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Original Article

Assessment of the Cytotoxic Activity of Alcoholic Extract of *Eucalyptus camaldulensis* on Breast Cancer Cell Line

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Abstract

The spread of different types of cancer has been on a rise in the recent century. The use of chemical medications develops drug resistance and causes serious side effects. *Eucalyptus camaldulensis* (*E. camaldulensis*) is one of the most famous herbal remedies considered owing to its anti-inflammatory effect and boosting the intimate immune system; moreover, it has demonstrated some anti-proliferative effects on cancerous cell lines. The current study assessed the cytotoxic activity of alcoholic extract of *Eucalyptus camaldulensis* (*E. camaldulensis*) at different concentrations of 6.125, 12.5, 25, 50, and 100 µg/mL on breast cancer cell line MCF-7. The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) analyses were employed to study the antioxidant efficiency of ethanolic extract of Eucalyptus camaldulensis. The results of Fourier transmission infra-red analysis and Phytochemical screening pointed to the presence of many active compounds in this extract, such as Tannins, Saponins, Phenolic compounds, Reducing sugar, Terpenoids, Steroids, Glucosides, Alkaloids, and Flavonoids. Furthermore, the results demonstrated that this extract significantly inhibited the growth of the MCF-7 cell line in a concentration-dependent manner, as compared to the control, and the cytotoxic activity of this extract elevated with an increase in the concentration. The results pointed out that *E. camaldulensis* can be considered a particularly valuable source of effective anti-proliferative and cytotoxic agents. The experimental findings demonstrated that *E. camaldulensis* extract possessed significant antioxidant efficiency and anti-proliferative effects on cancerous cell lines.

Keywords: Antioxidant, Anti-proliferative, Breast cancer, Crud extract, Ethanol

1. Introduction

Herbs have been used since ancient times for the improvement of the flavor and aroma of foods, as well as the maintenance of their nutritional value. One of the medicinal plants is Eucalyptus which is widely found in the Mediterranean region. The Eucalyptus species are used in many medical applications as antipyretic remedies for the symptoms of respiratory system infections, such as sinus congestion, flu, cold, astringent in dentistry analgesic, anti-inflammatory, and treatment of such diseases as bladder inflammation and diarrhea (1). Eucalyptus is cultivated for the production of many essential oils extensively used for medicinal purposes due to their high biological activities (2, 3).

The leaves of Eucalyptus are traditionally used to treat fungal infections and wounds due to their antimicrobial, analgesic, and anti-inflammatory properties (4). In addition, traditional herbal medicines are of great importance in developing countries, and 80% of the population relies on them for the provision of their basic health care needs (5). These compounds are known as secondary metabolites and have biological activities, such as modulating detoxification enzymes, prompting the immune system, reducing the aggregation of platelet, modulating the metabolism of

hormones, as well as antioxidant, antimicrobial, and anti-cancer activities (6).

Phytochemicals contain terpenoids, phenolics, and alkaloids (7). Many of these compounds, such as Alkaloids and Phenols, exert cytotoxic effects on cancer cells and have antioxidants activities. Cancer is a malignant disease characterized by abnormal division and differentiation of cells, leading to a dramatic increase in the number of dividing cells that later collect to produce the tumor or spread to the rest of the body tissue by blood or lymph. The cancer cell feeds itself through a method called the angiogenesis process in which the growth of a network of blood vessels will result in the creation of angiogenesis activators and reduce the production of angiogenesis inhibitors (8).

Eucalyptus camaldulensis is an agricultural crop which has been used as raw resources in plywood sheets, epoxy putty, and the solar industry. *Eucalyptus camaldulensis* timber is suited to railway sleepers, shipbuilding, fuel, and heavy buildings (9). It is an evergreen tree found in Pakistan, India, Australia, Nigeria, Egypt, and Iraq. In conventional treatments, *Eucalyptus camaldulensis* was commonly included as an anesthetic, antiseptic and astringent in several different conditions (10). Its new and young leaves are boiled with water in Iraq and flu treatment is carried out using decoction (11).

Eucalyptus extracts and essential oils have been very concerned about the chemical structure and biological processes in the nutrition and drug industries (12). Although some studies have been conducted on *Eucalyptus camaldulensis* as an antioxidant or anticancer compound (13), there is no research on the use of *Eucalyptus camaldulensis* extracts as antioxidant and anticancer agents. As scientists have reported, crown gall cancer reduction (induced by A. tumefaciens) on potato disks is obviously in line with natural products involved in anticancer test 3PS (leukemic mouse model).

