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Antioxidant and Cytotoxic Effect of Total Flavonoids from *Cuscuta Campestris Yunck*

[1]Rusul A. Sabea, [2]Abbas M. Ismail

[1,2]University of Baghdad, College of Science for Women, Department of Biology, Baghdad, Iraq.

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Abstract: The present study was conducted to investigate the antioxidant and cytotoxicity of purify flavonoids that isolated from *Cuscuta campestris*. Three solvent (methanol, Dichloromethane, ethyalacetate) were used to extracted flavonoid.were measured(128.5,85.1,98.3 mg Rutin / 100 gm) respectively, the type and quantity of flavonoid were detected by HPLC .The result showed that the plant extract contains flavonoids (Rutin ,Qurcetine, Kaempferol, Apigenin, Luteolin) (124.0, 130.5, 80, 9, 58.7, 51.5) µg/gm respectively. The antioxidant activities of purify flavonoids of *Cuscuta campestris* were screened using DPPH (2,2-diphenyl-1-picrylhydrazyl). the results revealed that the radical scavenging activity is effective and activity increase when the concentrations increase. The highest percentage of free radical scavenging is at of 200µg/mL (82.37) a concentration compared with control (13.20). Some species of *Cuscuta* possess anticancer activity on various cell lines (colon HT29, MCF10). Using MTT assay. The cytotoxic activity purify flavonoids extracted from *Cuscuta campestris.* The results showed that the administration of different doses (12.5,25,50,100,200) µg/mL after 72h of *Cuscuta campestris* resulted in decreased viability of(HT29) cells which have higher in inhibition rate compared with control (88.7,13.20) for HT29, MCF10 respectively. This plant may represent promising source of herbal treatment for colon cancer and is thus a safe and promising anticancer drug candidate.

Keywords*:* *Cuscuta*, HT29, MCF10, Flavonoid, cytotoxity.

Corresponding author: (Email**:** rusul.adel2302m@csw.uobaghdad.edu.iq).

Introduction

Phytochemicals, which are naturally occurring in plant. These substances are synthesized in various part of a plant such as the leaves, roots, stem, fruit, seeds and bark. Due to their potential medicinal value, plants are commonly screened for these compounds. Convolvulaceae family is a well-established plant family, contains numerous species with traditional applications in herbal medicine for various diseases (1). In this study, ***Cuscuta*** is Convolvulaceae family and is a group of 100- 170 species of yellow, orange, red or rarely green parasitic plants. *Cuscuta*, parasitic members of the Convolvulaceae family, pest and pathogen. These holoparasites utilize specialized structures called Haustoria to penetrate the vascular tissues of their hosts, disrupting nutrient transport and overall physiological functions. The resulting stress often culminates in poor plant performance or host death (2). *Cuscuta* sp (Convolvulaceae) is one of the most important parasitic wee., The genus name “Cuscuta” is believed to have originated from the Arabic word “kushkut,” which refers to a tangled strand of hair (3). These parasitic plants

lack true roots and leaves, and their slender, yellow-orange filaments enwrap host stems to extract nutrients. They are known under various common names depending on regional dialects according to the country and sometimes region, but the most common name is „Dodder", that probably derived from the old German word "dotter" which means „yolk"(4). Phytochemicals isolated from *Cuscuta* are flavonoids(5).

Flavonoids are ubiquitous in the plant kingdom, including within the Convolvulaceae family (6). These bioactive compounds are known to exhibit a wide range of beneficial pharmacological effects, such as antiviral, antibacterial, anti-inflammatory, antithrombotic, vasodilatory, and anti-allergic activities(7). Additionally, flavonoids and phenolic compounds found in various herbal plants possess significant antioxidant properties, contributing to their therapeutic potential (8). Phenolic compounds, in particular, demonstrate notable anti-inflammatory effects and have been shown to be effective in the management of various diseases, including those affecting the kidney and stomach (9). Phytochemicals, including flavonoids, are structurally diverse, and their distribution across plant species tends to be limited to certain taxa. Due to these distinct chemical characteristics, phytochemicals are frequently utilized as bio-diagnostic markers studies in chemotaxonomy, assisting in plant taxonomy by analyzing species-specific chemical characteristics. (10).

