Iraqi Journal of Science, 2026, Vol. 67, No. 8, pp: 4364-4377
DOI: 10.24996/ijs.2026.67.8.18

ISSN: 0067-2904

Anti-oxidant and Cytotoxic Activity of *Centaurium erythraea* Methanolic Extract: An *in vitro* Study Using MTT Assay

Asmaa Obaid Ismeel[1]*, Farah T.O. Al-Jumaili[2], Ruqaya Mohammed Al-ezzy[2]
[1]*Department of Applied Biological Sciences, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq*
[2]*Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq*

Received:22 /3/2024 Accepted: 6/ 9/2025 Published: 30/ 8/2026

Abstract

The *Centaurium erythraea* plant is widely used as a remedy for illnesses. The purpose of this study was to identify the key phytochemical components of the plant and assess how alcoholic extracts of those chemicals affected. *In vitro* anti-oxidant activity was measured using two methods: DPPH radical scavenging activity and reductive ability, to evaluate the cytotoxic activity of C. erythraea on two cancer cell lines (MCF-7 and HepG2) and a normal cell line (HdFn). A secondary metabolite study revealed that, depending on the solvent used, the entire plant contained varying amounts of tannins, glycosides, alkaloids, saponins, flavonoids, and polyphenols. Total flavonoid content was highest in the alcoholic extract (210.2±25.7 mg/g). *C. erythraea* showed efficacy in scavenging DPPH and ABTS radicals at all tested concentrations (200, 100, 50, 25 and 12.5 µg/ml). A breast cancer cell line was used to demonstrate dose-dependent cell viability. A plant that showed the most powerful cytotoxic action, with an IC_{50} value of 42.2 µg/ml, decreased the viability of the cells as the concentration increased. Additionally, a dose-dependent pattern was seen in cell viability in the liver cancer cell line (HepG2). By raising the quantity of *C. erythraea*, the cell viability is decreased. The lowest (HepG2) cell viability (%) was observed at 200µg/ml (44.98±6.45%), whilst the maximum (87.62±2.95%) was attained at 12.5 µg/ml. With an IC_{50} value of 47.6, *C. erythraea* demonstrated the most effective cytotoxic action and may offer a viable anticancer therapy when combined with specific medications.

Keywords: *Centaurium erythraea*; anti-oxidant; cytotoxicity.

الفعالية المضادة للاكسدة والسمية للمستخلص الميثانولي لنبات القنطريون خارج الجسم الحي باستخدام فحص *MTT*

اسماء عبيد اسماعيل[1]*, فرح تركي الجميلي[2], رقيه محمد العزي[2]
[1] قسم علوم الحياة التطبيقي, كلية التقنيات الاحيائية, جامعه النهرين
[2] قسم التقنيات الحيوية الجزيئية والطبية , كلية التقنيات الاحيائية, جامعه النهرين

* Email : Ruqaia.alezzy83@yahoo.com

الخلاصة

على نطاق واسع استخدم نبات *Centaurium erythraea* الكثير من الامراض. الغرض من هذه الدراسة هو تحديد المكونات الكيميائية النباتية الرئيسية للنبات وتقييم مدى تأثر المستخلصات الكحولية لتلك المواد الكيميائية. تم قياس نشاط مضادات الأكسدة في المختبر باستخدام طريقتين: وهما نشاط الكسح الجذري DPPH والقدرة الاختزالية وتقييم النشاط السام للخلايا على خطين من الخلايا السرطانية (MCF−7) وخطوط خلايا HepG2 بالإضافة إلى خط الخلايا الطبيعي HdFn كشفت دراسة المركبات الثانوية أنه اعتمادًا على المذيب المستخدم يحتوي النبات بأكمله على كميات مختلفة من التانين والجليكوسيدات، والقلويدات، والصابونين، والفلافونويدات، والبوليفينول. يحتوي المستخلص الكحولي على أعلى محتوى من إجمالي مركبات الفلافونويد (210.2±25.7 ملغم/جم). أظهر *Centaurium erythraea* فعاليته في التخلص من جذور DPPH و ABTS عند جميع التراكيز التي تم اختبارها (200، 100، 50، 25 و12.5 ميكروغرام/مل). تم إثبات ديمومة الخلية تعتمدعلى الجرعة من خلال خط خلايا سرطان الثدي. أظهر النبات أقوى تأثير سام للخلايا ضمن قيمة IC50 البالغة 42.2 ميكروغرام/مل قلل من حيوية الخلايا عند زيادة التركيز. بالإضافة إلى ذلك، شوهد بالاعتماد على الجرعة في تقليل قابلية ديمومة الخلايا في خط خلايا سرطان الكبد (HepG2). عن طريق زيادة كمية النبات، انخفضت ديمومة الخلية. ولوحظت أدنى نسبة ديمومة للخلية (HepG2) عند 200 ميكروغرام/مل (44.98±6.45%)، في حين تم الوصول إلى الحد الأقصى (87.62±2.95%) عند 12.5 ميكروغرام/مل. مع قيمة IC50 التبلغ 47.6، أظهر *Centaurium erythraea* التأثير الأكثر فعالية لقتل الخلايا وقد يكون علاجًا فعالاً مضادًا للسرطان عند دمجه مع أدوية محددة.

1. Introduction

Medicinal plants, sometimes referred to as medicinal herbs, have been recognized and used in traditional medical practices since ancient times [1]. Plants create a wide range of chemical compounds for defense and protection against diseases, fungi, insects, and herbivorous animals, among other uses. Herbal medicine has a long history that begins in prehistoric times. It comprises applying medicinal plants to cure ailments and enhance general health and well-being [2].

