Obtaining and Research of methanolic extract from eucalyptus plant

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Abstract

Because of its numerous medical characteristics, eucalyptus has been utilized for centuries as a medicinal plant. Methanol soxhlet extraction was used to assess the phytochemical content of Eucalyptus leaves. The methanolic extract maintained the greatest quantity of phytochemicals found in eucalyptus leaves. Also will clarify the structure of the biologically active compound using GC-MS technology. Examine the cytotoxicity of methanolic extract against cancer cell lines MCF7, HepG2, and PC3. This plant's extracts were also studied for their apoptosis and necrosis properties using high content screening (viability, nuclear, intensity, membrane potential, cytochrome releasing). The activity of methanolic compounds extracted from Eucalyptus will also be examined in order to analyze the extracts' potential as natural antioxidants. This is accomplished by examining the antioxidant properties of the extracts using DPPH. It is clear from the above. This study aims at the extraction of eucalyptus' major components from the leaves, the phytochemical screening of different compounds present in the leaves, and the study of the biological and pharmacological activity of these compounds.

Keywords: *Eucalyptus, Methanolic extract, GC-MASS.*

1. INTRODUCTION

Medicinal plants can help with a wide range of diseases, including cancer, bacterial infections, diabetes, and illnesses that cause inflammation. There are many different natural products in medicinal plants, like phenolic acids and flavonoids, which are highly valued for their anti-tumor and antioxidant properties. So, people think that substances that come from nature have fewer side effects than medicines like chemotherapy (1). The original species of Eucalyptus in Australia belongs to the Myrtaceae family, which has about 900 species (2). Essential oils can be made from a few different kinds of eucalyptus trees. Some species have stronger-smelling leaves than others, and the oils from different species can be used in very different ways (3). The Eucalyptus plant family contains biologically active compounds like steroids, tannins, polyphenolics, glycosides, terpenes, alkaloids, flavonoids, saponins, lignins, vitamin C, fatty acids, phenolics, triterpenoid flavones, anthocyanin, anthraquinone, coumarins, cardiac glycosides, terpenoids, and polyphenols (4) Even though EOs can be found in the leaves of more than 300 different species of Eucalyptus, less than 20 of them have been studied commercially for making EOs (5). Based on how they are made chemically, these EOs are complex mixtures of molecules that have anywhere from 20 to 80 different parts. terpenes and terpenoids make up most of the EOs made from eucalyptus leaves (6). A lot of research has been done on the chemicals, antioxidant power, and antibacterial properties of different

eucalyptus species (7). Eucalyptus oil is the most powerful antioxidant of all tree oils (8). Eucalyptus oil is the most powerful antioxidant of all tree oils (9). Chemicals in eucalyptus oil called terpinen-4-ol, -pinene, and -terpienene that fight cancer are called terpinen-4-ol, -pinene, and -terpienene (10). Terpinen-4-ol stops the cell cycle and causes necrosis in melanoma cells more than in other cells. It may also cause human melanoma cells to die by a process called caspase-dependent apoptosis, which has the potential to kill tumor cells. Researchers looked at how terpinen-4-ol affected cancer in human cell lines that had been exposed to cancer-causing chemicals (11). Terpinen-4-ol stopped the growth of colorectal, pancreatic, stomach, and prostate cancer in different ways (0.005-0.1%) depending on the dose. (12). The Myrtaceae family includes Eucalyptus camaldulensis. It is thought to contain compounds that have biological effects (13). The River Red Gum is a type of tree in the Eucalyptus genus. It is called Eucalyptus camaldulensis Dehn and is in the family Myrtaceae. This family has about 3800 species in tropical and subtropical areas all over the world. There are 140 genera in this family (14). Recent studies of the pharmacological and biological properties of E. camaldulensis leaf extract found that it is, among other things, antimicrobial, antioxidant, antifungal, anti-inflammatory, and anti-tumor (15). They showed that it makes sense to think that diseases start when people breathe in hot, wet E. camaldulensis vapors (16). Even though Eucalyptus camaldulensis has been studied as an antioxidant or anticancer compound (17), Eucalyptus Camaldulensis, the most common Eucalyptus genus in the Myrtaceae family, is widely planted in our area. Still, Eucalyptus camaldulensis leaves have many bioactive compounds like saponins, tannins, flavonoids, carbohydrates, and proteins (18).