Recent studies have extensively explored the functional role of flavonoids and phenolic acids as antioxidants has been increasingly recognized for their potential in disease prevention and management. These compounds are valuable not only for their antioxidant capacity but also as essential components of the human diet (11). As antioxidants, flavonoids and phenolic acids help protect biological systems by neutralizing reactive oxygen species (ROS), such as superoxide radicals, thereby mitigating the risk of oxidative stress-related diseases, including cancer. Flavonoids, in particular, constitute one of the most significant groups of bioactive compounds found both in the human diet and in plants.

Cancer stands as the second primary cause of death in the world. In 2020, an estimated (13, 29), million new cancer diagnoses and around 10 million deaths were recorded, which reflects global impact of cancer on humans of all kinds. especially since most current chemotherapy drugs are cytotoxic, causing damage to healthy tissues. Natural, non-toxic compounds have emerged as promising candidates in the field of cancer chemoprevention (12). drug Screening in colon cancer research has been extensively conducted using HT-29 cells. Moreover, the medicinal plants with anti-inflammatory properties, identified through ethnopharmacological knowledge have been investigation on this type of tumor cells, with focus on evaluating cytotoxity and effective concentration rang (13). Investigating medicinal plants, including toxicological and pharmacodynamics assessments, is crucial for pharmaceutical research and public health efforts. Based on their bioactive properties, scientists continue to explore various plant constituents and extracts with different solubility characteristics to identify and isolate specific biologically active phytochemicals that could serve as potential therapeutic agents for treating

diseases(14). This study aims to evaluate the antioxidant activity and cytotoxicity of flavonoid compounds extracted from Cuscuta using methanol as a solvent. The focus is specifically on their effects against human colon cancer cells (HT-29), with the goal of exploring their potential as natural anticancer agents.

Material and Methods

Plant Collection and identification:

The fresh plants of the parasitic plants were collected from different places in the University of Baghdad, College of Science for women and College of Agriculture, where the parasite was in a flowering period during the group collection stage from August to the end of September 2024. After that, they were dried at room temperature in dark, then ground.

Preparation of plant extract

The dry powder was extracted by soxhlet at ratio (weight volume) for each solvent (methanol, methylene chloride, ethyl-acetate) for 6-8 hour at 60-80 C. then the extract was filtered and dry(15).

Determination of total flavonoid content:

The total flavonoid concentration of the crude extract was assessed using the aluminum chloride colorimetric technique. In summary, 50 µL of crude extract (1 mg/mL ethanol) was diluted to 1 mL with methanol, combined with 4 mL of distilled water, followed by the addition of 0.3 mL of 5% NaNO2 solution. After 5 minutes of incubation, 0.3 mL of 10% AlCl3 solution was incorporated, and the mixture was permitted to stand for 6 minutes. Subsequently, 2 mL of 1 mol/L NaOH solution was added, and the final volume of the combination was adjusted to 10 mL using double-distilled water. The combination was permitted to rest for 15 minutes, and absorbance was recorded at 510 nm. The total flavonoid content was determined using a calibration curve and represented as mg of rutin equivalent per gram of dry weight (16).

Flavonoid purification

Extracts were solubilized in ethanol and subjected to treatment with 5% ethanolic KOH and hydrochloric acid. Observations concentrated on colorimetric alterations (17).

Quantitative and Qualitative of flavonoid by (HPLC):

Sample were analyzed by high performance liquid chromatography HPLC was performed using a SYKAM system (Germany). The mobile phase consisted of (Methanol, Distal water and formic acid) in a ratio of (70: 25: 5), separation by using a C18-ODS column (25 cm * 4.6 mm), with a UV detector set at 280 nm and flow rate 1.0 ml /min (18).

Antioxidant activity analysis

The DPPH radical scavenging activity was done following the method by (19). 50 µl of ***CUSCUTA*** flavonoids at various concentrations was poured into each well in the 96-well plate, mixed with 200 µl of DPPH solution (0.077 mmol/l), and then incubated in a dark room with room temperature (RT) for 30 min. Afterward, the mixture absorbance was measured at 517 nm using a microplate reader. Reference antioxidant used in all the assays was ascorbic acid. The radical scavenging activity of DPPH was expressed as a percentage and calculated according to the equation below:

% DPPH Scavenging Activity = (1-(As/Ac)) x 100

As) A control = absorbencies of control

Ac) A sample = absorbencies of sample.