In their study, Ashraf, Sarfraz (14) have also confirmed that considering the molecular mechanism, a potatoes disc test is a potent predictor of tumor activity. Despite the advancements in the detection, determination, and treatment of breast cancer, this malignancy is one of the deadliest tumors threatening the life of women. A comprehensive and efficient treatment for these patients includes systemic chemotherapy and endocrine treatment. For repetitive metastatic breast cancer (rMBC), one of the most viable treatment choices is systemic chemotherapy (15).

Some chemical compounds, such as acetate, crystallization substance, valeric aldehyde, and ethylic alcohol, are present in a small amount. cineole (60%-80%) is the most important and main material found in most species of eucalyptus (16). The leaves of Eucalyptus are the only used part in the treatment. The middle-aged leaves are more appropriate and can be used as a refrigerant, disinfectant, and vasoconstrictor. Moreover, Eucalyptus leaves contain tryneol, aliphatic aldehyde, eucalyptol (cineol), phenols, sesquiterpene alcohols, isoamyl alcohols, terpenes, and flavonoids (15). In light of the aforementioned issues, the present study amid to evaluated the cytotoxic activity of crude extract of *Eucalyptus camaldulensis* plant against breast cancer MCF-7 cell line. Furthermore, it assessed the antioxidant activities of various concentrations of solvent ethanolic extract of Eucalyptus camaldulensis.

2. Materials and Methods

2.1. Plant Materials: Preparation of Extract

Dried *Eucalyptus camaldulensis* plant was extracted by the soxhlet with ethanol 70%. The extracts were completely removed using a rotary evaporator and a semi-solid mass was obtained; thereafter, it was transferred to an oven to produce the crude extract and stored at 4°C until use (17).

2.2. Phytochemical Screening of Eucalyptus camaldulensis Extract

Phytochemical constituents, such as tannins, saponins, phlorotannins, phenolics, reducing sugar, terpenoid, steroid, glycosides, alkaloids, and flavonoids

of the crude extracts were analyzed. *Eucalyptus camaldulensis* extracts (2 ml each) were separately utilized for each analysis in a way that the formation of a precipitate, color change, or frothing indicates the presence of the phytochemicals mentioned above (18).

2.3. Fourier Transmission Infra-Red Assay

Fourier transmission infrared (FTIR) and UV Spectrum (Shimadzu) analyses were performed in the Lab of Iben Sena center/ University of Baghdad, Iraq.

2.4. Radical Scavenging Activity by DPPH

The evolution of scavenging activities by the utilization of the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical was performed. The DPPH radical antioxidant activity of the ethanolic extract was evaluated based on the technique published in the references (19). The DPPH radical has the highest absorptance at 515 nm and vanishes with degradation by a scavenging molecule. The DPPH• environment in methyl alcohol (0.006 mM) was synthesized every day, and 3.0 mL of this solution was combined with 100 µL of plant extract solution (6.125, 12.5, 25, 50, and 100 µg/mL). The incubation of each sample was at 37°C for 20 min., and absorbance was reduced at 515 nm. A solution of DPPH• (100 µL) of methyl alcohol as a blank solution was prepared for the evaluation of the absorption. The measurement was repeated in triplicate. The antioxidant activity was calculated according to Equation (1):

$$\text{Inhibition activity} = \frac{\text{Blank absorbance} - \text{Extract absorbance}}{\text{Blank absorbance}} \times 100 \quad (1)$$

2.5. Radical Scavenging Activity by ABTS

The antioxidant efficiency of *Eucalyptus camaldulensis* extract was also investigated utilizing the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay (20), according to reduce of ABTS+• radicals by scavengers of the plant *Eucalyptus camaldulensis* extract examined. The double deionized water was used to dissolve the ABTS compound (0.007 M). ABTS+• was generated through the chemical reaction of ABTS medium with 0.00245 M $K_2S_2O_8$, and the solution was placed in the dark at 303 K for 16 h. In the current investigation, ethyl alcohol was used to dilute the solution of ABTS+• to an absorbance of 734 nm. Ethyl alcohol as a reading of blank solution was used. Following that, 00 µL of Alcoholic extract of *Eucalyptus camaldulensis* was added to the solution (3 mL) of ABTS+•, at 303 K. All tested solutions were utilized after 10-60 min of preparation. Each experiment was conducted three times, and the mean was used. The inhibition performance of *Eucalyptus camaldulensis* solution against ABTS+• was evaluated regarding Equation (1).

