Original Article

Cytotoxic Effects of *Eucalyptus Camaldulensis Dehnh* Leaf Extract on KB Human Carcinoma Cell Line

Zohreh Jaafari-Ashkvandi [1], DMD, MSc; Nasrollah Erfani [2], MSc; Azadeh Hamedi [3], MSc; Somayeh Rezaeifard [4], MD Student; Shadi Tivay [5], MSc;

[1] Dept. of Oral and Maxillofacial Pathology, Biomaterials Research Center, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[2] Dept. of Immunology and Shiraz Institute for Cancer Researches, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
[3] Dept. of Pharmacognosy, School of Pharmacology, Shiraz University of Medical Sciences, Shiraz,
[4] Student Research Committee, Shiraz Institute for Cancer Research, School of Medicine, Shiraz, University of Medical Sciences, Shiraz, Iran.
[5] Dept. of Oral and Maxillofacial Pathology, Biomaterials Research Center, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.

KEY WORDS
Cell viability;
Plant Extracts;
Apoptosis;
Cell Proliferation;
Carcinoma;

Received: 23 November 2025;
Revised: 4 January 2025;
Accepted: 21 February 2026;

ABSTRACT

Background: The cytotoxic properties of isolated natural compounds from herbal medicines have been revealed in different cancer cell lines.

Purpose: The aim of this study was evaluating cytotoxic and apoptotic effects of *Eucalyptus camaldulensis Dehnh*. leaf extract on KB human carcinoma cell line.

Materials and Method: In this experimental study, an ethyl acetate extract of dried leaves of *Eucalyptus camaldulensis* was prepared and dried via a speed vacuum apparatus at 40°C. KB cells were treated with 0, 0.25, 0.5, 0.75 and 1 mg/mL extract. Cisplatin was used as the positive control. MTT assay and Annexin V/7-AAD staining were used to evaluate the cytotoxic and apoptotic effects.

Results: The IC_{50} values for the *Eucalyptus camaldulensis* extract were 0.75±0.07 mg/mL and 0.56±0.05 mg/mL at 24 and 48 hours, respectively. Apoptosis increased after 24 hours in a dose-dependent manner and a higher necrotic/late apoptotic population was detected after 48 h.

Conclusion: The ethyl acetate extract of *Eucalyptus camaldulensis* inhibited KB cell growth via the induction of early apoptosis and late apoptosis; therefore, it might be a potential anticancer agent. The identification and purification of active components are suggested to achieve more conclusive results.

Corresponding Author: Jaafari-Ashkavandi Z, Dept. of Oral and Maxillofacial Pathology, Biomaterials Research Center, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98-7136263193-4 Email: jaafari181@gmail.com

Cite this article as:

Introduction

Oral cancer is the main cause of death in patients with head and neck cancer, and more than 90% of these malignancies are oral squamous cell carcinoma (SCC). Some common benign oral diseases may progress and transform into this aggressive cancer. Surgical excision is the primary approach for treating SCC [1]. Radiotherapy and chemotherapy are often used as adjuvant therapies to increase the effectiveness of treatment [2]. All these approaches have serious side effects, including disability from surgery, chemical and radiation-induced toxicity, and suppression of the bone marrow. Most of these effects are related to nonspecific cell death. Moreover, recent studies have shown that some chemotherapeutic agents increase the likelihood of cancer metastasis [3-4].

Therefore, there is a growing interest in identifying novel anticancer compounds from natural sources.

Many recent studies have investigated the effects of natural and herbal substances on cancerous cells to discover alternative methods of treatment [5-7]. Plants of the Eucalyptus (E.) genus have been explored in the literature for their antioxidant, antiseptic, antibacterial and anti-inflammatory therapeutic properties as well as their potential effects on cancerous cells [6-7].

Over time, significant progress has been made in the extraction of plant secondary metabolites. Different extracts of E. spices are considered for their cytotoxic effects on a variety of cell lines, such as colon cancer [8-10]. A spray-dried water extract of *E. rubosta* was reported to have a greater toxic effect on pancreatic cancer cells than gemcitabine, the best chemotherapeutic agent for this type of cancer [11]. *E. camaldulensis* essential oil was also shown to have antioxidant and anticancer effects through the induction of apoptosis in a colorectal cancer cell line [12]. Moreover, this species showed antibacterial, antiviral, and anticancer properties in an *in vitro* study [8-9, 13]. Furthermore, another study proposed the combination of *E. camaldulensis* with a low dose of cisplatin as a new therapeutic concept to treat lung and breast adenocarcinoma [14]. The cytotoxic potential of *E. benthamii* essential oils on several tumor cell lines, including T-cell leukemia and the cervix, has been demonstrated [15]. The aqueous extract of *E. citriodora* reduced the number of cells in the G_0/G_1 phase of the cell cycle and induced apoptosis [16].