Cytotoxicity Assays

The HT-29 cell line and the normal MCF10 cell line were subjected to treatment with CUSCUTA flavonoids to assess the cytotoxic effects of these compounds. The MTT experiment was performed using 96-well plates. HT-29 cells were cultured at 1×10^4 cells per well and incubated for 24 hours. HT-29 cells were treated with the extract at various doses until confluence was achieved. After 72 hours of treatment, cell viability was assessed by removing the medium, adding 100 µL of 2 mg/mL MTT solution to the wells, and incubating for 2.5 hours at 37°C. Following the removal of the MTT solution, the resultant crystals were solubilized by the addition of 130 µL of DMSO and incubated at 37°C for 15 minutes with moderate agitation. Absorbance was quantified at 492 nm with a microplate reader, and the assay was conducted in triplicate. The cytotoxicity percentage (cell growth inhibition) was determined using the subsequent equation (19) (23):

Inhibition rate = (A - B) / A * 100, where A denotes the optical density of the control and B signifies the optical density of the experimental samples (24).

Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was employed to evaluate the impact of various Concentration in study parameters. significant difference LSD –Least test was used to compare the means and determine statistical significance (25).

Result and Desiccation

Total Flavonoids

The ***Cuscuta*** plant was extracted using three solvents, (methanol, methylene chloride, ethyalacetate). The total flavonoids of these solvent were (128.5, 85.1, 98.3) respectively.

Table (1):Total flavonoid in difference extraction.

Solvent / Plant	Methanol	Methylene chloride	Ethyalacetate
Cuscuta	128.5	85.1	98.3
L.S.D. value	12.712 *		
(P≤0.05).			

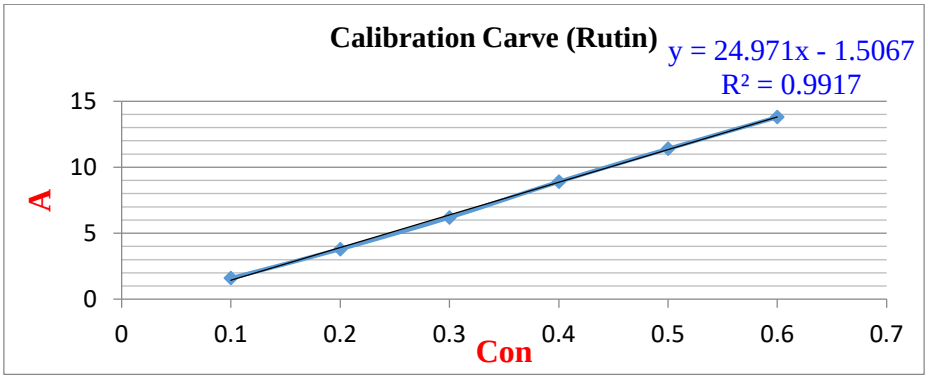

Figure (1): Calibration carve (Rutin).

The results showed that methanol extraction yielded the highest flavonoid concentration (128.5 mg/g), with a significant difference compared to the other solvents. There was also a significant difference between ethyl acetate (98.3 mg/g) and methyl chloride (85.1 mg/g), with the latter exhibiting the lowest extraction efficiency**.** According to the result methanol proved

to be the most efficient solvent for phenolic compound extraction. These results agreed with the (26)**.** Who found that polar solvent like methanol for extraction of flavonoid, that higher phenolic content is not always linked to high flavonoid concentration (27).

Qualitative and Quantitative analysis of flavonoids

The flavonoids were purified from methanolic extract, which is the highest total flavonoids. The qualitative and quantitative flavonoids value were detected by using HPLC. The result in ((table 2) showed that they are 5types of flavonoids in *cuscuta* plant (Rutin, Qurcetine, Kaempferol, Apigenin, Luteolin) which (124.0, 130.5, 80, 9, 58.7, 51.5)mg/L respectively (Figure 2-3-4-5-6-7). This result differs with the (28). That it does not contain compound (Hyperoside, Isorhamnetin) and agreed with it containing compound (Rutin,caempferol). This is attributed to the variation in environmental conditions like salinity, pH, temperature etc from one place to another (28).

Table (2): Quantitative analysis of flavonoids in *Cuscuta* methanolic extract.

