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Evaluating the Antioxidant Capacity, Phenolic Contents, and Anticancer Potential of *Caralluma europaea* Extracts

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Abstract

Background: *Caralluma europaea* (*C. europaea*) is a genus of shrubs in the Apocynaceae family, endemic to the Mediterranean region. Assessing the anticancer properties, total phenolic and flavonoid content, and antioxidant activity of different *C. europaea* extracts were the aim of this study.

Methods: To determine antioxidant activity, we used three free radical scavenging assays: DPPH (2,2-Diphenyl-1-picrylhydrazyl), hydrogen peroxide (H_2O_2), and nitric oxide (NO) assays. Total phenolic and flavonoid contents were measured using colorimetric methods. The anti-important activity was evaluated by MTT assay.

Results: Our findings showed that the efficacy of the extract in MCF7 cells decreased in the system containing chloroform, methanol, ethyl acetate, and water. The strength of the extract for U87 cells decreased according to methanolic, chloroform and water, where ethyl acetate was the strongest. The highest total phenolic and flavonoid content was found in methanol extract. All extracts showed a high scavenging activity of DPPH. Methanol extract showed the highest antioxidant activity in the NO assay of the solution, followed by ethyl acetate, chloroform, and aqueous extract. In the H_2O_2-based antioxidant activity assay, the methanol extract again showed the highest activity, followed by ethyl acetate, chloroform, and aqueous extracts.

Conclusions: Extracts of *C. europaea* have shown great potential as natural antioxidants and anticancer agents in this study, suggesting that they deserve further investigation into the food and pharmaceutical fields.

Introduction

Medicinal herbs have a significant role in improving human health and are widely utilized to treat a wide range of conditions, such as diabetes, cancer, and COVID-19 [1]. Medicinal plants and their derivatives provide a plethora of naturally occurring treatments with therapeutic qualities. In fact, according to the World Health Organization [2], 80% of people on the earth use various plant fractions and their active compounds as traditional remedies.

Secondary metabolites are a broad class of chemical substances produced by metabolic pathways originating from primary metabolism [3]. Although secondary metabolites are not required for an organism to survive, grow, develop, or reproduce, they frequently play a crucial role in defensive mechanisms against herbivores and other interspecies threats [4].

Plant-derived bioactive secondary metabolites, especially phenolic compounds, have attracted a lot of attention in the field of biology lately. The various groups of phytochemicals that these substances contain—such as flavonoids and phenolics, alkaloids and Saponins, steroids, and glycosides—are responsible for their bioactivities [3]. Numerous biological characteristics, such as antibacterial, antioxidant, anticancer, anti-inflammatory, and cardiovascular protective effects, are displayed by these substances. Phenolic chemicals are plant components whose redox characteristics are essential to their antioxidant function. The scavenging of free radicals was made easier by the hydroxyl groups found in plant extracts [5]. A broad class of secondary plant compounds known as polyphenols has two or more phenolic rings in its chemical structure [6]. Polyphenols not only possess antioxidant properties but may also contribute to human health through anti-inflammatory, anti-allergic, antihypertensive, neuroprotective, analgesic, antiviral, and antibacterial activities [7].

Antioxidants are essential for protecting the body from free radical-induced oxidative stress. Many antioxidants found in fruits, vegetables, and teas are derived from these foods and can be used by the body to counteract free radicals [8]. Antioxidant-rich compounds have the ability to scavenge free radicals, shielding cells from oxidative damage. Antioxidants are believed to be prevalent in several plant-based foods [9]. Free radicals, which include hydrogen peroxide, hydroxyl radicals, and superoxide radicals can have an adverse effect on almost all biological molecules, which include DNA, proteins, and carbohydrates. [10].

Pharmaceuticals derived from plants are an appealing option for treating cancer [11]. Because plants have a variety of anti-cancer molecules, these medications have shown promise in the treatment of several forms of cancer. Furthermore, as they are natural compounds, plant-based substances are frequently non-toxic [12]. Many studies have been done on the impact of different medicinal herbs on cancer in addition to chemotherapy. The National Cancer Institute (NCI) has looked into the possible anticancer activities of about 35,000 plant species [13]. The ability of more than 3,000 plants to target apoptotic pathways and function as potent anti-cancer medicines has been evaluated, demonstrating the plants' value in the battle against cancer [14]. These substances have antioxidant properties, inhibit carcinogens, stop cell division, stop the cell cycle, induce apoptosis and differentiation, stop angiogenesis, and overcome multidrug resistance, among other strategies to combat cancer [13].