Conventional medical systems tackle illnesses holistically by taking mind-body-physiology into account. For instance, according to Ayurveda philosophy, administering "a group of phytochemical compounds" can potentially influence immunological pathways and different disease targets, providing an immunomodulatory and adaptogenic effect [3].

Oxidative stress in the physiological system may be prevented or reduced by a class of naturally occurring substances known as anti-oxidants. Free radicals are constantly created because the body regularly consumes oxygen [4]. These free radicals cause cell damage in the body and are linked to a number of illnesses, including cancer, diabetes, heart disease, and macular degeneration. Anti-oxidants help prevent and repair the damage that free radicals cause to cells because they are good scavengers of these harmful substances [5].

Anti-oxidants are essential components in biological systems, protecting cells from damage by neutralizing harmful free radicals. Superoxide dismutase (SOD) is a significant anti-oxidant enzyme specialized in combating oxidative stress. Structurally, it is a metalloenzyme composed of distinct subunits, and it acts as a primary regulator of oxidative reactions within living cells [6].

The biological activity of medicinal plants is influenced by the phenolic compounds, vitamins, and trace elements present in them, as well as the volatile constituents of essential oils [7].

Centaurium erythraea, is a member of the Gentillaceae family. In traditional Moroccan medicine, this species is widely utilized to treat a range of conditions, including rheumatism, diabetes, hepatitis, allergies, and digestive disorders. It is also included in the traditional pharmacopoeias of various nations for the treatment of oxidative stress-related disorders, such as diabetes [8].

Numerous investigations have demonstrated the significant pharmacological properties of the plant's organic extracts, including antibacterial, diuretic, anticancer, antidiabetic, and anti-oxidant properties [9]. However, nothing is known about the biological effects of *C. erythraea* essential oils. The experiments that were conducted solely examined the essential oils at the flowering stage, yet the chemical components of the same plant alter throughout its phonological stages [10].

The presence of multiple kinds of secondary metabolites, including xanthonoids, terpenoids, flavonoids, phenolic acids, and fatty acids, was discovered by phytochemical analysis of *C. erythraea* [11].

Liver cancer is a highly challenging malignancy to cure and has one of the highest fatality rates worldwide. The highest incidences of liver cancer are found in Asia and Africa. Asian nations were the source of more than 75% of liver cancer cases. Liver cancer epidemics are on the rise everywhere, especially in the USA and India. The 11,773 cancer patients, 229 men (2.1%) and 92 women (0.8%) were reported to have hepatocellular carcinoma (HCC), according to the 2020 National Cancer Registry [12].

The main liver cancer, known as HCC, is more common in developing nations. The two main causes of HCC are reactive oxygen species-induced hepatocellular damage. Hepatocarcinogenesis is connected to the emergence of chronic inflammation. Responses of the human body to prevent hepatocarcinogenesis in a number of ways. Apoptosis, or programmed cell death, is one method by which the balance between cell division and death is maintained. During apoptosis, nuclear condensation and fragmentation are two of the most notable morphological alterations that occur [13].

The investigated project focused on the effects of C. *erythraea* alcoholic extracts on two different cell lines.

2. Materials and Methods

Plant material

The Iraqi Center for Herbs had earlier identified the dried *C. erythraea* plant, which was collected from the northern part of the country in December 2020. The entire plant was ground into a fine powder using an electric mill after the impurities were removed, and it was then sealed in bottles.

Alcoholic extraction

Using a Soxhlet extraction equipment, 20g of plant material was extracted with 250 ml of 70% methanol for six hours at 55 °C, following some changes according to Fua *et al.,* [14]. After filtering and evaporation, the alcoholic extract was kept at 4 °C up to needed.

The active phytochemicals determination of Centaurium erythraea plant

In congruence with Sticher [15], the qualitative phytochemical investigations of *C. erythraea* whole plant were carried out as follows:

- *Identification of Tannins*

A few drops of lead acetate solution (1%) were added to the extract. The formation of a white or gelatinous precipitate indicates the presence of tannins.

- *Identification of Glycosides*

After adding one milliliter of the plant extract to two milliliters of Benedict reagent and boiling for five minutes, the mixture was allowed to cool. The red deposit indicated the presence of polysaccharides.

-Identification of alkaloids (Dragangroff test)

In solution A, 60 mg of bismuth sub-nitrate is dissolved in 0.2 ml of HCl, whereas in solution B, 600 mg of potassium iodide KI is diluted in 1 ml of distilled water. After combining a plant aqueous extract with solutions A and B, the accumulation of orange to brown indicated the presence of alkaloids**.**

- *Identification of saponins*

The plant extract solutions will be agitated extensively as part of the detection procedure. Saponins are indicated as froth at the top of the extract.

- *Identification of Flavonoids*

By combining a small amount of plant extract solutions with a small amount of sodium hydroxide and waiting for the mixture to turn brilliant yellow, the presence of flavonoids was verified.

- *Detection of Polyphenolic Compounds*

Each plant extract solution received a few drops of a ferric chloride (3%) solution. The presence of polyphenolic chemicals is indicated by the formation of a brown precipitate during the reaction.

- *Determination of Total Flavonoids*

With minor adjustments, the $AlCl_3$ colorimetric method first presented in Sakanaka *et al.,* [16] was utilized to ascertain the plant extract's total flavonoid content. Methanol was used to create a rutin standard solution with concentrations of 2.5, 5, 10, 20, 40, and 80 µg/ml. Briefly, 1 ml of 5% ($NaNO_3$) solution and 5 ml of 50% methanol were used to dissolve 3.2 mg of each plant extract. 10 ml of 10% NaOH solution was added after 6 minutes, and the mixture was allowed to settle for 5 minutes after adding 1 ml of 10% ($AlCl_3$) solution. The mixture was vigorously shaken to bring it up to 50 ml with distilled water (DW). At 510 nm, the absorbance was immediately measured.