Different solvents are used in natural-product extraction because solvent polarity strongly determines which classes of secondary metabolites are recovered. Ethyl acetate, as a medium-polarity solvent, preferentially extracts mid-polar bioactive compounds such as phenolic acids and flavonoids (especially aglycones) and oxygenated terpenoids [17]. These compound classes are frequently associated with cytotoxic and antiproliferative effects and generally show good solubility in cell culture media, improving reliability of MTT-based cytotoxicity evaluation. Ethyl acetate fractions often exhibit enriched total phenolic and flavonoid contents compared with more polar aqueous fractions or nonpolar solvents, which supports its suitability for isolating mid-polar secondary metabolites [18]. In one study, results from six different fresh extracts of E. leaves revealed that all except methanolic extract, showed strong cytotoxicity against epithelial cancer cell lines [19].

Owing to the favorable effects of various plant extracts from the E. family on different cancer cell lines, the present study was conducted to investigate the cytotoxicity and apoptosis-inducing effects of *E. camaldulensis* leaf extracts on the KB human carcinoma cell line. This cell line is widely used as an *in vitro* model in oral cancer research, yet the cytotoxic and apoptosis-inducing effects of *E. camaldulensis* leaf extracts on this cell line have not been previously reported. Therefore, this study was designed to evaluate the cytotoxicity of *E. camaldulensis* leaf extracts and to determine whether the observed growth inhibition is mediated through apoptosis induction in KB cells. This investigation may provide preliminary evidence supporting the potential of *E. camaldulensis* as a source of natural anticancer agents for oral carcinoma and justify further mechanistic and *in vivo* studies.

Materials and Method

Preparation of *E. camaldulensis* leaf extract

For the preparation of *E. camaldulensis* leaf extract, fresh leaves of *E. camaldulensis* were collected from the Shiraz Garden Research Center in the winter and validated by a pharmacognosist at Shiraz University of Medical Science. The leaves were air-dried at room temperature for two weeks and then powdered via a cracking mill. Ethyl acetate was used as the solvent, and extraction was performed using a Soxhlet apparatus for 4 hours. The extract was concentrated in a rotary evaporator at 40°C. The mixture was subsequently dried in a speed vacuum apparatus at 40°C for 6 hours. The dried extract was stored at -20°C.

Cell Lines and Culture Medium

The KB cell line was prepared from the Pasture Institute cell bank in Tehran, Iran. Only authenticated KB cultures confirmed to be mycoplasma-free were included. The cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Wexford, Ireland); finally, they were cultured in a 5% CO_2 environment at 37°C.

MTT cell viability assay

KB cells were cultured in 96 flat-bottom microplate wells. Each well contained 10000 cells in 100 µL of cell culture media and was incubated for 24 hours at 37°C with 5% CO_2. To solve the ethyl acetate extraction in

RPMI-1640, we prepared a concentration of 3 mg/mL with 0.2% ethanol and placed it in an ultrasonic water bath for 15 minutes. The primary thick solution was diluted with 10% FBS-RPMI-1640 to different concentrations (0.25, 0.5, 0.75 and 1 mg/mL). The cells without treatment and with several concentrations (25, 50 and 100 µM) of cisplatin (Mylan Company, Greece) were considered negative and positive controls, respectively. After 24 hours of incubation, different concentrations of cisplatin and *E. camaldulensis* extract were added to the microplates. The cells were divided into two groups after 24 and 48 hours of incubation (37°C, 5% CO_2). Following incubation, 100 µl of 0.5 mg/ml MTT solution was added to the wells. The plates were incubated for 4 hours at 37°C, and the MTT solution was completely removed. Then, 150 µL of DMSO was added to the wells, which were incubated for 4 hours in 5% CO_2 at 37°C. The optical density (OD) of each well was measured via spectrophotometry at a wavelength of 492nm. Each experiment was independently repeated three times. The percentage of cytotoxicity was calculated with the following formula: 100-(OD of experimental cells)/ (OD of negative control cells) ×100. Finally, the concentrations that inhibited the viability of 50% of the cells (IC_{50}) were obtained. A t test was used to compare the cytotoxic effects of the extracts at various concentrations and for various durations of treatment.

