Exploring the Therapeutic Potential of *Tamarix aphylla* Extract: Antioxidant Properties, Antiproliferative Effects, and Cytotoxicity Assessment

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ABSTRACT

Background: The use of plants for cancer prevention and therapy has attracted more interest recently due to the large diversity of phytochemical components found in plants and their decreased adverse effects. **Objectives:** This study evaluated the cytotoxic capacity of a single medicinal plant species *Tamarix aphylla* from Al-Hilla, Babylon Governorate, Iraq, against two cell lines: One normal cell line (REF52) and one cancerous cell line (MCF-7) were used in the study. It also evaluated the antioxidant activity of the plant species. **Methodology**: In this study, the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used to evaluate cell toxicity. Additionally, several assays were employed to measure antioxidant activity, of which the DPPH radical-scavenging activity and the reducing power assay were the most significant. **Results**: When applied at the same concentrations, the *Tamarix aphylla* extract significantly affected MCF-7 more than REF52. The extract's impact on the two lines differed noticeably. *Tamarix aphylla* plants have demonstrated a significant antioxidant efficacy. **Conclusion**:This study found that the alcoholic extract of *Tamarix aphylla* exhibits a strong anti-free radical effect. In addition, inhibits the growth of MCF-7 breast cancer.

Keywords: Cytotoxicity, MCF-7, REF52, medicinal plants, *Tamarix aphylla* , antioxidant.
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INTRODUCTION

Cancer is a multifactorial disease in which an abnormal cell population expands and grows out of control, causing the illness to linger and spread. When genes controlling the cell cycle, oncogenes, and tumor suppressors are changed, aberrant proteins that support cell division and survival are expressed (1). As long as cancer is a major worldwide health concern, a great deal of research focusing on complementary and alternative medicine will likely be done. Natural active ingredients extracted from medicinal plants can be used to prevent and treat a wide range of ailments and health conditions (2). Certain plant molecules that are categorized as secondary metabolites exhibit bioactive properties that may be used to treat a variety of disorders across several fields (3), including chronic diseases (4, 5). In recent decades, medicinal plants have been explored as potential cancer treatments because of their safety (6). Reports indicate that many plants contain relatively high levels of bioactive secondary metabolites with potential for use as anticancer drugs (7). Members of the family *Tamaricaceae* are commonly referred to as "salt cedar" or "tamarisk". They are easily recognized by their needle-like leaves, which are covered in salt produced by salt glands (8). While *Tamarix* species are distributed in temperate regions, their ability to thrive in hot, dry conditions has made them best known. *Tamarix* species are cultivated in arid regions to stabilize sand dunes (9). However, they are considered invasive plants in humid areas because they impede the growth of other species, making their presence undesirable (10). Several important phytochemicals have been reported in many studies of various *Tamarix* species, including polyphenolic substances such as phenolic acids, flavonoids, and tannins. Many studies have shown that extracts of *Tamarix aphylla* leaves exhibit antimicrobial effects on multidrug-resistant bacteria. The identified Phytochemicals in *T. aphylla* extract are reported to be biologically important and need further investigation to develop safe and cheap drugs (11).

METHODOLOGY

1. Plant collection and pretreatment

The leaves of the *Tamarix* plant were collected in October 2023 from the areas surrounding the ruins, which are located in the city of Al-Hilla, Babylon Governorate, Iraq, and were certified by a taxonomy expert, Dr. Sakina Al-Halawi, Biology Department, University of Baghdad, College of Science.

2. Plant (extracts) preparation

The plant samples were cleaned twice: once with running tap water and once with sterile water. Afterward, they were stored at room temperature in the dark for three weeks until the plant was dried, after which they were ground to a fine powder. The powder was then placed in sterile polythene bags and stored in the laboratory after passing through a 0.5 mm sieve. To extract the active ingredients from the plants.

3. Ethanolic Extraction by Soxhlet

The plant leaves were dried at ambient temperature for three weeks. An electric grinder was used to grind the plant's dried leaves into powder. About 50 g of powdered plant leaves was placed in the thimble of the Soxhlet apparatus for indirect hot-continuous extraction with 70% ethanol for 6-7 hours at 70 °C. The extract was dried in an oven at 50°C. The residue was weighed and kept in a dark, cool place for further analysis (12).