The medicinal plant *C. europaea* is a member of the Order Gentianales, Subfamily Asclepiadoideae, and Family Apocynaceae. According to Bushnak et al. [15] it is distributed through Algeria, Tunisia, Egypt, Jordan, Spain, Italy, and Morocco. *C. europaea* is a perennial succulent that grows gradually and forms mats. Its stems are flat to concave, four-angled (tetragonal), and can climb or sprawl up to 25 cm in height. Their color might be either bright green, dark green, or dark blue-green [16].

According to Dra et al. [17], this medicinal plant's aerial parts are frequently used as a powder or drink to treat goiter, diabetes, and cysts. The anticancer, antioxidant, antibacterial, and anti-inflammatory qualities of *C. europaea* have been confirmed by numerous scientific investigations [18]. Furthermore, *C. europaea* stems are used orally along with milk or water as a meal to treat diabetes [16]. Research has indicated that various anti-tumor chemicals are present in *C. europaea*. These substances consist of Saponins, triterpenoids, and flavonoids. It has been demonstrated that these substances lessen and limit the growth of cancer cells. Furthermore, it has been discovered that *C. europaea* lowers the expression of several genes linked to the development of cancer. Additionally, it has been discovered that this plant inhibits the growth of new blood vessels, which is necessary for the metastasis of cancer cells [19], exhibits anti-inflammatory properties, lowers oxidative stress, and has immunomodulatory effects [20].

The present study aimed to assess the total phenolic and flavonoid contents, in vitro antioxidant activity, and antiproliferative activities of the *C. europaea* extract.

Methods

Plant materials and extraction

From Wadi Almujib in the Al-Karak region (Elevation: 789.471, Latitude: 31° 44' 5.988'' N, Longitude: 35° 81' 7489'' E), the aerial sections of *C. europaea* were collected in May 2022. The fresh samples were washed with tap water to remove any dirt and then allowed to

dry at room temperature before being ground into a fine powder. The Soxhlet extraction apparatus was used for 48 hours using a variety of solvents (methanol, chloroform, ethyl acetate, and distilled water) to get the appropriate amount of powdered plant components. The solvent was completely evaporated at low pressure in a rotating vacuum evaporator, producing crude extracts that were collected and stored at -20 °C for further testing.

Scavenging radical Activity by the DPPH method

The ability to neutralize free radicals was assessed by performing a DPPH test. Five milliliters of a 0.04 % radical DPPH solution were added to the extract-containing solutions. After 30 minutes in the dark, the mixture was vortexed. The optical density, also known as OD, was determined at 517 nm [21]. Ascorbic acid was utilized as a positive control.

The activity of DPPH scavenging (%) was determined using the subsequent formula: Scavenging activity (%) = [(AC - AS) / AC] x 100%.

Where AC stands for the absorbance of the control and AS reflects the absorbance of the sample. In addition, the IC_{50} values were calculated using the Dose-Response-Inhibition method, and a dose-response curve was generated in Graph Pad Prism Software Ver. 8.0.

Scavenging Activity by Nitric oxide method

A mixture of different concentrations of *C. europaea* extracts dissolved in dimethyl sulfoxide (DMSO) and sodium nitroprusside (5 m M) in phosphate-buffered saline was added. The mixture was then incubated at 25 °C for 150 minutes [22]. The same amount was used in a control experiment, but no test extracts were used. 0.5 ml of each of the incubated solutions was mixed with 0.5 ml of the Griess reagent (1% sulfanilamide, 0.1% N-(1-Naphthyl)ethylenediamine dihydrochloride, and 2% H_3PO_4) at regular intervals. The absorbance at 546 nm indicates the presence of the chromophore produced by the interaction between nitrite and Griess reagent. The amount of nitrite ions in the test liquids was determined using potassium nitrite as a reference.

Scavenging activity (%) = [(AC - AS) / AC] x 100%]. Where AC is the absorbance of the control and AS is the absorbance of the sample.