Annexin V/7-AAD Apoptosis Assay

150000 KB cells were treated with different concentrations of *E. camaldulensis* extract (0, 0.25, 0.5 and 1mg/mL) for 48 hours. The untreated cells (0mg/mL of extract) were considered the negative control group. Necrosis and apoptosis were determined with a PE Annexin V Apoptosis Detection Kit. According to the manufacturer's instructions, 2 µL of Annexin V (An. V), and 2µL of 7-AAD (7-A) staining solution was added to each test tube containing binding buffer and cells. After 15 minutes of incubation in the dark, the cells were analyzed via a flow cytometer (BD FACSCalibur (USA)) and then analyzed via FlowJo software. Viable cells were An. V- and 7-A-negative; the cells in early apoptosis were An. V positive and 7-A negative; the cells in late apoptosis were both An. V and 7-A positive; and the necrotic cells were An. V negative and 7-A positive.

Results

The MTT cell viability assay revealed the cytotoxic effects of different concentrations of *E. camaldulensis* extract, and the results are shown in Figure 1. The IC_{50} values for the *E. camaldulensis* extract were 0.75±.07 and 0.56±.05 mg/mL at 24 and 48 hours, respectively, and the IC_{50} values for cisplatin were 18.1 and 8.7µg/mL at 24 and 48 hours, respectively. Both agents inhibited cell growth in a time- and dose-dependent manner. Student's T- test revealed a significant difference in the cytotoxic effects of the extract after 24- and 48-hour treatments compared with those of the untreated cells at all concentrations (p= 0.01) and between the 24-hour-treated cells and the 48-hour-old cells at the IC50 concentrations (p=0.02).

The cytotoxicity increased from 6.7% to 90.9% at 24 h and from 8.1% to 91.7% at 48 h, while the concentration increased from 0.25 to 1 mg/mL (Figure 1A). The cytotoxicity of cisplatin increased from 3.8% to 75.2% at 24 hours and from 35.7% to 91.5% at 48 hours as the concentration increased from 7.5 to 30µg/mL (Figure 1B). The results of Ann. V/7-A staining of cells treated with *E. camaldulensis* extract for 24 hours is shown in Figures 2A and 3A.

The percentage of early apoptotic cells increased from 12.8% to 24.1% within 24 h, while the concentration increased from 0.25mg/mL to 1mg/mL

The highest rate of late apoptosis/secondary necrosis

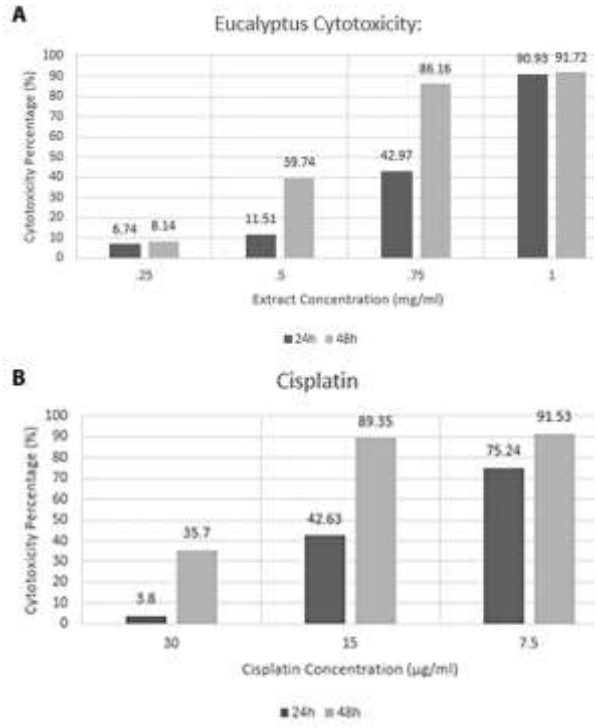

Figure 1: A: Cytotoxic effects of *E. camaldulensis* extract on KB cells after 24 and 48 hours, **B:** Cytotoxic effects of Cisplatin on KB cells after 24 and 48 hours