4. Quantitative determination of the extract by the HPLC technique

For HPLC analysis, 5g of the ethanolic extract by residue was dissolved in 10 mL of 70% ethanol, followed by filtration through a 0.22 µm Millipore filter. The sample was analyzed by high-performance liquid chromatography (HPLC) to identify the active component of the *T. aphylla* (12).

The mobile phase:

0.1% H_3PO_4 in D.W.

Gradient program:

Flow Rate = 0.8 mL/min

Detector = 210 nm

Temperature = Room temperature

Volume Injected = 20µl

Column = C18

The concentration for each *T. aphylla* compound was calculated as follows:

The concentration of the sample = (Area of sample / Area of standard) × Concentration of standard (mg/L).

5. Assessment of Anti-oxidant Activity *in vitro*

The antioxidant activity of the *Tamarix aphylla* ethanolic extract was assessed *in vitro* through two evaluations: Ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity.

5.1 Ferric reducing antioxidant power (FRAP)

A. Solutions: The solutions were prepared according to (13). The method described by Fu *et al.* was adopted to evaluate the Ferric reducing antioxidant power (FRAP), in which 1 mL of each concentration of the plant extract (0.08, 0.16, 0.32 and 0.64 mg/mL) was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1.5 mL of 1% potassium ferricyanide, and then incubated at 50°C for 20 minutes. Then, 1 mL of 10% trichloroacetic acid was added to the mixture to stop the reaction. The mixture was centrifuged for 10 minutes at 3000 rpm, and 2.5 mL of the supernatant was mixed with 2 mL of distilled water and 0.5 mL of freshly prepared 1% ferric chloride. After that, the absorbance was measured at 700 nm. The same procedure was applied to the Trolox solutions (standards). All tests were done in.

Trolox, a water-soluble analog of vitamin E, was used as a standard for comparison with the ethanolic extract of *T. aphylla*. Various concentrations of Trolox (0.08, 0.16, 0.32, and 0.64 mg/mL) were prepared by dissolving the necessary amounts in distilled water.

5.2 DPPH Radical Scavenging Activity (14)

- **Preparation of DPPH (1,1-Diphenyl-2-picryl-hyrazyi) Solution:** About 4.3 mg DPPH powder was dissolved in 3.3 mL of DMSO-methanol (1:9 v/v), and the solution was kept protected from light by covering the test tubes with aluminum foil.
- **Preparation of Plant Extract**: Four concentrations (0.0625, 0.125, 0.250, and 0.500 mg/mL) were prepared by dissolving the required weight of ethanolic extract in 1-2 drops of DMSO, and then the volume was made up with distilled water.
- **Preparation of Ascorbic Acid (Vitamin C) Solution:** Similar concentrations of the plant extract were prepared.

B. Method

The antioxidant activity of plant ethanolic extract and the standard (vitamin C) was assessed on the basis of the radical scavenging effect of the stable DPPH free radical, and the method of (15) was followed. An aliquot of 0.1 mL of the extract or standard at a concentration of (0.0625, 0.125, 0.250, and 0.500 mg/mL) was added to 3.9 mL of DPPH solution in a test tube. After incubation at 37°C for 30 minutes, the absorbance of each solution was determined at 517nm using a spectrophotometer. All measurements were made in triplicate. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}\right) \times 100$$

6. Cytoxic Assay

This *in vitro* cytotoxicity assay was performed to investigate the potential cytotoxic effect of *T. aphylla* against tumor cell lines (MCF-7) and normal cell line REF-52. The cell lines were propagated and maintained as described by (16)

The MCF-7 and REF-52 cells were cultured in complete RPMI 1640 medium in a humidified atmosphere containing 5% CO_2 at 37°C until they reached 80% confluence. The growth medium was removed, and the adhesive cells were washed twice with PBS solution . Two to three mL of trypsin-EDTA solution was added to the flask, and the flask was inverted to completely cover the monolayer, with gentle shaking. The flask was incubated at 37°C for 1-2 minutes until the cells were detached from the flask surface. Trypsin was inactivated by adding complete RPMI-1640 medium, followed by distributing the cell suspension to other flasks containing fresh complete RPMI medium. Cultured flasks were incubated at 37°C in 5% atmospheric CO_2 incubator. The required cell concentration was obtained using the trypan exclusion cell counting method by mixing 1 volume of cell suspension with 1 volume of trypan blue stain. After 3 min waiting, the cells were counted microscopically using a hemocytometer and applying the formula:

Total Cell Count mL-1 = Cell count x Dilution Factor (Sample Volume) x 10^4

MTT Protocol

The cytotoxic effect of *T. aphylla* was performed by using the MTT ready-to-use kit, Intron Biotech (Korea)

• After incubation, the medium was removed, and two-fold serial dilutions of the extract (62.5, 125, 250, 500, 1000 µg /mL) were added to the wells.

• For every concentration, triplicates were employed along with the controls, which were cells cultured in serum-free media. The plates were incubated for the chosen exposure length of 24 hours at 37°C with 5% CO_2.

• Ten microliters of the MTT solution were applied to each well following exposure. The plates underwent a 4-hour incubation period at 37°C and 5% CO_2.

• After incubation, a liquor of 100 µL of solubilization was applied to each well and allowed to sit for five minutes.

• Absorbance was measured using an ELISA reader set to 575 nm. To determine the IC_{50}, which represents the concentration of a substance required to reduce cell viability by 50% in each cell line, statistical analysis was performed on the optical density data."

RESULTS

1. Determination of Crude Extract by High-performance liquid chromatography (HPLC)

In HPLC, qualitative identifications have been made by comparing the retention times of the analyzed samples, obtained under identical chromatographic conditions, with those of authenticated reference standards. According to the HPLC analysis of the *T. aphylla* ethanolic extract, it was found that leaves contain fourteen different flavonoids (higher concentration was 4-hydroxybenzoi acid) as shown in Figure (1).

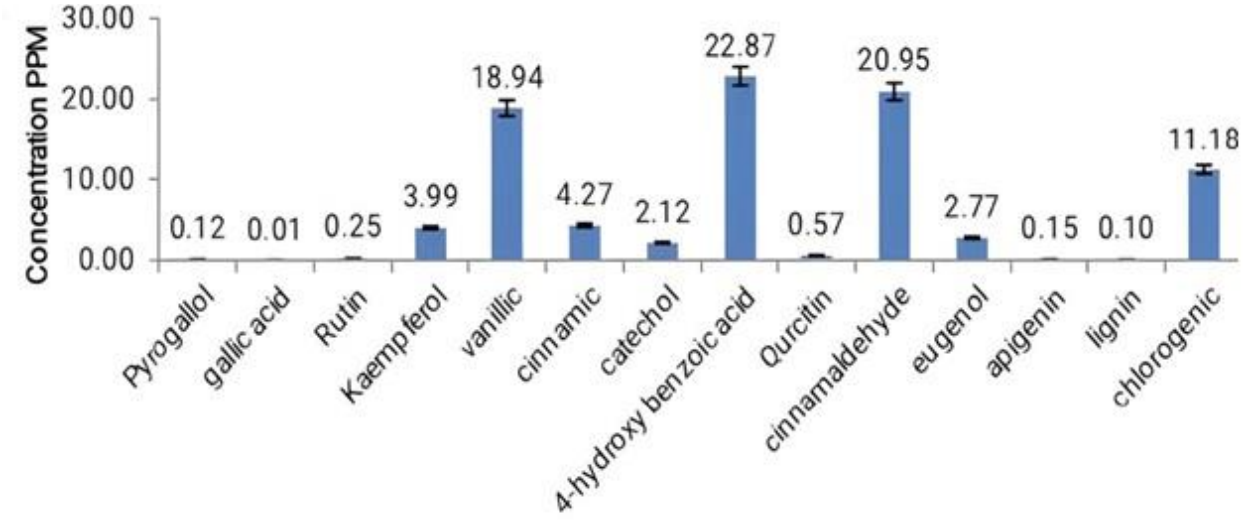

Figure (1): Histogram for Flavonoid compounds and their concentration in the *T. aphylla* HPLC assay

2. Antioxidant and Radical Scavenging Activity

The ethanolic extract of *Tamarix aphylla* was assessed *in vitro* for its antioxidant and radical-scavenging properties by measuring its Ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity.