No.	Active compound	Concentration (mg/L)
1	Rutin	124.0
2	Qurcetine	130.5
3	Kaempferol	80.9
4	Apigenin	58.7
5	Luteolin	51.5

Result chromatography (Uncal - F:\ cuscuta)

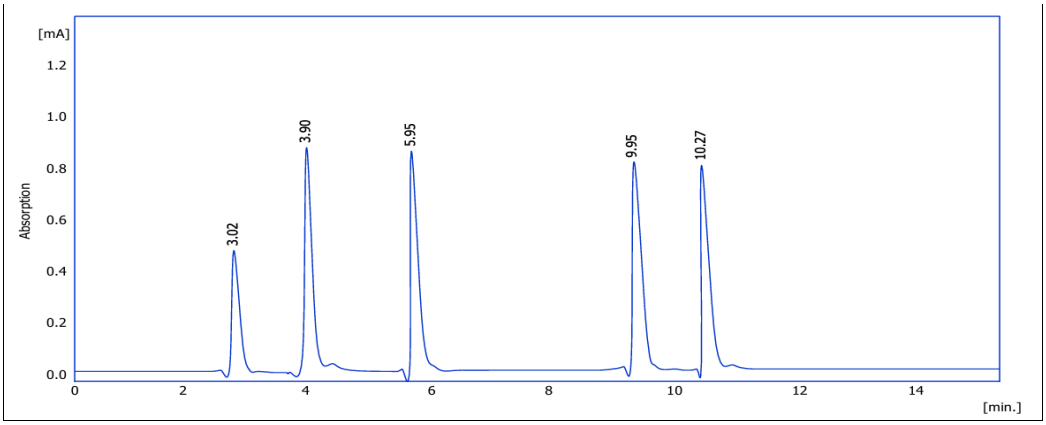

Figure (2): HPLC chromatography of *cuscuta* flavonoids.

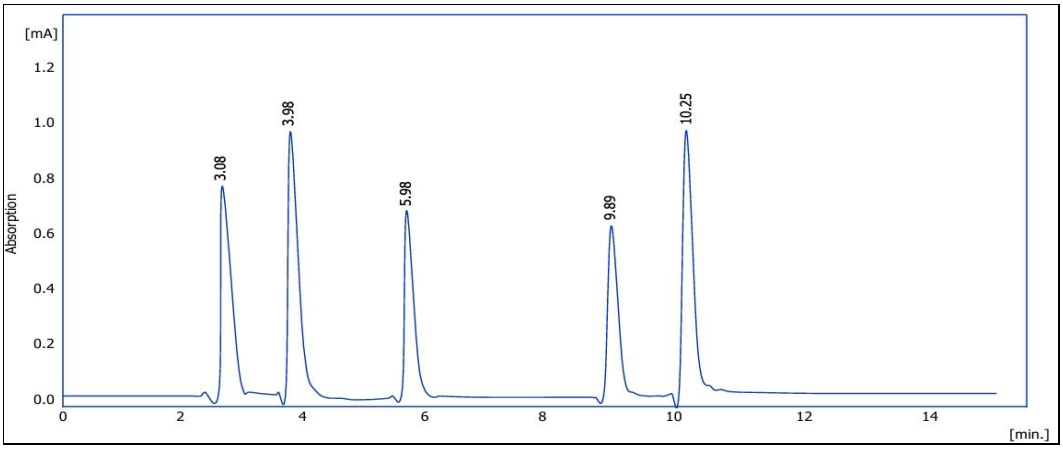

Figure (3): Result HPLC chromatography of stander mix.

Antioxidant activity

The table (3) indicate the effect of purified flavonoids to scavenging the free radicals. Five different concentration were examined (12.5, 25, 50, 100, 200)µg/mL, which gave the higher value (88.38,92.40) respectively indicated at (200)µg/mL compared with ascorbic acid and the lower value(26.27,56.60)indicated at (12.5) µg/mL concentration(Figure4).These results agreed with the (29).that refer to *cuscuta* can be used as antioxidant. These bioactive compounds exhibit significant reducing activity against DPPH radical. The potent antioxidant impact of the purified flavonoids may be attributed to their high capacity to donate hydrogen atoms, efficient metal chelating ability and scavenge reactive oxygen species such as hydrogen peroxide and superoxide (30).

Table (3): Effect of concentration in Scavenging activity.

Concentrations µg/mL	Mean scavenging activity ±SD	
	Plant	Ascorbic acid
12.5	26.27±0.217	56.60±2.37
25	38.57±0.9838	65.30±2.63
50	48.47±1.856	83.60±3.14
100	72.47±1.819	89.20±0.40
200	88.38±1.401	92.40±1.704
L.S.D. value	7.894 *	8.063 *
Means having with the different letters in same column differed significantly. * (P≤0.05).		