2.6. Detection of the Toxicity: Maintenance of Cell Cultures

Breast cancer MCF-7 cell line, from the unit of Cell Bank of Iraq biotech, was reserved in RPMI-1640 supplemented by 10% fetal bovine serum, streptomycin, and penicillin antibiotics (100 units/mL). Cells were passaged by Trypsin-EDTA and re-seeded at 50% confluence for two weeks at 37 °C (21).

2.7. Cytotoxicity Assays

Cytotoxic effects were determined by the MTT test. The ethanolic extract of *Eucalyptus camaldulensis* was used at different concentrations (6.125, 12.5, 25, 50, and 100 µg/mL). Cell lines were cultured at 1×10^4 cells following overnight incubation, the monolayer was achieved, and the extract was then applied to the cells. After 72 h, the viability of the cells was measured by the addition of 28 µL of 2 mg/mL solution of MTT after removing the medium and incubating the cells at 37°C for 1.5 h. Subsequently, the MTT solution was eliminated, and the remaining crystals were solubilized in the wells by adding 130 µL of DMSO then incubated at 37°C for 15 min with shaking (22). The absorbency was measured by a microplate reader at 492 nm. The percentage of cytotoxicity was determined using the following equation (2):

$$\text{Inhibition activity} = \frac{\text{Control optical density} - \text{Sample optical density}}{\text{Control optical density}} \times 100 \quad (2)$$

3. Results and Discussion

3.1. Phytochemical Screening

Preliminary Phytochemical Screening of *Eucalyptus camaldulensis* extract revealed that the phlorotannins, reducing sugar, and steroids were absent in Ethanolic extract of *E. camaldulensis*. On the other hand, the active compound present in the alcoholic extract of *Eucalyptus camaldulensis* include Tannins, Saponins, Phenolic compounds, reducing sugar, Terpenoids, Steroids, Glucosides, and Alkaloids. Wagner's test and Flavonoids Ferric chloride test are positive as displayed in table 1.

Table1. Preliminary Phytochemical Screening of *Eucalyptus camaldulensis* extract

Types of Active compound	Presence in /alcoholic
Tannins	+ve
Saponins	+ve
Phenolic compounds	+ve
Reducing sugar	-ve
Terpenoids	-ve
Steroids	+ve
Glucosides	-ve
Alkaloids Wagner's test	+ve
Types of Active compound	Presence in /alcoholic

3.2. Fourier Transmission Infra-Red Assay

As presented in Figure 1, the high severe band 3317-3340 cm-1 refers to (OH) groups; moreover, o band 1350,1037 cm-1 signifies the incidence of asymmetrical patterns to the CH3 groups of alcoholic composite, and 1618/cm-1, band refer to the incidence of C=C group (23).

3.3. Anticancer activity of *Eucalyptus camaldulensis*

The cytotoxic activity was examined as illustrated in figure 2. Ethanolic extract of *Eucalyptus camaldulensis* treatment with some different concentrations (6.125, 12.5, 25, 50, and 100 µg/mL) indicated there is a decrease in the percentage of MCF-7 cell viability and an increase in the cytotoxic activity on the MCF-7 cells.

The cytotoxic activity of Ethanolic extract of *Eucalyptus camaldulensis* elevates with the increased concentration of the extract. The results indicated that the treatment of the cells with *Eucalyptus camaldulensis* significantly inhibited the growth of cells with an increase in extract concentration. The results pointed out that *Eucalyptus camaldulensis* is a valuable source of effective cytotoxic and anti-proliferative agents. The crud extract of *Eucalyptus camaldulensis* demonstrates apoptosis-inducing and proliferation-inhibiting effects in the human body, killing cancer cells. In the present study, the anti-tumor effect of breast cancer cells (MCF-7 cell line) was investigated using the crude alcoholic extract of *the Eucalyptus camaldulensis* plant. The induction of apoptosis by anticancer drugs through their antitumor effects against cancer cells is a significant phenomenon in cancer chemotherapy (24).