2.1 Ferric reducing antioxidant power (FRAP)

The ethanolic extract of *T. aphylla* showed significantly more potent reducing power ($P \leq 0.001$) than vitamin C. The plant extract showed a considerable increase in ferric-reducing antioxidant power (p) at a concentration of 0.64 mg/mL, yielding a reducing power of 1.395. In contrast, vitamin C at the same concentration exhibited a reducing power of 0.211. Antioxidant activity had been significantly increased with an increase in the plant concentration.

Table (1): Reductive Ability of *Tamarix aphylla* ethanolic extract and vitamin E

Concentration	Reductive Ability Absorbance (Mean ± SD)		P ≤
(mg/mL)	*Tamarix aphylla* extract	Vitamin E	
0.08	0.501 ± 0.010^{C}	0.108 ± 0.001^{CD}	0.001
0.16	0.849 ± 0.015^{B}	0.114 ± 0.004^{C}	0.001
0.32	1.204 ± 0.013^{B}	0.132 ± 0.007^{B}	0.001
0.64	1.395 ± 0.023^{A}	0.211 ± 0.015^{A}	0.001

Different letters: Significant difference ($P \leq 0.001$) between means of columns.

3.1 DPPH Radical Scavenging Activity

At the four tested concentrations (0.0625, 0.125, 0.250, and 0.500 mg/mL), the ethanolic extract of *T. aphylla* demonstrated significantly greater DPPH radical scavenging activity compared to vitamin C. The estimated radical scavenging activity of the plant extract was consistent at 0.250 and 0.500 mg/mL, showing values of 89.00 ± 2.00% and 78.66 ± 3.51%, respectively. However, at lower concentrations of 0.0625 and 0.125 mg/mL, activity was significantly higher, with values of 42.33 ± 5.03% and 62.66 ± 4.50%, respectively ($P < 0.05$). Variations in vitamin C activity were also observed across the four concentrations, with a notable difference particularly between the 0.250 and 0.500 mg/mL concentrations Table (2).

Table (2): DPPH radical scavenging activity of *T. aphylla* ethanolic extract and vitamin C

Concentration	DPPH Radical Scavenging Activity (Mean ± SD; %)		P ≤
(mg/mL)	*Tamarix aphylla* extract	Vitamin C	
0.0625	42.33 ± 5.03^{B}	39.66 ± 2.52^{A}	0.001
0.125	62.66 ± 4.50^{B}	41.33 ± 10.01^{A}	0.001
0.25	78.66 ± 3.51^{A}	48.33 ± 8.50^{A}	0.001
0.5	89.00 ±2.00	53.00 ± 10.53^{A}	0.001

Different letters: Significant difference ($P \leq 0.05$) between means of columns.

3.2 Cytotoxic effect of *T. apylla* on MCF-7 cells *in vitro*

The cytotoxic effects of *T. apylla* ethanolic extract on the two lines REF52 and MCF7, assessed by MTT assay, are shown in Figures (1) and (2).

Figure (3) indicated that there were significant antioxidant changes ($P<0.001$) at the concentrations of 125 and 250 µg/mL, while no significance appeared at plant extract concentrations of 62.5, 500, or 1000 µg/mL ($P>0.001$).

Figure (3) showed a marked decrease in cell viability, especially in MCF-7 breast cancer cells, in comparison with the control for both lines, with IC50 on both lines