Scavenging Activity by Hydrogen Peroxide Method

The free radical scavenging capacity of *C. europaea* herbal extracts against hydrogen peroxide was assessed using a technique described by Al-Awaida et al. [23]. Various extract volumes (pH 7.4) were mixed with 3.4 ml of 0.1 M solution of phosphate buffer and 600µl of the hydrogen peroxide solution. Ascorbic acid was used as a standard compound. The amount of H_2O_2 in the product mixtures was measured by measuring their absorbance at 230 nm after 10 minutes of incubation [24].

The following equation was used to calculate the percentage of inhibitory effects (%): Scavenging activity (%)= [(AC - AS) / AC] x 100%]. Where AC is the absorbance of the control and AS is the absorbance of the sample.

Determination of total phenolic content

The TPC for *C. europaea* was calculated using the Folin-Ciocalteu technique with a few minor modifications [25]. Following three to five minutes, a mixture of 100 µl plant extract, 475 µl 5% sodium carbonate solution, and 475µl 50% Folin- Ciocalteu reagent was added. The combined solution was left in the dark and at room temperature for one hour. A UV-visible spectrophotometer was used to measure the resultant solution's absorbance at 724 nm. Total phenols (TPs) were expressed as Gallic acid equivalents per gram of dried plant extract (g/g) using a standard curve.

Determination of total flavonoid content

The total flavonoids of *C. europaea* were evaluated using the aluminum chloride assay [26]. An aliquot of extracts (0.5 ml) with been placed in separate test tubes was mixed with 2 ml of distilled water and 0.15 ml of sodium nitrite (5% $NaNO_2$, w/w). After that, the mixture was left to stand for six minutes. After that, 0.15 ml of 10% aluminum trichloride ($AlCl_3$) was added, and six minutes were given for the mixture to settle. After that, 2 ml of sodium hydroxide (NaOH, 4% w/v) was added, and distilled water was used to eventually bring the mixture's volume to 5 ml. At 510 nm, the absorbance was measured after a 15-minute incubation period. As a blank, distilled water was used. The rutin standard curve was utilized to ascertain the overall flavonoid concentration in every extract. The results were given in terms of milligrams (mg QE) of quercetin equivalent for each gram of dry extract.

The growth conditions of cell culture

The MCF7 cell line was generously donated by the American University of Madaba Molecular Biology Laboratory. It was originally acquired from the American Type Culture Collection (ATCC; USA). The MCF7 cells were maintained as adherent monolayer cultures in RPMI 1640 medium (Euroclone, MI, Italy). The medium was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Euroclone, MI, Italy), 100 u/mL, and 100 g/mL of penicillin and streptomycin (Euroclone, MI, Italy). A 25 cm^2 or 75 cm^2 attached-cap culture flask with a venting system was used to cultivate each type of cell (Corning Inc., New York). The cells were then cultured in a tissue culture incubator using the ESCO Cell Culture CO_2 Incubator

(Esco Technologies, Inc., USA) at 37 °C in a 90% humidified environment with 5% CO_2. The disposable, commercially pre-sterilized equipment used in cell culture was paired with cell culture-grade fluids. All cell culture operations were performed in a biological safety cabinet manufactured by Heil-Force in China. All equipment was cleaned with 70% ethanol before being used in the biological safety cabinet (Promega, USA).

Cell harvesting (passaging)

When adherent cells on a flask surface are examined using inverted light microscopy at 80% confluence (Optika, Italy), The cells that were stuck to the flask surface were extracted by aspirating the media and rinsing them with laboratory-made phosphate buffer saline (PBS). The cells were then freed from the incubation medium and treated with 0.25% trypsin (Euroclone, MI, Italy). Following a 5-7-minute incubation period at 37 °C, the flasks were examined using an inverted light microscope (Optika, Italy).

Cell Viability and Counting

To find out how many viable cells were present, an aliquot of detached cells was stained with Trypan blue solution (Sigma-Aldrich, UK) at a 1:1 ratio to the cell suspension. Under an inverted microscope, cells were counted using a counting chamber for microscopes. Trypan blue cannot pass through the membrane of living cells, hence the brighter cells represented viability while the darker blue cells represented death.