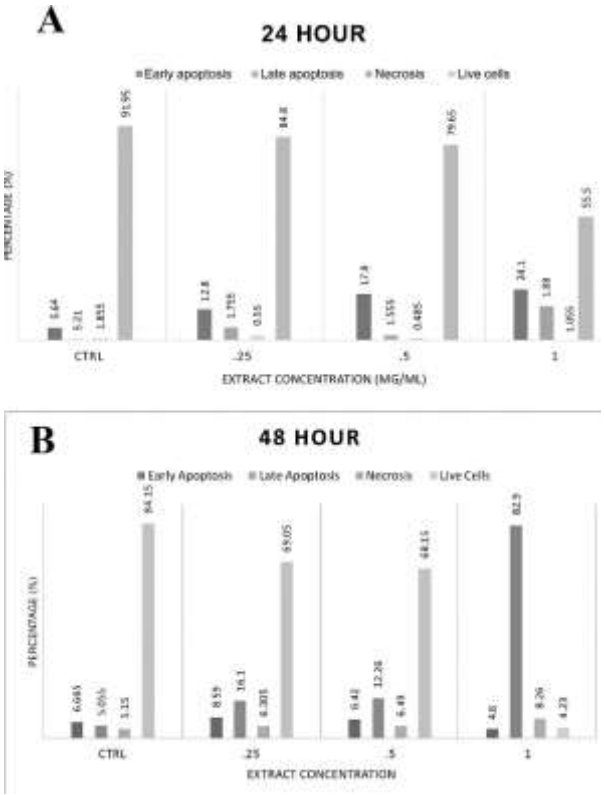

Figure 2: Results of apoptosis assay in KB cells treated by *E. camaldulensis* extract, **a:** after 24 hours, **b:** after 48 hours

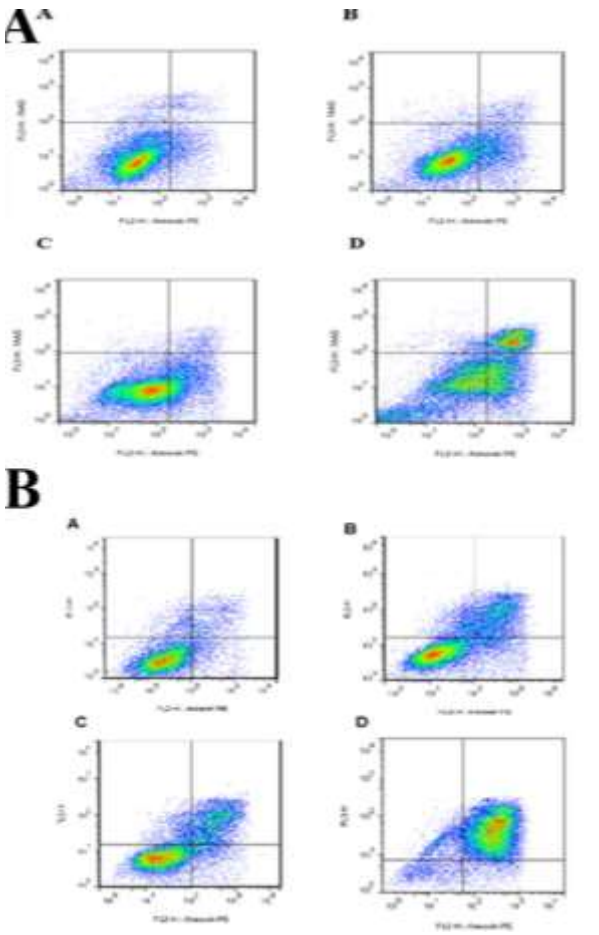

Figure 3: **a:** 24-hour, **b**: 48 hour- Annexin V/7AAd assay: A, apoptosis and necrosis in untreated KB cells; B, in 0.25 mg/mL; C, in 0.5mg/mL, D, in 1 mg/mL concentrations of *E. camaldulensis* extract

was found with the 1 mg/mL extract after 24 h (1.6%). The results of An. V/7-A staining of the *E. camaldulensis* extract after 48 hours are presented in Figures 2B and 3B. At higher concentrations, a marked shift toward the An. V+/7-AAD+ quadrant was observed, indicating enhanced late apoptosis/secondary necrosis, while the An. V−/7-AAD+ population (primary necrosis) remained comparatively lower. The percentage of early apoptotic cells decreased from 8.6% to 4.6% at 48 h, while the concentration increased from 0.25 mg/mL to 1 mg/mL. The highest rate of late apoptosis/secondary necrosis was associated with the 1 mg/ml extract after 48 hours (8.26%).