Anti-oxidant activities of Centaurium erythraea plant

Anti-oxidant activity of *C. erythraea* was *in vitro* assessed through two evaluations, which were DPPH radical scavenging activity and reductive ability.

- *DPPH radical scavenging activity*

This approach was evaluated in accordance with Sanja *et al.,* [17]. The total free radical scavenging capability of the methanolic extracts from different plant quantities was evaluated using the stable DPPH radical, which has an absorption maximum at 515 nm, with little modification to the previously published technique. The absorbance of the DPPH radical in

the absence of an anti-oxidant or a blank was measured. The ability to scavenge the DPPH radical was calculated using the formula below.

$$\text{DPPH Scavenged } (\%) = \left(\frac{(AB - AA)}{AB}\right) \times 100$$

- *ABTS radical scavenging assay*

The ability of plant materials to scavenge free radicals was ascertained according to Jing *et al.,* [18] using the ABTS radical cation decolorization assay. The cation radical ABTS•+ was created by reacting 2.45 mM potassium persulfate (1:1) with 7 mM ABTS in water. It was then stored for 12 to 16 hours at room temperature in the dark before being utilized. Subsequently, the ABTS•+ solution was diluted with methanol, and the absorbance was determined at 734 nm, which was 0.7. After a 30-minute initial mixing time (5µm) of the plant extract and a 3.9 µm diluted ABTS•+ solution was mixed together, the absorbance was determined. A solvent blank was calculated for each experiment. A minimum of three measurements were obtained for each. The percentage of absorbance inhibition was calculated at 734 nm:

$$\text{ABTS} \cdot + \text{ scavenging effect } (\%) = \left(\frac{(AB - AA)}{AB}\right) \times 100$$

Cytotoxic activity of Centaurium erythraea on breast (MCF-7) and liver (HepG2) cancer cell lines

The cytotoxic activity of *Centaurea erythraea* was evaluated using two cancer cell lines, MCF-7 (breast cancer) and HepG2 (liver cancer), alongside the normal human dermal fibroblast cell line (HdFn). The experiment was conducted at the Biotechnology Research Center, Al-Nahrain University.

- *Cell line culture and maintenance*

After suspension in complete RPMI-1640 medium, cells were cultured in flasks at 37°C for 24 hours in a humidified incubator containing 5% CO_2. Once cells reached approximately 80% confluence, the growth medium was discarded, and adherent cells were washed twice with phosphate-buffered saline (PBS). Subsequently, 2–3 mL of trypsin-EDTA solution was added to each flask, gently agitated to fully cover the cell monolayer, and incubated at 37°C for 1–2 minutes to facilitate cell detachment. Trypsin activity was then halted by adding complete RPMI-1640 medium, and the resulting cell suspension was transferred to fresh flasks containing new complete medium. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2. The required cell concentration was determined using the trypan blue exclusion method. One volume of cell suspension was mixed with an equal volume of trypan blue stain and incubated for 3 minutes, after which viable cells were counted microscopically using a hemocytometer according to the following formula:

Dilution factor = (sample volume) x cell count equals total cell count mL^{-1}.

- *Assessment of cytotoxic activity of cell line culture and maintenance*

For twenty-four hours, the cells were cultivated in culture flasks at 37°C in a humidified environment with 5% CO_2.They were suspended in full RPMI-1640 media. The growing medium was removed, and the connected cells were rinsed twice with PBS solution once the cells had reached 80% confluence. To fully cover the monolayer, shake the flask gently after adding two to three milliliters of trypsin-EDTA solution. The flask was incubated for one to two minutes at 37°C until the cells separated from the sample's surface. Complete RPMI-1640 medium was added to halt trypsin activity, and the cell suspension was then transferred to other flasks with brand-new complete RPMI medium.Culture flasks were incubated at 37°C in 5% atmospheric CO_2 incubator [19].

The required concentration of cells was obtained using the trypan blue stain exclusion cell counting method, which involves combining an equal amount of trypan blue stain with one volume of cell suspension. After three minutes of waiting. The hemocytometer was used to count the cells under a microscope, and the formula was applied:

Total Cell Count mL^{-1} = Cell count x Dilution Factor (Sample Volume) $x10^4$

- *Assessment of cytotoxic activity of Centaurium erythraea on breast and liver cell line using MTT Assay*

A ready-to-use kit called 3-4, 5-Dimethylthiazol-2-yl-2, 5-Diphenyltetrazolium Bromide (MTT) to examine the cytotoxic effects of *C. erythraea* on the cell lines MCF-7, HepG2, and normal HdFn. According to the IC50 obtained, reactive oxygen species (ROS) production from MCF7 was calculated using ready to use kit (MnSOD) Kit.

- *Statistical analysis*

Statistical significance was assessed as $p \leq 0.05$. Version 6 of GraphPad Prism was used to calculate statistical significance and express data as mean±standard deviation (Graph Pad Software Inc., La Jolla).

3. Results and Discussion

Phytochemical screening and total flavonoids

The different phytochemical components found in alcoholic extracts of the complete C. erythraea plant are shown in Table 1. Total tannin and glycoside components were found to be abundant in the alcoholic extract. Compounds including flavonoids, polyphenols, alkaloids, and saponins were identified.

The extract exhibited the highest concentration of total flavonoids (210.2±25.7 mg/g), as indicated in Table 2.

Table 1: Evaluation of *Centaurium erythraea* extract's total flavonoids and major bioactive phytochemical components

Compounds	Alcoholic extract
Tannins	+++
Glycosides	+++
Alkaloids	+
Saponins	+
Flavonoids	++
Polyphenols	++

Table 2: Concentrations of total flavonoids compounds in *Centaurium erythraea* extract

Concentration (mg/g)	**Alcoholic extract**
Total Flavonoids	210.2±25.7

Anti-oxidant and Free Radical Scavenging Activity

Anti-oxidant and free radical scavenging activity of *C. erythraea* were assessed in vitro by measuring the activity of DPPH radical scavenging and ABTS assay.

-DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of *C. erythraea* was effective at all evaluated concentrations (200, 100, 50, 25 and 12.5 µg/ml). The concentrations of 100 and 200 mg/ml of *C. erythraea* showed higher radical scavenging activity (76.54±3.307and 62.08±1.011%)

respectively when compared with the other concentrations (50, 25, and 12.5) µg/ml that possessed DPPH radical scavenging activity of (48.57±2.800, 40.78±3.919 and 27.70±1.351%, respectively) when compared with Vitamin-C (Table 3 and Figure 1).

Table 3: DPPH radical scavenging activity of *Centaurium erythraea* alcoholic extract and vitamin-C

Concentration (µg/ml)	Vitamin-C(control)	Plant extract
200	75.77±2.655	76.54±3.307
100	61.96±1.270	62.08±1.011
50	48.57±2.800	52.74±3.143
25	36.19±1.381	40.78±3.919
12.5	19.64±7.559	27.70±1.351

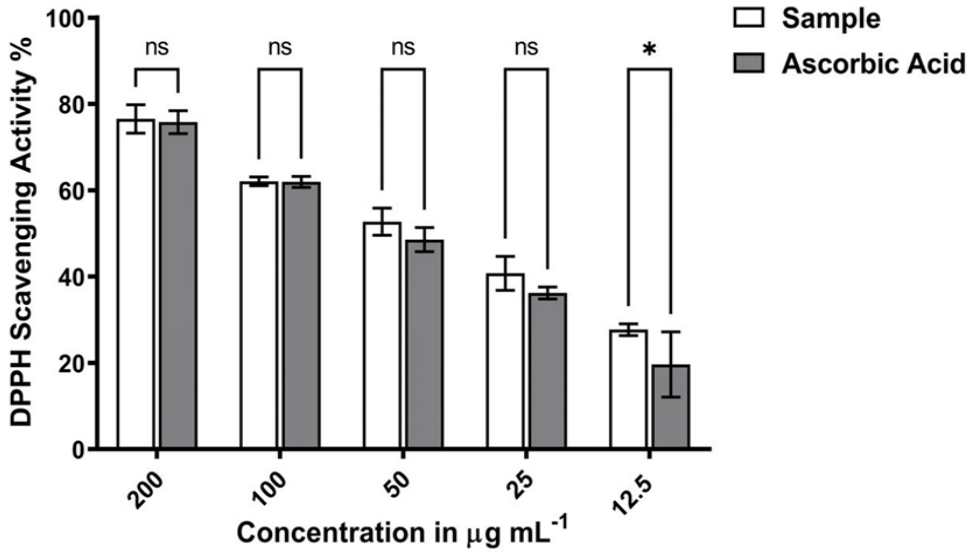

Figure 1: DPPH radical scavenging activity *Centaurium erythraea* alcoholic extract and ascorbic acid

- *ABTS Radical Scavenging Activity*

The ABTS radical scavenging activity of *C. erythraea* was significant at all examined concentrations (200, 100, 50, 25, and 12.5 µg/ml). The concentrations of 200 and 100 mg/ml of *C. erythraea* showed higher radical scavenging activity (65.12±3.089 and 50.50±1.011%), respectively, when compared with the other concentrations (50, 25, and 12.5) µg/ml that possessed ABTS radical scavenging activity of (41.17±3.143, 28.05±4.135 and 16.13±1.351%, respectively) when its compared with vitamin C (Table 4 and Figure 2).

Table 4: ABTS radical scavenging activity of *Centaurium erythraea* alcoholic extract and vitamin C.

Concentration(µg/ml)	Vitamin C (control)	Plant extract
200	76.03±4.028	65.12±3.089
100	64.03±1.007	50.50±1.011
50	57.60±2.207	41.17±3.143
25	39.00±1.732	28.05±4.135
12.5	22.90±1.836	16.13±1.351

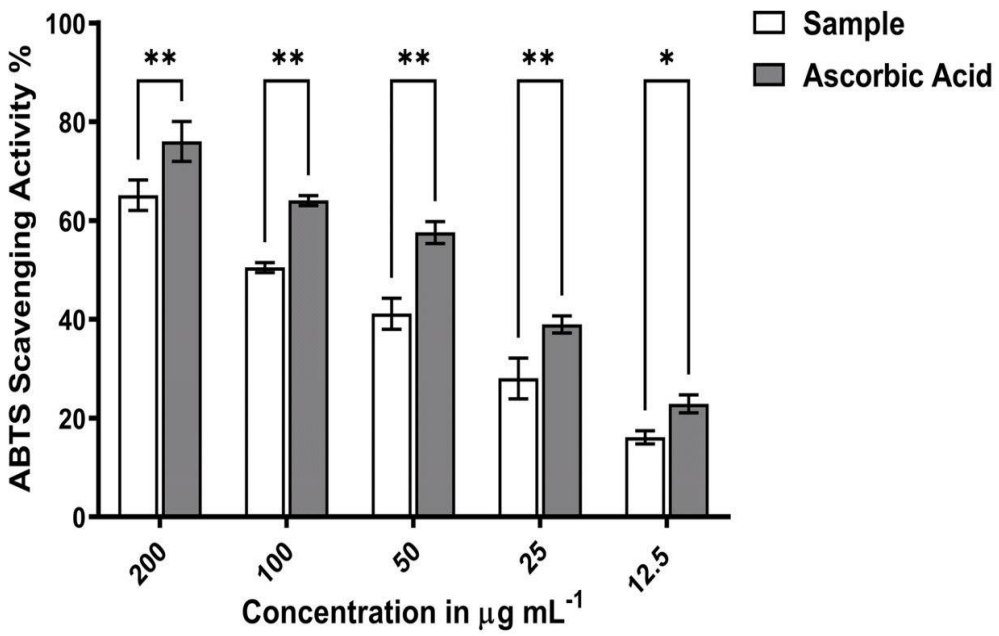

Figure 2: ABTS radical scavenging activity of *Centaurium erythraea* alcoholic extract and vitamin C.