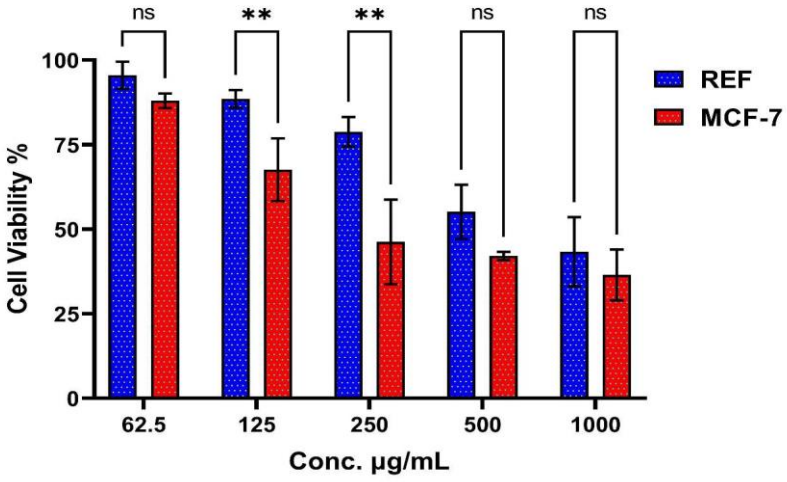

Figure (2): Histogram for Cell Viability in REF and MCF-7

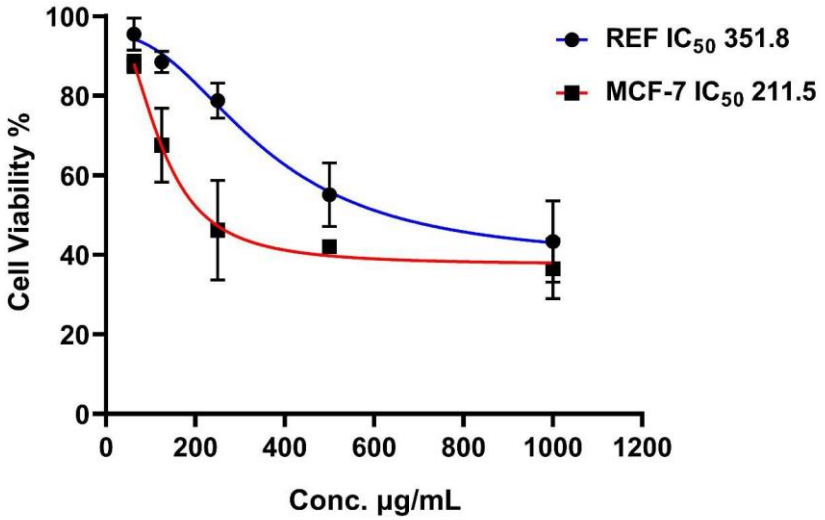

Figure (3): Cytotoxic effect of *T. aphylla* on the viability of human tumor cell lines MCF-7 and normal cell line REF.

DISSCUSION

The results were consistent with prior literature on HPLC analysis of *T. aphylla* extracts. *In vitro* evaluation of the radical-scavenging and antioxidant capabilities of the ethanolic *T. aphylla* extract was carried out by measuring its DPPH radical-scavenging activity and reductive ability.

Compared with controls, for each vitamin E and C, respectively, *T. aphylla* exhibits a higher yield of crude extract, which increases its reductive capacity and DPPH radical-scavenging activity. This conclusion is supported by the findings of *T. aphylla*, which grows in Iraq and utilizes an ethanolic extract. The potent antioxidant and DPPH radical scavenging activity of *T. apylla* is probably due to the plant's high tannin and flavonoid concentration (15), and it has been suggested that these constituents possess antioxidant activity (16). The ability of DPPH radical scavenging improved with the content of phenolic compounds, including flavonoids, phenolic acids, and phenolic diterpenes. Phenolic chemicals, which function as antioxidants and eliminate free radicals, are among the most significant substances included in the extracts. It is undeniably true that phenolic compounds protect cells from oxidative damage, thereby dramatically reducing cancer cell growth (17). Because they can neutralize free radicals generated during the process by which normal cells become cancerous, these extracts are high in phenolic compounds, which helps explain their harmful effect on cancer cells. Numerous biological investigations of various Tamarix species have been conducted. The present investigation found that the ethanolic extract (EE) of *T. aphylla* effectively inhibited MCF-7 cells' proliferation at doses of 125 and 250 µg/mL. The methanolic extract of *T. aphylla* inhibited the proliferation of MCF-7 breast cancer cells in a concentration-dependent manner (18). However, at 500 *µ*g/mL, the methanol extract of *T. aphylla* induced a 70% death rate, according to a recent study that examined its cytotoxic potential (19). Further toxicological studies using *T. phylla* extract at doses up to 2000 mg/kg did not find any harmful effects (20). The effects of several plant extracts on mouse lymphoma, rat hepatoma, and rat glioma cell lines were examined in separate investigations. Notably, the alcohol extract contained potent anticancer compounds with a significant cytotoxic effect at 10 *µ*g/mL, resulting in a 58.9% survival rate (21). Furthermore, the *T. africana* shoot extract effectively inhibited the proliferation of A-549 lung cancer cells, with an IC50 value of 34 *µ*g/mL (22). Previous studies on *T. aphylla* have revealed significant quantities of flavonoids, polyphenols, and tannins (23, 24). It was found that some phenolic compounds from the family Tamaricaceae exhibited cytotoxic activity when tested for their ability to induce cell death in several cancer cell lines (25). These are compounds that are derivatives of ferulic acid. Aphyllin, a glycosylated form of isoferulic acid, has been shown to possess a unique capacity to scavenge radicals and enhance human keratinocyte survival. (26). Furthermore, studies have demonstrated that