Discussion

Chemotherapy drugs have cytotoxic effects on normal cells that lead to systemic problems and drug resistance in cancer patients [20]. Currently, many natural products and phytochemicals have attracted the attention of researchers for drug development against cancer. This study focused on the cytotoxic effects of ethyl acetate extraction from *E. camaldulensis* leaves on the KB cell line and its ability to induce apoptosis after 24 and 48 hours of treatment. One study revealed that different extracts of E.leaf had cytotoxic effects on different cell lines. Among them, the ethyl acetate extract showed the greatest cytotoxic effect [19]. Therefore, ethyl acetate was used for this study.

According to the results of the MTT cytotoxicity assay, the E. extract suppressed KB cell growth in a dose- and time-dependent manner, although this inhibition was not as potent because this extract was composed of several compounds. Some major compounds in E. extracts are 1-8 cineole, polyphenols, terpinen 4-OL, tannins, etc. 1-8 cineole, which is known as eucalyptol, suppresses Survivin and S-phase and activates the p_{38} protein. Furthermore, it increases apoptosis by increasing the levels of poly (ADP–ribose) polymerase (PARP) and caspase-3 [21-22]. This natural product has been shown to have anticancer effects via its ability to inhibit proliferation and reduce β-catenin expression in head and neck SCC cells [22]. Some polyphenols have anticancer effects through the regulation of signaling path-

ways, such as mitogen-activated protein kinase (MAPK), nuclear factor KB, and activator protein-1 [23-24]. Another study reported that *E. globulus* essential oil exhibited cytotoxic effects on colon and liver cell lines only at high doses [25].

The results showed that after 24 h, the E. leaf extract induced apoptosis without any significant necrosis. After 48 hours, an increase in late apoptosis was observed. There is a balance between cell proliferation and cell death, which leads to apoptosis in damaged cells. Apoptosis is a type of planned cell death that plays an important role in physiological cell growth and hemostasis. Inadequate apoptosis plays a basic role in tumor progression [26]. One of the most important features of malignancy is low rates of apoptosis. Anticancer agents exert their growth inhibitory effects through two mechanisms: increasing apoptosis and necrosis and reducing cell proliferation. The regulation of apoptosis is one of the main goals of anticancer drug development [27].

In one study, *E. camaldulensis* essential oil induced apoptosis through significant upregulation and downregulation of *BAX* and *BCL-2* expression, respectively [12]. According to the study of the HepG2 cell line by Shen *et al.* [16], the aqueous extract of E. reduced the speed of the cell cycle in the G_0/G_1 stage and induced apoptosis. Moreover, Falih *et al.* [28] demonstrated that E. oil significantly induced the caspase 9-dependent pathway, as well as the caspase 8-dependent pathway, to a lesser extent.

After 24 h, the E. extract induced early apoptosis with less late apoptosis/ secondary necrosis. However, after 48 hours, more late apoptosis/ necrosis was induced. The necrosis mechanism causes immunologic cells to enter the tumor site and enhances the effects of chemotherapy drugs. The inflammatory response may damage tissues; induce the release of mitogens and cytokines; and increase cell growth, proliferation and metastasis [29].

Like *E. camaldulensis* extract, many chemotherapeutic drugs that are routinely used in head and neck cancer treatment, such as cisplatin, Taxol and fluorouracil, induce both necrosis and apoptosis [30]. Low concentrations of cisplatin help induce apoptosis via cell shrinkage and condensation and fragmentation of nuclear chromatin. Necrosis induction occurs at high concentrations of cisplatin via cytosolic swelling and damage to the plasma membrane [31-32]. Taxol is another chemotherapeutic drug that keeps cells in the G_2 phase of mitosis, inhibits proliferation, and induces apoptosis. At high concentrations, the drug increases the polymerization of microtubules and bundle formation, which prevents the cell from entering the S phase and induces necrosis [33]. In this study, the higher necrotic fraction observed at 48 h, may reflect progression from early apoptosis to late apoptosis/secondary necrosis, which is commonly reported following prolonged exposure or higher intracellular stress, particularly when apoptotic bodies are not cleared *in vitro*.

Conclusion

The results of the present study showed that the ethyl acetate extract of *E. camaldulensis* increased cell cytotoxicity in the KB cell line via the induction of both early and late apoptosis. The results of this study were in line with those of previous studies on the effects of E. extract on several cancer cell lines. Furthermore, identifying the active component of the ethyl acetate extract of *E. camaldulensis* and investigating its effects on animal models of SCC may be considerable.