- *Cytotoxic Effect of Centaurium erythraea In Vitro Using MTT Assay*

Breast cancer cell lines represented a dose-dependent decline in the viability of cells. Raising the concentration resulted in a decrease in cell viability. The decline in the percentage of (MCF-7) cell viability was observed at 200µg/ml and reached (53.40±3.31 %) At 12.5 µg/ml, the greatest cell viability was observed as 95.60±0.76%. Within an IC50 value of 42.2 µg/ml, *C. erythraea* demonstrated the most effective cytotoxic action by a considerable amount. Nonetheless, the impact of *C. erythraea* complex on the HdFn normal cell line yielded an IC50 of 105.5 µg/ml, with cell viability ranging from 80.75±1.62 to 95.80±2.68 for 200 to 12.5 µg/ml, respectively. (Table 5 and Figure 3).

Table 5: Cytotoxic effect of *Centaurium erythraea* extract on HdFn normal cell line and MCF-7.

Concentration of plant extract µg/ml	MCF-7(Mean±SD)	HdFn(mean±SD)
200	53.40±3.31	80.75±1.62
100	63.23±2.99	84.45±2.60
50	73.19±4.88	92.21±0.47
25	85.92±4.14	95.33±1.99
12.5	95.60±0.76	95.80±2.68

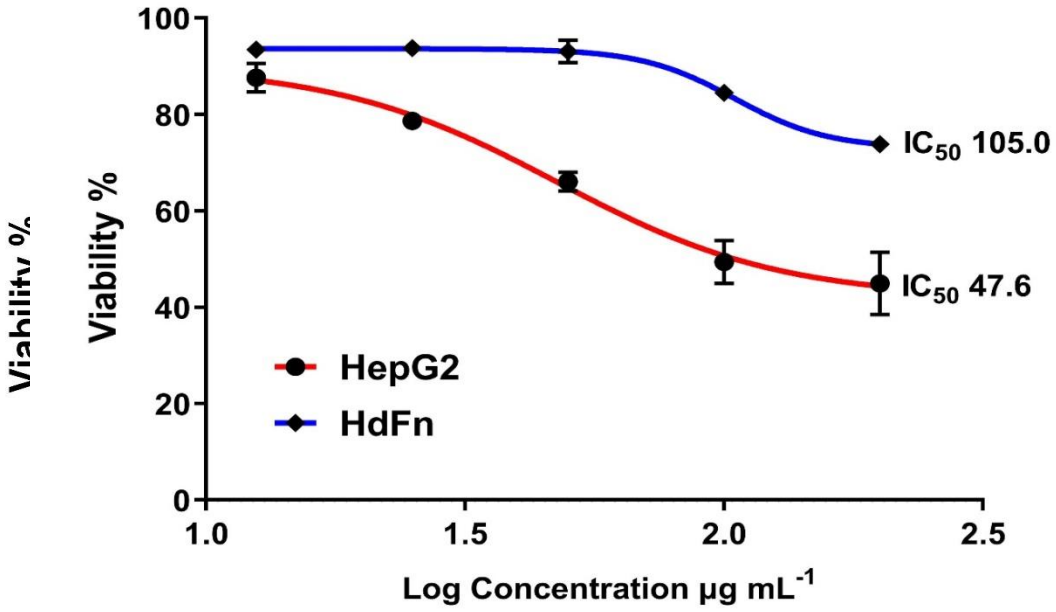

Figure 3: Cytotoxic effect of *Centaurium erythraea* extract on HdFn normal cell line and MCF-7.

Liver cancer cell line (HepG2) demonstrated a dose-dependent decline in cell viability. By raising the concentration of *C. erythraea*, the cell viability is decreased. 200µg/ml (44.98±6.45%) showed a decreasing percentage of (HepG2) cell viability, whereas 12.5 µg/ml (87.62±2.95%) showed the maximum percentage. With an IC50 value of 47.6 µg/ml, *C. erythraea* had the most intense cytotoxic action. However, the action of *C. erythraea* on the HdFn normal cell line yielded an IC50 of 105.5 µg/ml, and the cell viability was 73.84±0.70 and 93.48±1.36 µg/ml for (200 and 12.5 µg/ml), respectively (Table 6 and Figure 4).

Table 6: Cytotoxic effect of *Centaurium erythraea* extract on HdFn normal cells and HepG2 cells.

Concentration of plant extract µg/ml	HepG2(mean ±SD)	HdFn(mean±SD)
200	44.98±6.45	73.84±0.70
100	49.42±4.48	84.49±1.80
50	66.09±1.97	93.06±2.34
25	78.67±1.58	93.79±0.58
12.50	87.62±2.95	93.48±1.36