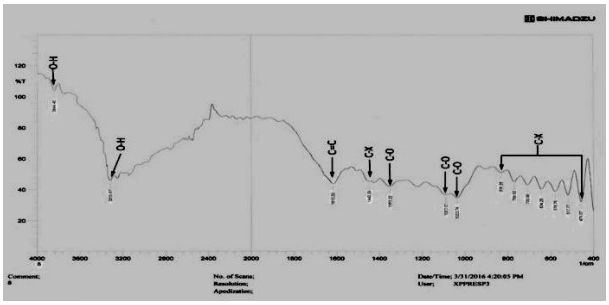

Figure 1. Fourier transmission infra-red analysis of Eucalyptus camaldulensis alcoholic crud extract

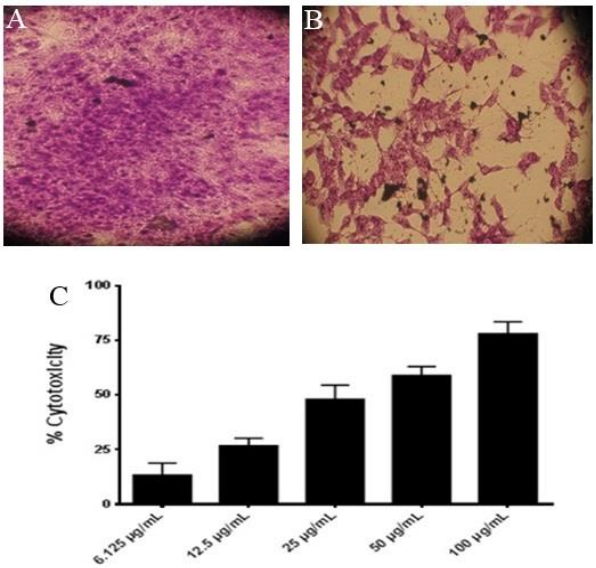

Figure 2. Cytotoxic effect of *Eucalyptus camaldulensis* on breast cancer cell line

In comparison to the typical round nuclei of the control, crud extract-treated cells show up condensed and fragmented nuclei. It was noticed that the level of apoptotic cell demise was maximum. The current study supported the use of this plant due to its significant effects on cancer cell lines and suggested that it can be used to combat cancer; therefore, further in vitro and in vivo studies are needed to shed more light on the mechanisms of cell death (25).

Cancer is one of the leading causes of death across the globe. Resistance to the drugs used in anticancer therapies highlights the urgent need to conduct further research to discover plant-derived substances that act as anticancer. Many plants, vegetables, herbs, and spices might have potential uses in medicine as a source for the prevention of cancer; therefore, further studies are required to identify the biological properties and therapeutic potential of these plants (26). The results of the present study are in agreement with those obtained by Salomons, Smets (27) who revealed that the cell viability decrease with an increase in extract concentration. Moreover, in vitro studies proved that ethanolic extract of *Eucalyptus camaldulensis* leaves exerts a cytotoxic effect on some types of leukemia, such as human chronic myelogenous leukemia K562 cells.

Eucalyptus contains many active compounds, such as alkaloids, polyphenols, flavonoids, tannins, steroids, sterols, glycosides, and fatty acids. Some of these compounds, such as phenols and alkaloids, have significant antioxidants and anti-cancer activities; moreover, the apoptosis of cancer cells can be induced by phenol. In the same way, anti-mutagenic and anti-cancer effects have been reported for flavonoids. Moreover, they have a protective effect against cancer by their effect on signal transduction in angiogenesis and cell proliferation (28). Furthermore, the results of this study are in line with those indicated by Sun, Heilmann (29) who worked on the K562 cell line and found that 50 µg/ml had the highest cytotoxic effect and it is the best concentration in destroying the cancer cells.

The alcoholic extract of *Eucalyptus camaldulensis* demonstrated the inhibitive performance (94.51% of DPPH inhibition) at the highest investigated concentration. The results of ABTS which started from 67.6%-97.1% (as various concentrations of alcoholic extract of *Eucalyptus camaldulensis*) was better than that of DPPH analysis. The alcoholic extract of *Eucalyptus camaldulensis* possessed the highest scavenging activity of 97.1% regarding the highest utilized concentration. Scavenging activities of alcoholic extract of *Eucalyptus camaldulensis* depended on the concentration of plant extract and also the conditions of the utilized measurements.