Acknowledgments

This research program was funded by Vice-chancellery of Shiraz University of Medical Sciences (Grant # 95-01-03-13257). The authors are grateful to Dr. M. Vosoughi at Dental Research Development Center of Shiraz Dental School for statistical analysis. This manuscript is based on the undergraduate thesis of Sh. Tivay.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Almangush A, Mäkitie AA, Triantafyllou A, de Bree R, Strojan P, Rinaldo A, et al. Staging and grading of oral squamous cell carcinoma: An update. Oral Oncol. 2020; 107:104799.

[2] Patel G, Dalwadi C. Cytotoxicity and Cellular Uptake of 5-Fluorouracil Loaded Methylcellulose Nanohydrogel for Treatment of Oral Cance. Lett Appl NanoBioSci. 2021; 10: 1904-1918.

[3] Karagiannis GS, Condeelis JS, Oktay MH. Chemotherapy-induced metastasis: mechanisms and

translational opportunities. Clin Exp Metastasis. 2018; 35: 269-284.

[4] Huang SH, Hahn E, Chiosea SI, Xu ZY, Li JS, Shen L, et al. The role of adjuvant (chemo-)radiotherapy in oral cancers in the contemporary era. Oral Oncol. 2020; 102: 104563.

[5] Wang S, Long S, Deng Z, Wu W. Positive Role of Chinese Herbal Medicine in Cancer Immune Regulation. Am J Chin Med. 2020; 48: 1577-1592.

[6] Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. J Sci Food Agric. 2018; 98: 833-848.

[7] Abiri R, Atabaki N, Sanusi R, Malik S, Abiri R, Safa P, et al. New Insights into the Biological Properties of Eucalyptus-Derived Essential Oil: A Promising Green Anti-Cancer Drug. Food Rev Int. 2021: 38: 598-633.

[8] Abiri R, Atabaki N, Sanusi R, Malik S, Abiri R, Safa P, et al. New Insights into the Biological Properties of Eucalyptus-Derived Essential Oil: A Promising Green Anti-Cancer Drug. Food Rev Int. 2022; 38(sup1): 598-633.

[9] Grewal K, Joshi J, Rathee S, Kaur S, Singh HP, Batish DR. Chemical Composition and Potential of Eucalyptus camaldulensis Dehnh. Essential Oil and Its Major Components as Anti-inflammatory and Anti-leishmanial Agent. J Essent Oil Bear Plants. 2022; 25: 419-429.

[10] Ben Hamouda S, Zakraoui O, Souissi S, Bouzeyen R, Essafi M, Essafi-Benkhadir K. Deciphering the Mechanisms Underlying the Antitumor Effects of Eucalyptus Essential Oil and Its Component 3-Cyclohexene-1-Methanol Against Human Colon Cancer Cells. Int J Mol Sci. 2025; 26: 8876.

[11] Vuong QV, Hirun S, Chuen TL, Goldsmith CD, Munro B, Bowyer MC, et al. Physicochemical, antioxidant and anti-cancer activity of a Eucalyptus robusta (Sm.) leaf aqueous extract. Ind Crops Prod. 2015; 64: 167-174.

[12] Elham T, Saba G, Maryam S, Nasrin Seyyed S, Fatemeh Firouzi A, Masoud H, et al. Inhibition of Colorectal Cancer Cell Line CaCo-2 by Essential Oil of Eucalyptus camaldulensis Through Induction of Apoptosis. Acta Med Iran. 2020; 58: 260-265.

[13] Hanif H, Elikaei A, Vazini H, Mohammadi A. Anticancer and Antibacterial Effect of Eucalyptus Camaldulensis, in Vitro. Med Laboratory J. 2021; 15: 26-32.

[14] Nasser M, Damaj R, Merah O, Hijazi A, Trabolsi C, Wehbe N, et al. Potency of Combining Eucalyptus camaldulensis subsp. camaldulensis with Low-Dose Cisplatin in A549 Human Lung Adenocarcinomas and MCF-7 Breast Adenocarcinoma. Medicines (Basel). 2020; 7: 40.

[15] Döll-Boscardin PM, Sartoratto A, Sales Maia BH, Padilha de Paula J, Nakashima T, Farago PV, et al. In Vitro Cytotoxic Potential of Essential Oils of Eucalyptus benthamii and Its Related Terpenes on Tumor Cell Lines. Evid Based Complement Alternat Med. 2012; 2012: 342652.