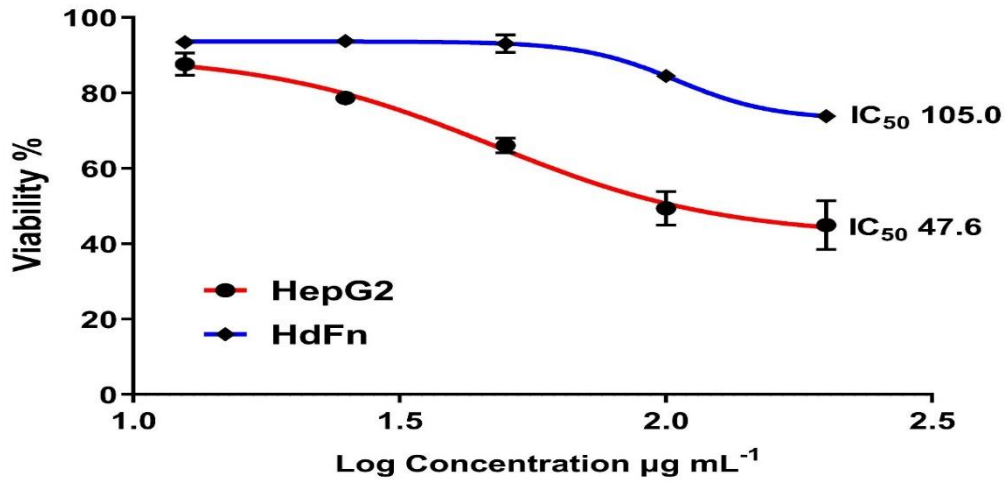

Figure 4: Cytotoxic effect of *Centaurium erythraea* extract on HdFn normal cells and HepG2 cells.

- *Detection of the Reactive Oxygen Species (ROS) generation using Manganese superoxide dismutase (MnSOD) Kit*

In this test, the MCF-7 cancer cell line was treated with *C. erythraea* at two concentrations (100 and 200 µg/ml) in addition to a negative control for 24 hours at 37 ˚C.

According to the results listed in Table 7, *C. erythraea* increased the ROS induction in the MCF-7cell line after 24 hours of treatment, and the mean of ROS induction was (729.7±53.61 and 869.0±20.22) at concentrations of (100 and 200 µg mL^{-1}), respectively. This increase in ROS was significantly different compared to untreated control cells (457.3±73.04) (Figure 5).

Table 7: The standard deviation of various concentrations of *Centaurium erythraea* on the generation of ROS in MCF-7 cells

Con µg mL^{-1}	Intensity (Mean ± SD)
Control negative (DMSO)	552.0±23.64
Control positive (Taxol)	1898±160.2
200	1211±57.83
100	739.0±24.98

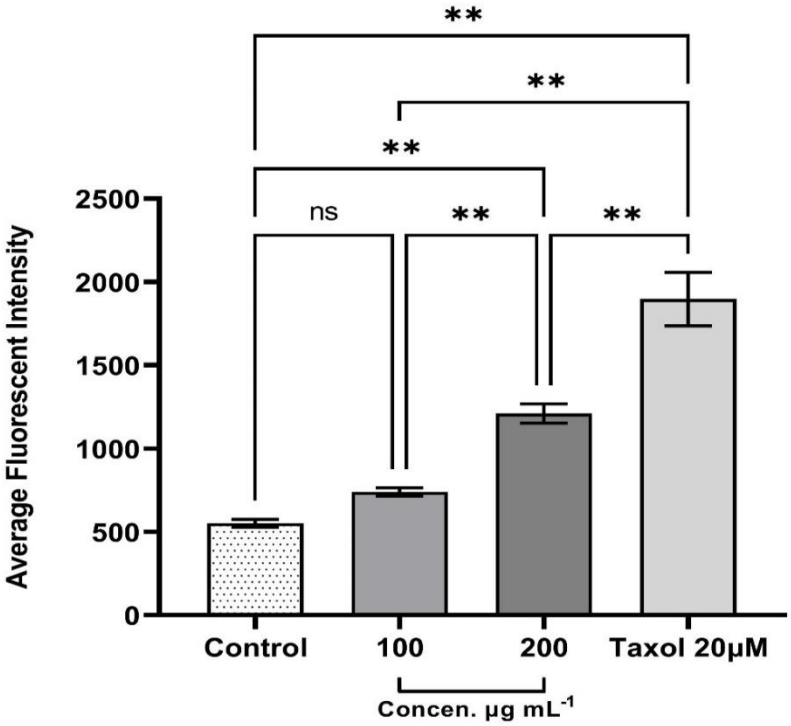

Figure 5: The mean intensity of different *Centaurium erythraea* concentrations on ROS induction in MCF-7 cells

Many secondary metabolites are found in plants, such as vitamins, terpenoids (including carotenoids), phenolic compounds (such as phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins), nitrogen-containing compounds (such as alkaloids, amines, and betalains), and other endogenous metabolites [20, 21].

Flavonoids and phenolic acids are important types of secondary metabolites that have the ability to improve plant scent, coloring, and flavor as well as scavenge free superoxide radicals and reduce cancer risk [22]. Phenolic agents offer protection against plant diseases that infiltrate plants. Phenol and flavonoids have been shown to possess cytotoxic, anti-inflammatory, anti-ulcerative, anti-oxidant, and anti-depressant qualities. Consuming phenolic substances lowers the chance of developing oxidative stress-related degenerative diseases, such as obesity, diabetes, and cardiovascular disease [23].

A key characteristic of the majority of bioactive substances with anti-aging and anti-carcinogenic qualities is their anti-oxidant activity [24]. Numerous metabolic pathways could accomplish these goals, such as preventing the production of free radicals, delaying the initiation and/or breaking chain propagation reaction, increasing the capacity of detoxifying enzymes like glutathione transferase (GST) and superoxide dismutase (SOD), and producing de novo anti-oxidants and adaptations where the signal for the production and reaction of free radicals is present and transporting the anti-oxidant to the appropriate location [25].

Among the many anti-oxidant, anticancer, and antibacterial compounds found in *C. erythraea*, also referred to as specialized metabolites, include tannins, flavonoids, alkaloids, and terpenoids, which are not found in synthetic compound libraries 2020 [26].