ellagitannins possess exceptional host-mediated antiangiogenic and anticancer properties (27). Tamarixetin was discovered to have a cytotoxic effect on leukemia cells (28).

CONCLUSION

The research findings can be condensed into the following conclusion points based on the data collected: These investigations lead researchers to the conclusion that *T. aphylla* is a promising plant for a wide range of pharmacologically active flavonoid compounds (29, 30). Phenolic chemicals abound in the Tamarix plant, and its alcoholic extract has a strong anti-free radical effect. After 24 hours of in vitro exposure, the alcoholic extract of *Tamarix* inhibits the growth of MCF-7 breast cancer cells, with little effect on the normal cell line REF52 at the specified concentrations, as shown by the MTT assay. Extracts from *T. aphylla* contain several components that are of significant medical importance. Plant extracts contain phytochemicals, which are a significant source of natural compounds with excellent cytotoxic properties. This work serves as a proof of concept for the idea that numerous pharmacological lead candidates with diverse biological activities can be obtained from medicinal plants.

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الكشف عن الإمكانات العلاجية لمستخلص نبات الاثل: تقييم الخصائص المضادة للأكسدة، التأثيرات المضادة للتكاثر، والسمية الخلوية

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قسم التقنيات الاحيائية النباتية، كلية التقنيات الاحيائية، جامعة النهرين، العراق

الخلاصة

خلفية عن الموضوع: لقد اجتذب استخدام النباتات للوقاية من السرطان وعلاجه المزيد من الاهتمام في الآونة الأخيرة بسبب التنوع الكبير في المكونات الكيميائية النباتية الموجودة في النباتات وانخفاض آثارها الضارة. **الهدف من الدراسة:** قيمت هذه الدراسة القدرة السمية للخلايا لنوع واحد من النباتات الطبية الاثل من الحلة، محافظة بابل، العراق، مقابل خطين من الخلايا: خط خلية طبيعي وخط خلية سرطانية واحد. كما تم تقييم النشاط المضاد للأكسدة للمستخلص النباتي. **طرق ومواد العمل:** تم استخدام اختبار **MTT** (بروميد 3-(4،5-ثنائي ميثيل ثيازول-2-يل)-2،5-ثنائي فينيل تترازوليوم) لتقييم سمية الخلايا. بالإضافة إلى ذلك، تم تطبيق عدة اختبارات لقياس نشاط مضادات الأكسدة، وكان من بين أهم هذه الاختبارات اختبار نشاط كسح الجذور الحرة **DPPH** واختبار القدرة الاختزالية. **النتائج:** عند تطبيقه بنفس التركيزات، أثر مستخلص نبات الاثل بشكل كبير على خط الخلايا السرطانية (MCF-7) أكثر من خط الخلابة الطبيعي (REF52). اختلف تأثير المستخلص على الخطين بشكل ملحوظ عن بعضهما البعض. أثبتت نباتات الاثل مستوى كبير من الفعالية المضادة للأكسدة. **الاستنتاجات:** خلصت هذه الدراسة إلى أن المستخلص الكحولي لنبات الاثل له تأثير قوي مضاد للجذور الحرة. بالإضافة إلى ذلك يمنع نمو سرطان الثدي MCF-7.

الكلمات المفتاحية: السمية الخلوية ، MCF-7، REF52، النباتات الطبية، مضادات الأكسدة.