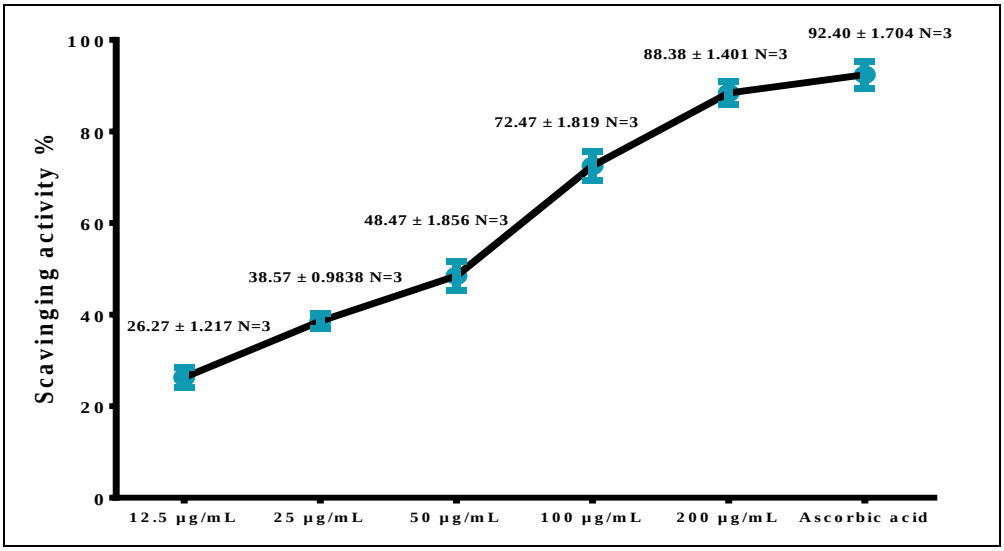

Figure (4): Antioxidant activity of *Cuscuta* using DPPH assay. Data are represented as mean ± SD.

The antioxidant properties of flavonoids are attributed to their high concentration and potent free radical scavenging activity (31). These secondary metabolites exhibit their antioxidative effects through mechanisms such as neutralization, absorption, and quenching of (ROS). This activity is primarily due to their redox potential, the conjugated ring structure, and the presence of functional groups such as the carboxyl group, which facilitate electron transfer and free radical stabilization. Consequently, flavonoids play a critical role in cellular defense against oxidative stress and

contribute to various biological and pharmacological benefits (32).

Cytotoxicity activity

MTT assay were down to exam the activity of flavonoids to inhibited HT-29 cells line after 72h compared with the normal cell line MCF-10(table4) indicated that the highest inhibition rate (88.37) achieved at (200) µg/mL concentration will the lowest inhibit at (12.5) µg/mL concentration (17.47) Compared with the control cell line (MCF-10) which have the highest inhabit (13.20) at (200) µg/mL concentration.

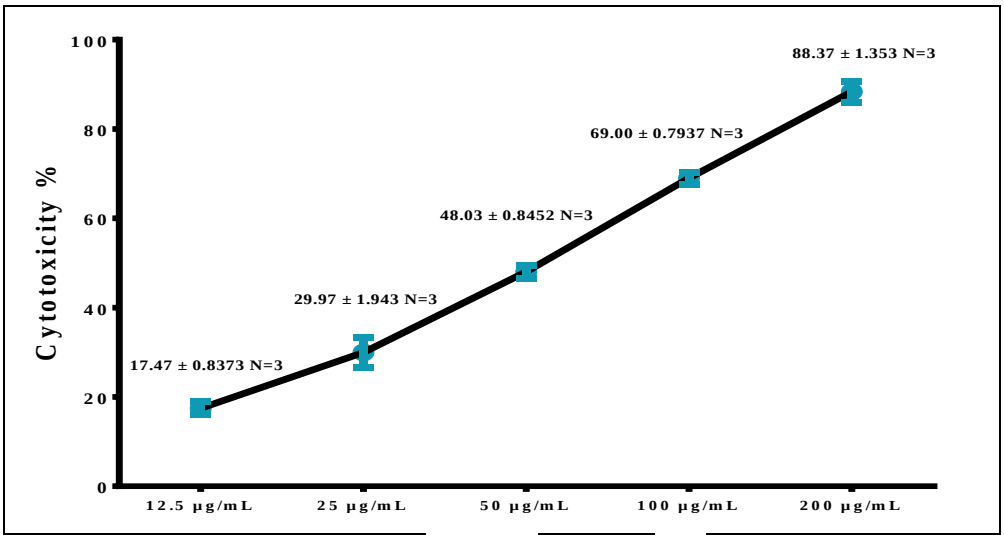

Figure (5): Cytotoxicity effect of (*Cuscuta*) in HT-29 cells. Exposure time 72hr.