Many reported techniques are available for the in vitro calculation of total antioxidant power, which could be divided into two kinds: hydrogen atom transfer testing (HAT) and electron transfer testing (ET). The HAT-based testing, such as the ORAC test, uses a dynamic reaction system in which the scavenger and substrate compete for radicals generated thermally. It is known and certain that there is one sufficient and comprehensive technique to identify the efficiency of a particular substance and study its ability as an antioxidant. The types of action and mechanisms of different antioxidants must be taken into account where more than one type of measurement method must be performed on the strength of antioxidants (30).

In the present research, the free radical removal ability of a plant extract was determined using DPPH and ABTS techniques (Figures 3 and 4). The DPPH and ABTS measurements have been used extensively and with multiple concentrations of plant extract to determine the viability of the plant extract as an antioxidant since it requires relatively standard equipment and delivers rapid and reproducible results. In fact, comparative studies of six recently published methods of determining antioxidant capacity have demonstrated that DPPH and ABTS techniques are

faster, easier, and more real (31). The ABTS scale is a technique of particular interest in plant extracts since wavelength absorption at 734 nm eliminates color interference (32). Moreover, this test is easy to perform, like the DPPH and ABTS assays (33).

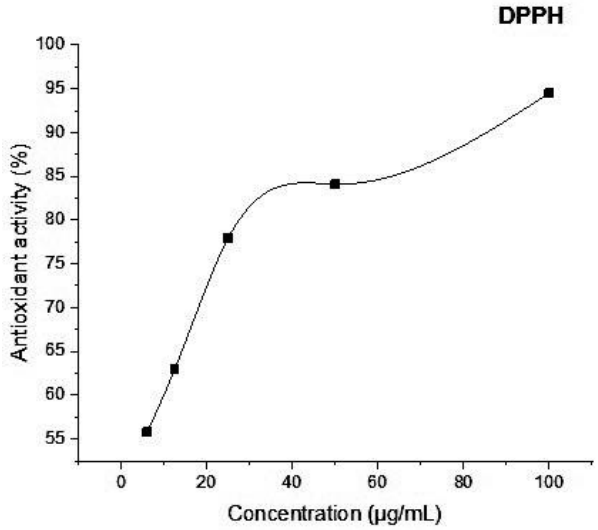

Figure 3. Antioxidant activity of alcoholic extract of *Eucalyptus camaldulensis* using DPPH

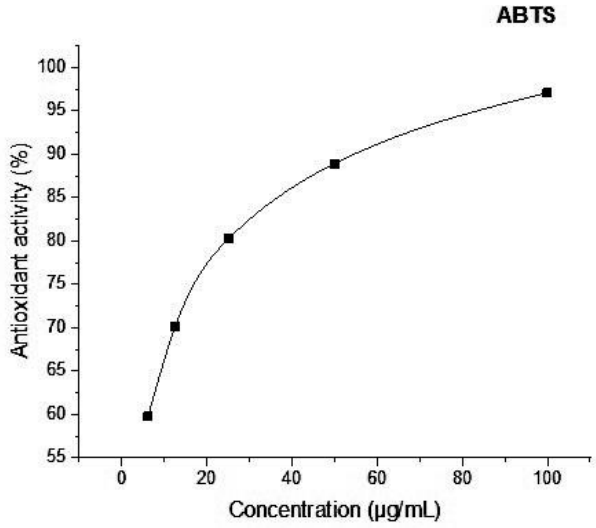

Figure 4. Antioxidant activity of *Eucalyptus camaldulensis* alcoholic extract using ATBS

As evidenced by the findings of the present study, it may be concluded that *the Eucalyptus camaldulensis* plant can be used as a probable source of innate cytotoxic potential. Nevertheless, further research is needed for the identification of biological activity for the compounds present in this plant. The ethanolic extract demonstrated a wide range of potentially promising antioxidant efficiencies; moreover, ethanolic showed a significant scavenging effect using all measurement techniques.

Authors' Contribution

Study concept and design: W. H. M.
Acquisition of data: S. A. S.
Analysis and interpretation of data: W. H. M.
Drafting of the manuscript: A. h. H.
Critical revision of the manuscript for important intellectual content: W. H. M.
Statistical analysis: N. N. H.
Administrative, technical, and material support: W. H. M.

Conflict of Interest

The authors declare that they have no conflict of interest.

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