Cell cytotoxicity assay MTT

Plant extracts have been evaluated for their capacity to inhibit cell proliferation in MCF7 and fibroblast cells using the Cell Titer Non-Radioactive Cell Proliferation Assay Kit® (Promega, USA) based on the manufacturer's instructions. The reduction of the yellow tetrazole 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) in the mitochondria of cells that are alive produces the purple formazan used in this colorimetric experiment. Following being placed at a count of 10^3 cells per well in 96-well plates, the cells were given 24 hours to multiply. Then, horizontal 100 µL/well drug of dilutions that ranged from 400 to 1.56 g/mL were added. Each plate had a positive control of 1% DMSO and control of cells in plain media. The concentrations were added in triplicate. Subsequently, the cells were cultured for 72 hours at 37 degrees Celsius. Following a four-hour incubation period at 37 °C, 100 µL of stop solution was added to each well, and the media was removed from the wells and replaced with fresh media (100 L per well) at the conclusion of the incubation period. The absorbance was determined at 570 nm using a plate with 96-wells reader (Multiscan FC microplate photometer; Thermo Scientific).

Statistical analysis

The IC_{50} values were calculated statistically using Graph Pad Prism, eighth edition (Graph Pad Software, Inc., San Diego, CA, USA). The data was evaluated via the student's t-test, one-way analysis of variance (ANOVA), and Tukey's multiple comparison test, with a level of significance of $P < 0.05$. The results are presented as the mean SEM. The numbers indicate the means of three separate trials.

Results

The effects of Doxorubicin and different extracts of C. *europaea* on various cancer cell lines

Table 1 displayed the effects of doxorubicin and different extracts of C. *europaea* on three distinct cell lines: human skin-fibroblast cells (HDFa), brain cancer cells (U87), and breast cancer cells (MCF 7). According to the IC_{50} values, brain cancer cells are significantly more sensitive to doxorubicin (0.05µM) than breast cancer cells (0.352µM) or human skin-fibroblast cells (9.00µM).

On the MCF 7 cell line, the *C. europaea* chloroform extract exhibited the strongest anti-proliferative impact. At this concentration, it was able to inhibit cell growth by 50%, as indicated by its IC_{50} value of 44.20±4.12 µg/ml. With an IC_{50} value of 53.93 ± 3.60 µg/ml, the methanolic extract had the second-highest anti-proliferative impact, while the ethyl acetate extract had a high IC_{50} value, measuring 79.75 ± 26.72 µg/ml. With an IC_{50} value higher than 400 µg/ml, the aqueous extract had the least anti-proliferative activity, suggesting that it was unable to impede cell proliferation at this particular dose (Table 1).

Regarding the cytotoxic effects of various *C. europaea* extracts on the U87 cell line, the IC_{50} value of the ethyl acetate extract exhibited the strongest cytotoxic impact (203.49±11.05µg/ml) (Table 1). The second most effective was the methanolic extract (317.95±25.65 µg/ml). The aqueous and chloroform extracts have IC_{50} values higher than 400 µg/ml, indicating less efficacy (Table 1). The impact of various *C. europaea* extracts on the HDFa cell lines indicated that the methanolic extract exhibited the maximum cytotoxic activity (IC_{50} of 312.74 µg/ml), while the ethyl acetate, chloroform, and water extracts had IC_{50} values of 382.77, 374.21, 374.21 µg/ml, respectively.

Extracts	MCF7	U87	HDFa
Methanol	53.93±3.60	317.95±25.65	345.74
Chloroform	44.20±4.12	> 400	374.21
Ethyl acetate	79.75±26.72	203.49±11.05	382.77
Water	> 400	> 400	> 400
Doxorubicin	0.352	0.05	9.00

Mean ± SEM, n=3.

Table 1: The values of IC_{50} (µg/ml) of Doxorubicin and different *C. europaea* extracts against MCF-7, U87, and HDFa cell lines.

The total phenols and flavonoid contents of various extracts of *C. europaea*

Based on the Folin-Ciocalteu method, the total phenolic content of *C. europaea* has been examined in methanolic, chloroform, ethyl acetate, and water extracts. The methanolic extract had the highest phenolic concentration, measuring 0.13±0.01 mg/g. Phenolic concentration was lowest in the chloroform extract at 0.05±0.01 mg/g. The phenolic content of the ethyl acetate and aqueous extracts was found to be 0.12±0.02 mg/g and 0.06±0.01 mg/g, respectively (Table 2).

