cartilaginea* leaf extract was added into the plates using ten graduated concentrations (three replicates), while six wells served as controls where media or 0.5% DMSO (HiMedia Laboratories Private Limited, Mumbai, India) were added. Each plate was then incubated for 24 hrs.

To assess cell viability, the media in wells were withdrawn and replaced with 100 µl of fresh RPMI 1640 medium devoid of phenol red. Then, 10 µl of the MTT stock solution (12 mM) were added to each well, including the control wells. After that, the plate was incubated for 4 hrs at 37°C with a 5% CO_2 atmosphere. After incubation, 85 µL of the medium in each well was replaced with 50 µL of DMSO, thoroughly mixed, and incubated for 10 min at 37°C.

A microplate reader (Sunrise, TECAN US, Inc., USA) was used to measure the absorbance of the mixture at 590 nm to determine the number of viable cells, and the viability percentage of cells was calculated. A dose–response curve was generated by plotting cell survival against extract concentrations for HepG2 cells treated *with C. cartilaginea*, and the IC_{50} was estimated from the curve.

2.4. Statistical analysis

Data were analyzed using GraphPad Prism software, version 8.0 (GraphPad Software, Inc., San Diego, USA). The results were expressed as mean ± standard deviation (SD). Cell viability and growth inhibition percentages were calculated compared to untreated control wells. Dose-response curves were generated by plotting the percentage of viable cells against the extract concentration. The IC_{50} values were determined by non-linear regression analysis.

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3. Results

3.1. Cytotoxic activity of *C. cartilaginea* leaf extract against the HepG2 cell line

Figure 1 shows that the IC_{50} of *C. cartilaginea* leaf extract against the HepG2 cell line was 167.81±6.23 µg/mL.

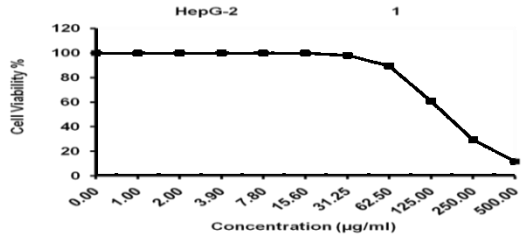

Figure 1: Survival curve of the HepG2 cell line after treatment with different concentrations of *C. cartilaginea* leaf extract

Table 2 shows the percentage viability and inhibition of HepG2 cells following treatment with different concentrations of *C. cartilaginea* leaf extract.

Table 2: Percentage viability and inhibition of the HepG2 cell line in different *C. cartilaginea* leaf extract concentrations

Conc. (µg/mL)	Cell viability (%)	Growth inhibition (%)	SD (±)
500	11.78	88.22	1.46
250	29.43	70.57	2.19
125	60.71	39.29	2.35
62.5	89.42	10.58	1.06
31.25	98.15	1.85	0.39
15.6	100	0.00	NA
7.8	100	0.00	NA
3.9	100	0.00	NA
2	100	0.00	NA
1	100	0.00	NA
0	100	0.00	NA

SD, standard deviation; NA, not applicable.

3.2. Cytotoxic activity of vinblastine sulfate against the HepG2 cell line

Figure 2 shows that the IC_{50} of vinblastine sulfate against the HepG2 cell line was 3.04±0.22 µg/mL.

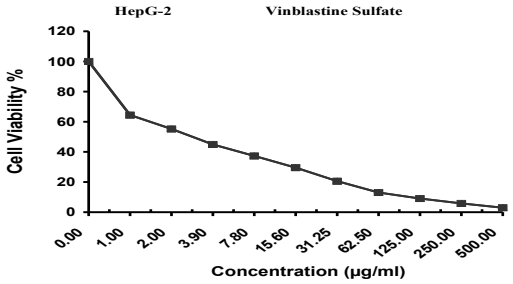

Figure 2: Survival curve of the HepG2 cell line after treatment with different concentrations of vinblastine sulfate

Table 3 shows the percentage viability and inhibition of HepG2 cells following treatment with different concentrations of vinblastine sulfate.

Table 3: Percentage viability and inhibition of HepG2 cell line in different vinblastine sulfate concentrations

Conc. (µg/mL)	Cell viability (%)	Growth inhibition (%)	SD (±)
500	2.94	97.06	0.28
250	5.84	94.16	0.63
125	9.06	90.94	0.57
62.5	13.04	86.96	0.79
31.25	20.61	79.39	1.14
15.6	29.55	70.45	2.04
7.8	37.31	62.69	1.30
3.9	44.91	55.09	1.18
2	55.24	44.76	2.08
1	64.49	35.51	1.25
0	100	0	0.0

SD, standard deviation; NA, not applicable.

4. Discussion

This study showed that *C. cartilaginea* leaf extract had a weak cytotoxic activity against HepG2 cells, with an IC_{50} value of 167.81 µg/mL, compared to the markedly lower IC_{50} of 3.04 µg/mL for vinblastine sulfate as a reference cytotoxic agent. This result is consistent with a previous study that showed that the methanolic extract of *C. cartilaginea* flowering parts has no cytotoxic activity against HepG2 cells, with IC_{50} of >50 µg/mL.[11] The study also reported no cytotoxic activity of that extract against MCF-7, WEHI-164, A-549, and MDBK cell lines, with IC_{50} values similarly exceeding 50 µg/mL.[11] Mothana et al.[10] reported similar findings for the methanolic extract of *C. cartilaginea* leaves, which exhibited no cytotoxic activity against A-427, 5637, and MCF-7 cell lines, with IC_{50} values of >50 µg/mL.

In contrast, Sonbol et al.[13] found that the ethanolic extract of *C. cartilaginea* fruits has a significant cytotoxic activity with an IC_{50} value of <20 µg/mL after 72 hrs of treatment against 22RV1 cell line. On the other hand, Latif et al.[12] reported that the leaf methanolic extract had a moderately cytotoxic effect, with an IC_{50} value of about 182.15 µg/mL against the Vero cell line and about 72.16 µg/mL

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against the Hep2 cell line. Moharram et al.[4] also reported that the methanolic extract of *C. cartilaginea* twigs had the highest cytotoxic activity against the A-549 cell line, with an IC_{50} value of 57.5 µg/mL, followed by the stem extract with an IC_{50} value of 240 µg/mL. Similarly, Thamer et al.[6] found that the ethanolic extract of *C. cartilaginea* leaves had a significant cytotoxic activity with IC_{50} values of about 59.58, 39.14, and 102 µg/mL against HCT-116, MCF-7, and RD cell lines, respectively.

The observed variation in the cytotoxic activity of extracts derived from various parts of *C. cartilaginea* against various tumor cell lines may be attributed to differences in the geographical origin of the plant material, which can influence the composition and concentration of bioactive constituents and their biological effects. On the other hand, such differences may reflect selective in vitro cytotoxic activity of plant extracts toward specific cancer cell lines. Furthermore, some cytotoxic mechanisms may not be adequately detected by the MTT assay, which could result in an underestimation of anticancer activity.

5. Conclusion

Despite the widespread traditional use of *C. cartilaginea* leaves in cancer treatment, the hydromethanolic extract of its leaves exhibits a weak cytotoxic activity against the HepG2 cell line compared with the standard cytotoxic drug and prior reports on other plant parts. These findings suggest its limited potential as a direct anticancer agent. Further studies on other parts of the plant, different solvents, and purified compounds are recommended.

Conflict of Interest

The authors declare no conflict of interest associated with this article.

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