Table (4): Cytotoxity effect of *Cuscuta* inHT-29.

Concentration µg/mL	Means of cytotoxicity inHT-29	Means of cytotoxicity in MCF-10
12.5	17.47±0.8373 d	2.580±0.8660 d
25	29.97±1.943 d	5.120±0.6963 cd
50	48.03±0.8452 c	7.490±0.89927 bc
100	69.00±0.7937 b	10.47±0.5877 ab
200	88.37±1.353 a	13.20±1.018 a
L.S.D. value	8.027 *	3.174 *
Means having with the different letters in same column differed significantly. * (P≤0.05).		

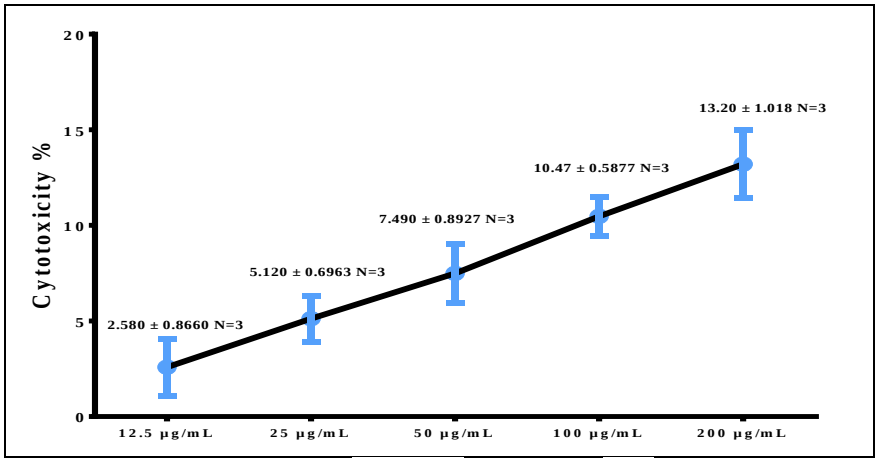

Figure (6): Cytotoxicity effect of (*Cuscuta*) in MCF-10 cells. Exposure time 72hr.

The results shown that CUSCUTA flavonoids significantly inhibit HT-29 cells while exhibiting no effects on normal MCF-10 cells (Figure 5-6). The MTT experiment demonstrates that Cuscuta inhibits cell growth in a way contingent upon exposure duration and dosage. These findings are corroborated by prior research indicating that Cuscuta extract can impede cancer cell proliferation and induce apoptosis and cell cycle arrest in cancer cells (33). Flavonoids demonstrate several anticancer actions, including the modulation of antioxidant enzymes that neutralize reactive oxygen species (ROS), activation of cell cycle arrest, reduction of cancer cell growth and invasiveness, and encouragement of apoptosis. Numerous chemotherapeutic drugs elicit apoptosis via the caspase-dependent pathway, a process integral to cancer suppression (34). The findings of this study concur with (35) in inhibiting the activity of cancer cells. Consequently. The advantageous properties of flavonoids stem from their capacity to transition from antioxidants to pro-oxidants, facilitating apoptosis, inhibiting tumor cell development, and reducing inflammation. Flavonoids can directly scavenge reactive oxygen species (ROS) and chelate metal ions, owing to their capacity to stabilize free radicals through the presence of phenolic hydroxyl groups. The indirect antioxidant effects of flavonoids involve the activation of antioxidant enzymes, inhibition of pro-oxidant enzymes, and the stimulation of both antioxidant and phase II detoxifying enzyme synthesis. Flavonoids exhibit both antioxidant and pro-oxidant properties that contribute to their anticancer effects (36).

Conclusion

The study demonstrated that the methanolic extract of Cuscuta contains active flavonoid compounds with notable antioxidant properties and significant cytotoxic effects against human colon cancer cells (HT-29). These findings highlight its potential as a natural source for the development of anticancer agents. The results support the importance of medicinal plants in the search for safer and more effective therapeutic alternatives. Further phytochemical and biological investigations are recommended to confirm the mechanism of action and evaluate its long-term safety.

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