The results of the total flavonoid content of *C. europaea* were found to be 0.85 ± 0.04, 0.76 ± 0.01, 0.46 ± 0.01, and 0.27 ± 0.10 mg/g for the methanolic, ethyl acetate, aqueous, and chloroform extracts, respectively (Table 2).

Extracts	Total Phenolic (mg/g)	Total Flavonoid (mg/g)
Methanol	0.13±0.01	0.85±0.04
Chloroform	0.05±0.01	0.27±0.10
Ethyl acetate	0.12±0.02	0.76±0.01
Water	0.06±0.01	0.46±0.01

Mean ± SEM, n=3.

Table 2: Total phenolic and flavonoid contents (mg/g) in different extracts of *C. europaea*.

Antioxidant activity:

Effect of DPPH radical scavenging capacity of four extracts of *C. europaea.*

The data shows the DPPH-scavenging capacity of four extracts (methanolic, chloroform, ethyl acetate, and water) and ascorbic acid (as a positive control) (Figure 1). The IC_{50} values (µg/ml) show the concentration of each extract required to scavenge 50% of the DPPH radical (Figure 1A).

Among the *C. europaea* extracts, the chloroform extract exhibited the strongest antioxidant activity, with an IC_{50} value of 194.20±5.29 µg/ml. The aqueous extract came in second, with an IC_{50} value of 216.80±4.34 µg/ml. Comparing the IC_{50} values of the methanolic and ethyl acetate extracts to the chloroform and aqueous extracts, they were 254.01±7.41 and 236.71±10.38 µg/ml, respectively, showing lesser antioxidant activity.

It is noteworthy that the positive control, vitamin C (ascorbic acid), had a significantly lower IC_{50} value of 64.36±1.81 µg/ml than the *C. europaea* extracts (Figure 1A). This suggests that vitamin C has far more effective antioxidant activity.

At doses ranging from 500 µg/mL to 4 µg/mL, the percentage of DPPH scavenging activity (%) for each extract is displayed in Figure 1B. All four extracts demonstrated strong DPPH scavenging activity at the maximum concentration (500 µg/mL); the methanolic extract showed 98% scavenging activity, while the aqueous, ethyl acetate, and chloroform extracts all showed 100% scavenging activity. The extracts' ability to scavenge DPPH declined as their concentration did. The aqueous and ethyl acetate extracts were in order of decreasing DPPH scavenging activity, with the chloroform extract showing the highest overall. Out of the four extracts, the methanolic extract exhibited the least amount of scavenging action.

Effect of NO radical scavenging capacity of four extracts of *C. europaea*

The results of the NO radical scavenging assay for four distinct extracts made from the plant species *C. europaea* are displayed in Figure 2A. With an IC_{50} of 17.38 ± 0.91 µg/ml, the methanolic extract of *C. europaea* has the maximum effect, followed by the ethyl acetate extract (IC_{50} of 22.22 ± 1.23 µg/ml), the chloroform extract (IC_{50} of 26.50 ± 1.22 µg/ml), and the aqueous extract (IC50 of 32.83 ± 1.99 µg/ml). Additionally, the IC_{50} of ascorbic acid was 25.17 ± 1.73 µg/ml. As a result, the *C. europaea* methanolic extract has the greatest impact, followed by the aqueous, chloroform, ethyl acetate, and ascorbic acid extracts.

Figure 2B. displayed the findings of an investigation conducted to ascertain the proportion of NO scavenging activity (%) displayed by diverse extracts extracted from *C. europaea* at varying doses. The chart makes it clear that the type of extraction solvent and extract concentration have an impact on the percentage of NO scavenging activity. The aqueous extract had the lowest NO scavenging activity, while the methanolic extract showed the highest activity, followed by chloroform and ethyl acetate. All of the solvents' NO scavenging activities declined as the extract concentration dropped. Methanolic extract (61%) and aqueous extract (45%) showed significantly different NO scavenging activities at a concentration of 500 µg/ml.