By boosting the tumor-suppressor protein p53, flavonoids activate the caspase-mediated signal transduction pathways, which in turn cause cell apoptosis. Numerous reports have

shown that the anticancer activity of flavonoid metal complexes is higher than that of the equivalent free flavonoids [27].

In order to absorb an electron and become stable, free radicals quickly react with other substances. The targeted molecule becomes a free radical after losing an electron. This has anthelmintic, hypotensive, antipyretic, and antidiabetic properties and initiates a chain reaction that disturbs living cells. It is used topically to heal wounds [28].

The integrity of the genetic makeup depends on several repair mechanisms, such as excision repair, photoreactivation repair, post-replication repair, error-prone repair, and error-free repair, and such mechanisms may be considered as a target for medicinal plants or their products in terms of enhancement [29].

From the aerial parts of *C. erythraea,* the following were found: centauroside, ursolic acid, maslinic acid, dimethyl acetal, secologanin, erythraeaxanthone I, erythraeaxanthone II, and demethyleustomin. Spectroscopic methods, including mass spectrometry and NMR, were employed to identify the chemicals. The XTT test was used to assess the cytotoxic potential of undescribed compounds against human breast cancer MCF-7 and MDA-MB-453 cell lines, as well as murine fibroblast 3T3-L1 cell lines. It was discovered that erythraeaxanthone II exhibited the strongest cytotoxic effects [30,31].

4. Conclusion

In conclusion, *C. erythraea* contained different amounts of tannins, glycosides, alkaloids, saponins, flavonoids, and polyphenols, which exhibited anti-oxidant activity and showed the most potent cytotoxic action against two different cell lines. This combination may offer a viable anticancer therapy when combined with specific medications.

Disclosure and conflict of interest

Conflict of Interest: The authors declare that they have no conflicts of interest.

Acknowledgement

Thanks, and appreciation to all staff of the College of Biotechnology, Al-Nahrain University, in Iraq. For their support and help in completing this work.

References

[1] S. Borse , M. Joshi, A. Saggam, V. Bhat, S. Walia , A. Marathe, S. Sagar, P. Chavan-Gautam, A. Girme, L. Hingoraniand G. "TilluAyurveda botanicals in COVID-19 management: An in silico-multitarget approach," *plos one*, vol. 11, no 16, 2021.

[2] P. Yudharaj, Shankar, R. Sowjanya, B. Sireesha, E. Ashok, R. Naik, and P. Jasmine, "IMPORTANCE AND USES OF MEDICINAL PLANTS – AN OVERVIEW," *International Journal of Preclinical & Pharmaceutical Research*, vol. 7,no.2,pp. 67-73, 2016.

[3] P. Hooda, R. Malik, S. Bhatia ,A. Al-Harrasi, A. Najmi, K. Zoghebi, M.A. Halawi, H.A. Makeen, and S.Mohan, "Phytoimmunomodulators: A review of natural modulators for complex immune system," *Heliyon*. vol. 10, no. 1,2023 .

[4] F.T.O. Al-Jumaili, M. Hamid, and A. Abed , "Assessment of active constituents and total flavonoids of Ammi majus plant extracts on immunological and kidney protective activities in mice," *Revista bionatura*,vol. 8,no.1,pp.1-12,2023.

[5] S.M. Jolić, I.R. Redovnikovic, K. Markovic, , Đ.I. Šipušic, and K. Delonga, "Changes of phenolic compounds and anti-oxidant capacity in cocoa beans processing," *International Journal of Food Science and Technology*,vol. 46, no.9,pp.1793–1800,2011.

[6] I.G. Munteanu and C. Apetrei "Analytical Methods Used in Determining AntioxidantActivity:AReview, " *International Journal of Molecular Sciences*,vol. 25,no.22,pp.3380,2021.

[7] M.N.N. Lima, , J.S. Costa, , B.A. Guimarães, J.J.S. Freitas, W.N. Setzer, J.K.R. Silva, and J.G.S. Maia ,"Chemometrics of the composition and anti-oxidant capacity of Hyptis crenata essential oils from Brazil," *Molecules* ,vol. 21,no. 28,pp. 3371,2023.

[8] M. Milos., M. Anja Tolic, J. Rajic , M. Mihailovic , J.A. Jovanovic , A. Uskokovic , N. Grdovic , M.B. Đorđevic , D. Misic , B. Siler , M. Vidakovic, and S. Dinic, "Centaurium erythraea methanol extract improves the functionality of diabetic liver and kidney by mitigating hyperglycemia-induced oxidative stress," *Journal of Functional Foods*,vol. 90, pp. 104975,2022.

[9] S.S. Salman, R.M. Al-Ezzy, and A. Soussi, "green Synthesis, Analysis, and Characterization of Nano-silver-Based Conyza Canadensis (SYN: Erigeron Canadensis) Extract, "*Chemical Methodologies*, vol. 8, pp. 856-873, 2024.

[10] N.Y. Paniagua-Zambrana and R.W. Bussmann, "Centaurium erythraea Rafn GENTIANACEAE. In: Paniagua-Zambrana, N., Bussmann, R., *(eds) Ethnobotany of the Andes. Ethnobotany of Mountain Regions*," *Springer Cham*,2020.

[11] H. Akşit , S. Gözcü, and A. Altay, "Isolation and cytotoxic activities of undescribed iridoid and xanthone glycosides from Centaurium erythraea Rafn. (Gentianaceae)," *Phytochemistry*, vol. 205,pp. 113484,2023.

[12] P. Valentão, E. Fernandes, F. Carvalho, P. B. Andrade, R. M. Seabra, and M. L. Bastos, "Anti-oxidant Activity of Centaurium erythreae Infusion Evidenced by Its Superoxide Radical Scavenging and Xanthine Oxidase Inhibitory Activity," *Journal of Agricultural and Food Chemistry* ,vol. 49,no. 7,pp. 3476–3479,2001.