[16] Shen KH, Chen ZT, Duh PD. Cytotoxic effect of Eucalyptus citriodora resin on human hepatoma HepG2 cells. Am J Chin Med. 2012; 40: 399-413.

[17] Gębalski J, Małkowska M, Wnorowska S, Gawenda-Kempczyńska D, Strzemski M, Wójciak M, et al. Ethyl acetate fraction from eleutherococcus divaricatus root extract as a Promising Source of Compounds with Anti-Hyaluronidase, Anti-Tyrosinase, and Antioxidant Activity but Not Anti-Melanoma Activity. Molecules. 2024; 29: 3640.

[18] Lagu IJL, Nyamai DW, Njeru SN. Phytochemical analysis, *in vitro* and *in-silico* study of antiproliferative activity of ethyl acetate fraction of *Launaea cornut-a* (Hochst. ex Oliv. & Hiern) C. Jeffrey against human cervical cancer cell line. Front Pharmacol. 2024; 15: 1399885.

[19] Bhagat M, Sharma V, Saxena AK. Anti-proliferative effect of leaf extracts of Eucalyptus citriodora against human cancer cells in vitro and in vivo. Indian J Biochem Biophys. 2012; 49: 451-457.

[20] Huang CY, Ju DT, Chang CF, Muralidhar Reddy P, Velmurugan BK. A review on the effects of current chemotherapy drugs and natural agents in treating non-small cell lung cancer. Biomedicine (Taipei). 2017; 7: 23.

[21] Murata S, Shiragami R, Kosugi C, Tezuka T, Yamazaki M, Hirano A, et al. Antitumor effect of 1, 8-cineole against colon cancer. Oncol repo. 2013; 30: 2647-2652.

[22] Roettger A, Bruchhage KL, Drenckhan M, Ploetze-Martin K, Pries R, Wollenberg B. Inhibitory Effect of 1,8-Cineol on β-Catenin Regulation, WNT11 Expression, and Cellular Progression in HNSCC. Front Oncol. 2017; 7: 92.

[23] Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and Anti-Inflammatory Role of Polyphenols. Nutrients. 2018; 10: 1618.

[24] Boukhatem MN, Sudha T, Darwish NHE, Chader H, Belkadi A, Rajabi M, et al. A New Eucalyptol-Rich

Lavender (Lavandula stoechas L.) Essential Oil: Emerging Potential for Therapy against Inflammation and Cancer. Molecules. 2020; 25: 3671.

[25] Khazraei H, Shamsdin SA, Zamani M. In Vitro Cytotoxicity and Apoptotic Assay of Eucalyptus globulus Essential Oil in Colon and Liver Cancer Cell Lines. J Gastrointest Cancer. 2022; 53: 363-369.

[26] Obeng E. Apoptosis (programmed cell death) and its signals: A review. Braz J Biol. 2021; 81: 1133-1143.

[27] Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol. 2020; 17: 395-417.

[28] Falih SMJ, Al-Saray ST, Alfaris AA, Al-Ali AAA. The synergistic effect of eucalyptus oil and retinoic acid on human esophagus cancer cell line SK-GT-4. Egypt J Med Hum Genet. 2022; 23: 70.

[29] Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol Immunol. 2021; 18: 1106-1121.

[30] Kitamura N, Sento S, Yoshizawa Y, Sasabe E, Kudo Y, Yamamoto T. Current Trends and Future Prospects of Molecular Targeted Therapy in Head and Neck Squamous Cell Carcinoma. Int J Mol Sci. 2020; 22: 240.

[31] Zhou J, Kang Y, Chen L, Wang H, Liu J, Zeng S, Yu L. The drug-resistance mechanisms of five platinum-based antitumor agents. Front Pharmacol. 2020; 11: 343.

[32] Fang CY, Lou DY, Zhou LQ, Wang JC, Yang B, He QJ, et al. Natural products: potential treatments for cisplatin-induced nephrotoxicity. Acta Pharmacol Sin. 2021; 42: 1951-1969.

[33] Urbaniak A, Piña-Oviedo S, Yuan Y, Huczyński A, Chambers TC. Limitations of an ex vivo breast cancer model for studying the mechanism of action of the anticancer drug paclitaxel. Eur J Pharmacol. 2021; 891: 173780.