Effect of H_2O_2 radical scavenging capacity of four extracts of *C. europaea*

Out of the four extracts, the methanolic extract exhibited the strongest radical scavenging ability, as evidenced by its lowest IC_{50} value of 33.29±3.75 µg/ml (Figure 3A). The extracts with the next highest activity were the ethyl acetate (37.93± 1.06 µg/ml), chloroform (44.51±0.76 µg/ml), and aqueous (49.61±2.15 µg/ml). The well-known antioxidant ascorbic acid was also tested as a reference, and its IC_{50} value (25.17±1.73 µg/ml) was found to be lower than that of the methanolic extract, suggesting that the former is less efficient than the latter (Figure 3A).

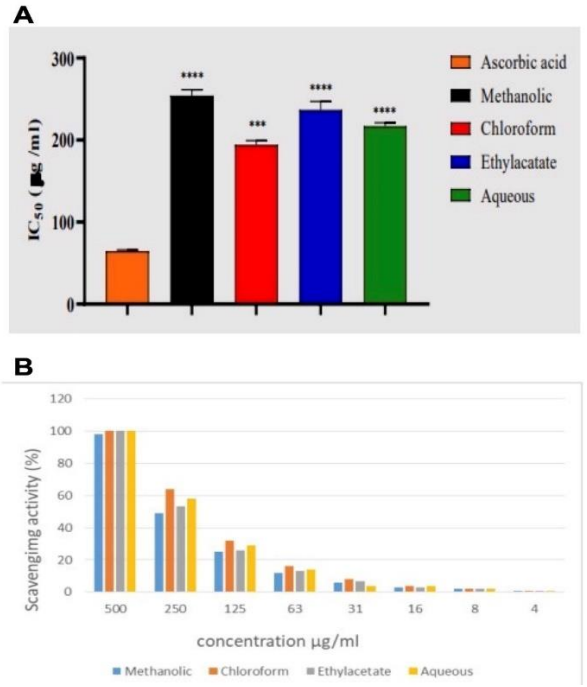

Figure 1: DPPH radical scavenging by *C. europaea* extracts. A. IC50 values (µg/mL). B. The percentage of radical activity. The results are presented as a mean ± SEM (n=3). Data were analyzed using one ANOVA test, then followed by Tukey's multiple comparison tests, with a level of significance of P<0.05. *** P ≤ 0.001 and ** P ≤ 0.0001, respectively.

The percentage of antioxidant scavenging activity of *C. europaea* extracts on H_2O_2 at various solvent concentrations is displayed in Figure 3B. The findings show that *C. europaea* extracts have antioxidant activity, with the strength of this activity varying according to the concentration of the extracts and the extraction solvent. The methanol and ethyl acetate extracts have the best antioxidant activity at the maximum concentration (100 µg/ml), with percentages ranging from 91% to 95%, respectively. At the same concentration, the antioxidant activity of the water and chloroform extracts is comparatively lower, ranging from 91% to 45% and 89% to 87%, respectively. The percentage of antioxidant activity drops along with the extracts' concentration. For example, the methanol and ethyl acetate extracts exhibit substantially less antioxidant activity at the lowest concentration (25%) than at the maximum concentration (with percentages ranging from 14% to 10%), respectively. On the other hand, with percentages ranging from 4% to 26%, the water and chloroform extracts show the least antioxidant activity at the lowest concentration.