[13] T. Jeyaraj, S. W. Nimesha, S. S. Kanishka , R. Umapriyatharshini, H. T. Kamani, T.Ira, and R. S. Sameera, "Cytotoxicity against Human Hepatocellular Carcinoma (HepG2) Cells and Anti-Oxidant Activity of Selected Endemic or Medicinal Plants in Sri Lanka," *Advances in Pharmacological and Pharmaceutical Sciences,*vol. 9,pp.640-688,2022.

[14] W. Fua, J.Chena, Y. Caia, Y. Leia, L. Chenb, L. Peic, D. Zhoua, X. Lianga, and J. Ruana, "Anti-oxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.) Ching," *Journal of Ethnopharmacology*, vol.130, pp. 521-528, 2010.

[15] O. Sticher, "Natural product isolation", *Natural Product Reports*,vol. 25,no.3,2008.

[16] S. Sakanaka, Y. Tachibana, and Y. Okada, "Preparation and anti-oxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha) ," *Food Chemistry*,vol. 89,no.4,pp. 569–575,2005.

[17] S.D. Sanja, N.R. Sheth, N.K. Patel, P. Dhaval, and P. Biraju,"Characterization and evaluation of anti-oxidant activity of portulaca oleracea," *International Journal of Pharmacy and Pharmaceutical Sciences*,vol. 1,pp. 74-84,2009.

[18] G. Jing, Audrey, S. K. Hui, and Z. Weibiao, "Enhancing health benefits of bakery products using phytochemicals," *Advances in Food and Nutrition Research*, vol. 99,pp.239-281,2022.

[19] R. I. Freshney, "Culture of animal cells: a manual of basic and specialized applications,"*National Cancer Institute*, vol. 51, no.5,pp.1417–23 ,2015.

[20] [20] H.W. Zhang, J.J. Hu, R.Q. Fu, L. Xin, Z. Yan-Hao, L. Jing, L. Lei , L. Yu-Nong, D. Qin, L. Qing-Song, O. Qin, and Ga. Ning, "Flavonoids inhibit cell proliferation and induce apoptosis and autophagy through down regulation of PI3Kγ mediated PI3K/AKT/mTOR /p70S6K/ULK signaling pathway in human breast cancer cells," *Scientific Reports*,vol. 8,no.1,pp.11255,2018.

[21] A.M. Salam and C.L. Quave, "Opportunities for Plant Natural Products in Infection Control,"*Current Opinion in Microbiology*, vol. 45,no.12,pp.189–194,2018.

[22] M. Reyes-Farias and C. Carrasco-Pozo, "The Anticancer Effect of Quercetin: Molecular Implications in Cancer Metabolism," *International Journal of Molecular Sciences*, vol. 20,no.13,pp.3177,2019.

[23] S. sen and S. K. Samanta, "Medicinal plants, human health and biodiversity: a broad review," *Advances in Biochemical Engineering/biotechnology* ,vol. 147,pp. 59-110,2015.

[24] Y. Wang, X.J., Chen, J.P. Cao, X. Li and C.D. Sun," Citrus flavonoids and their anti-oxidant evaluation," Critical Reviews in Food Science and Nutrition, vol. 6362, no. 14, pp.3833-3854,2022.

[25] J. Božunovic´, S. Živkovic´, U. Gašic´, J. Glamocˇlija, A. C´ iric´, D. Matekalo, B. Šiler, M. Sokovic´, Ž. Tešic´, and D. Mišic´, " In Vitro and in Vivo Transformations of Centaurium erythraea Secoiridoid Glucosides Alternate Their Anti-oxidant and Antimicrobial Capacity," *Industrial Crops and Products*,vol. 111,pp.705–721,2018.

[26] J. Zhou,L. Wang, J. Wang, and N. Tang,"Synthesis,characterization,antioxidative and antitumor activities of solid quercetin rare earth(III) complexes," *Journal of Inorganic Biochemistry*,vol. 83,pp.41-48,2001.

[27] D. Singh, H. Tanwar, B. Jayashankar, J. Sharma, S. Murthy, S.Chanda, S.B. Singh, and L. Ganju, "Quercetin exhibits adjuvant activity by enhancing Th2 immune response in ovalbumin immunized mice," *Biomed Pharmacother*,vol. 90,pp.354-360,2017.

[28] H. YIN and L. XU, "Free radical lipid peroxidation: mechanisms and analysis ," *Chemicals Reviews*,vol. 111,no. 10,pp. 5944-72,2011.

[29] H. A. Ibrahim and N. H. Zaki, "The Biological Activity of Some Extracted Proteins from Bacillus spp. Isolated from Soil Against Pathogenic Bacteria," *Al-Mustansiriyah Journal of Science*, vol. 30, no. 4, pp. 29–38, 2020, doi: 10.23851/mjs.v30i4.685.

[30] M. R. Kachmar. , O. A. P. Oliveira, A. Gil-Izquierdo, R. Domínguez-Perles, A. Ouahbi, K. El Badaoui, P.B. Andrade, and F. Ferreres , "HPLC-DAD-ESI/MSn phenolic profile and in vitro biological potential of Centaurium erythraea Rafn aqueous extract," *Food Chemistry,*vol. 278,no 3,pp. 242-233,2019.

[31] N. El. Menyiy, F. Guaouguaou, A. Baaboua, N. El Omari, D. Taha, N. Salhi, M. Shariati, T. Aanniz, T. Benali, G. Zengin, M. El-Shazly, I. Chamkhi, and A. Bouyahya, "Phytochemical properties, biological activities and medicinal use of Centaurium erythraea Rafn," *Ethnopharmacol,* vol. 10,pp.276-114171,2021.