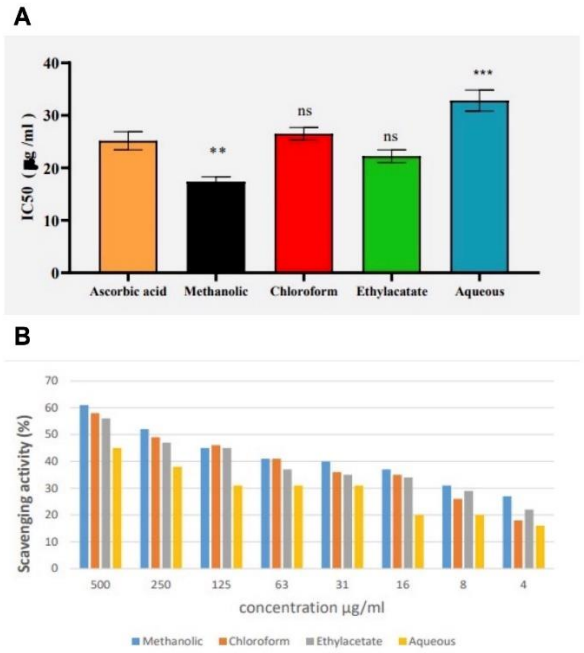

Figure 2: Nitric oxide detoxification by *C. europaea* extracts. A. The IC_{50} concentrations (µg/mL). B. The percentage of nitric oxide radical scavenging. The results are presented as the mean ± SEM (n=3). Data was evaluated via one ANOVA, then with Tukey's multiple comparison tests, with a significance level of P<0.05. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001, respectively.

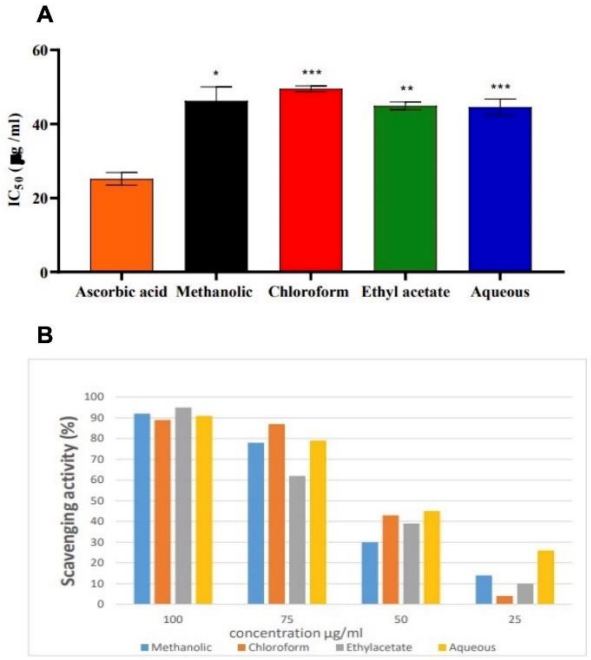

Figure 3: The H_2O_2 radical scavenging of *C. europaea* extracts. A. IC_{50} values (µg/ml). B. percentage of H_2O_2 scavenging activity. Data was evaluated via one ANOVA, then with Tukey's multiple comparison tests, with a significance level of P<0.05. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001. ns indicates non-significant values.

Discussion

Since cancer is one of the deadliest diseases in the world and effective preventive therapeutic strategies are needed, novel biological therapies have been the focus of extensive research. Plant medicines can be used as building blocks for pharmaceuticals or current or traditional medicine. Models of anticancer activity provide valuable insights into the identification of plant extracts with potential anticancer properties for future research [18] Along with the chemical and immunological properties of interest has attracted the attention of many researchers. These effects can be attributed to phytochemicals such as glycosides and pregnane steroids [28].

MTT assay revealed that the *C. europaea* extract exhibited moderate cytotoxicity in a dose-dependent manner when tested on MCF-7 cells. Adding these plants to herbal remedies can help prevent or reduce the risk of oxidative stress-related disorders such as breast cancer. The findings of our study highlight the potential benefits of Jordanian *C. europaea* as a potential novel anticancer agent. According to the results, the type of drug used may affect the anti-MCF7 cell activity of several *C. europaea* extracts. This suggests that the efficacy of *C. europaea* extracts' action against MCF7 is significantly affected by the type of solvent used. According to Amrati et al. [29], nonpolar molecules in the chloroform extract may have anti-proliferative effects. According to previous studies, *C. europaea* is likely to contain phenolic compounds and active ingredients. For example, De Luca et al. [30] found quercetin along with other phenolic compounds in the methanolic extract of C. europaea. Methanolic, chloroform, ethyl acetate, and water extracts were used to evaluate the resistance of *C. europaea* to U87. These results indicate that the ethyl acetate extract was more effective than the methanolic extract in inhibiting U87 cell growth. IC50 values larger than 400 indicated that the extracts and chloroform were ineffective against U87 cells. Consequently, of the four extracts examined in this work, the ethyl acetate extract appeared to be the most effective, suggesting that it would be the best option for future research in C on the antimicrobial activity of *C. europaea*. Polar solvent extraction of the polar substance Saponins from *C. europaea* has been found to significantly inhibit the growth of K562 and HL60 cell lines [29].

The results of this study suggest that the *C. europaea* extract can be safely applied to normal cells such as fibroblasts, as it has little effect on human skin fibroblast proliferation. The effects of the extract on MCF7, U87, and human skin-fibroblast cells are variable. U87 and human skin-fibroblast cells were less sensitive to various extracts than MCF7 cells, which were probably the most sensitive. Notably, there were no visible changes in cell morphology or viability, indicating that the extracts were safe to use on normal cells such as fibroblasts. This indicates that the extract can be safely used to treat cancer by acting as an anticancer agent without any cytotoxicity. The DPPH scavenging activities of four *C. europaea* isolates are compared.

These data suggest that the ability of *C. europaea* to extract DPPH involves both polar and non-polar molecules. With an IC50 of 64.36±1.81 64.36±1.81 µg/ml , the reference compound ascorbic acid exhibits significantly higher activity. According to Zozoli et al. [31], the methanolic and aqueous extracts of *C. europaea* showed greater IC50 values than chloroform, indicating that the scavenging activity of the aqueous extracts was lower than that of the ascorbic acid reference chemical. This may be because the chloroform contains more metabolites than the methanolic, and these compounds have been shown to have good scrubbing properties.

The most remarkable nitric oxide scavenging activity was exhibited by the methanolic extract of C. europaea, which exhibited an IC50 value of 17.38 ± 0.91, much lower than that of ascorbic acid of 25.17 ± 1.73. The ethyl acetate extract was found to have an IC50 of 22.22 ± 1.23, placing it between the methanolic and chloroform extracts, while the chloroform extract had an IC50 of 26.50 ± 1.22. Last but not least, compared to ascorbic acid, the aqueous extract of *C. europaea* showed a significantly higher IC50 of 32.83 ± 1.99. The highest nitric oxide scavenging activity was observed in the methanolic extract of *C. europaea*, according to case studies. This is consistent with our findings, indicating a high scavenging capacity of nitric oxide by the methanolic extract of *C. europaea*. Four different extracts of *C. europaea* (methanolic, chloroform, ethyl acetate, and water) extracts were used to investigate the degradation activity of H_2O. These data suggest that, among all the extracts, the methanolic extract of *C. europaea* probably has more adsorptive capacity as an antioxidant than ascorbic acid.

Depending on the type of extract used, *C. europaea* has very different phenolic concentrations. The phenolic content of the methanolic extract is the highest, while the water content is the lowest. In another study, Bourhia et al. [18] found that the methanolic extract of *C. europaea* had a total phenolic content of 0.10±0.03 mg/g. The total phenolic content of the ethyl acetate extract was determined to be 0.05 mg/g, while the total phenolic content of the chloroform extract was 0.07±0.02 mg/g. The four extracts have the potential to serve as a useful source of *C. europaea* flavonoids. The findings of the study are consistent with previous research findings [32]. Among them, the methanolic extract of *C. europaea* had the highest flavonoid content and the aqueous extract the least. Plant species and origin may have different biological activities and

chemical composition, which in turn may influence the extraction process and the control agents used [33]. Using UHPLC analysis, kaempferol, luteolin, kaempferol-3-O-hexose deoxyhexose, trans-ferulic acid, and syringic acid were detected in polyphenolic extracts of C. europaea. These flavonoids and phenolic acids may be responsible for the cytotoxic properties of the plant [29].

In conclusion, the findings illustrate that *C. europaea* methanolic extract inhibits the proliferation of brain and breast cancer cells (in vitro) with minimal damage to healthy cells. Furthermore, *C. europaea* possesses potent antioxidant activity through its ability to scavenge DPPH, Nitric oxide, and H_2O_2 substances. This suggests that *C. europaea* may provide a valuable source of bioactive chemicals for the synthesis of novel medications targeting cancer and other disorders brought on by free radical damage.

Author Contributions

The data collection and experiments were done by Makarem Al-Awaida and Hamzeh Al-Ameer. All authors contribute to the statistical analysis. Wajdy Al-Awaida, Hamdi Uysal, and Omar Atrooz formulated ideas, research goals, and aims. Omar Atrooz supervised the research, and prepared, revised, and edited the article.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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