2. Material and Methods

2. Material

2.1. Collection of plant

Fresh leaves of eucalyptus camaledonsis (age about 10 years) were collected from the south of Iraq in maysan city in 20 June. Fresh leaves were washed with water and then dried under sunlight after that put in the room for 17 days at room temp. 25±2C then grinding to a fine powder. This fine powder was used for the extraction. 900 g of the dried powder leaves were used for the extraction.

2.2. Chemical solvent

The biggest benefits of Soxhlet extraction are that high temperatures speed up mass transfer and fresh solvents can be reused (which further improves the transfer equilibrium). Because of these benefits, this method is more efficient at extracting than the other traditional methods. Polar compounds are extracted with methanol.

2.3 Method

2.3.1 Preparation of Extracts by the Soxhlet Method

A soxhlet apparatus was used to get the plant powder out of the plant. With a soxhlet apparatus and a methanol solvent, the dried, powdered plant material was taken out. In the Soxhlet machine, 250 ml of solvent was poured into each of the six chambers, which each held 150 g of eucalyptus powder. The temperature at which the water was boiling was kept at 67 oC and then dropped to 60 oC. The flask with the extraction solvent in it was heated until it started to bubble. An extra 6 hours were added to the extraction. After the extract was made, the solvent was taken away. The part of the solid that didn't dissolve in water stayed in the thimble and was thrown away. The extract was eventually taken out of the distillation flask and put through filter paper. The filtrate was put in the beaker and

put in a water bath at 67 oC to get rid of the solvent and get a dark green semi-solid extract. In the end, 14.5 g of a semi-solid extract was got.

2.3.2 GC-Ms analysis

On a GC Clarus 500 Perkin Elmer system with an AOC-20I auto sampler and a gas chromatograph connected to a mass spectrophotometer, GC-MS analysis was done under the following conditions: Column Elite-1 fused silica capillary column (30 mm 0.25 mm I.D., 100% dimethyl polysiloxane) worked in electron impact mode at 70 eV with helium (99.999%) as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 0.5 µ l. (split ratio of 10:1). The injector is at 250 °C, and the ion source is at 280 °C. The temperature of the oven was set to go from 110°C (no change for 2 minutes) to 200°C (10°C/min) to 280°C (5°C/min) to 280°C (no change for 9 minutes). The mass spectra were taken at 70 eV, with a scan interval of 0.5 s and fragments from 45 to 450 da. It took 60 minutes to run the whole GC.

2.3.3 MTT Cytotoxicity Assay

The anticancer activities of Eucalyptus leaf methanolic extracts are evaluated as shown below.

2.3.3.1. Cell Lines

1. MCF-7 Cell Line
2. PC3 Cell Line
2. HePG2 cell line

4. HdFn cell: from a fibroblast cell

2.3.3.2 Cytotoxic effect of eucalyptus leaf methanolic extract

This in vitro cytotoxicity method was performed to investigate the possible cytotoxic effect of the extract against the tumor cell lines MCF-7, HePG2, and PC3, with less effect against HdFn normal cells.

2.4. Antioxidant assay

Prieto et al. evaluated the total antioxidant activity of E. camaldulensis(19). Oyaizu's protocol predicted the reducing ability (20), but Zhang et al.'s method slightly modified it. A calibration curve was conducted in each case to express the results as mg of ascorbic acid equivalents per gram of dry plant sample (21). The ability of plant extracts to scavenge hydrogen peroxide was also tested. (22)

3. Result and discussion

3.1. Results

3.1.1. Soxhlet methods

The Soxhlet method produces a 14.5 gram, dark green, semi-solid methanol extract. As shown in table (1) below.

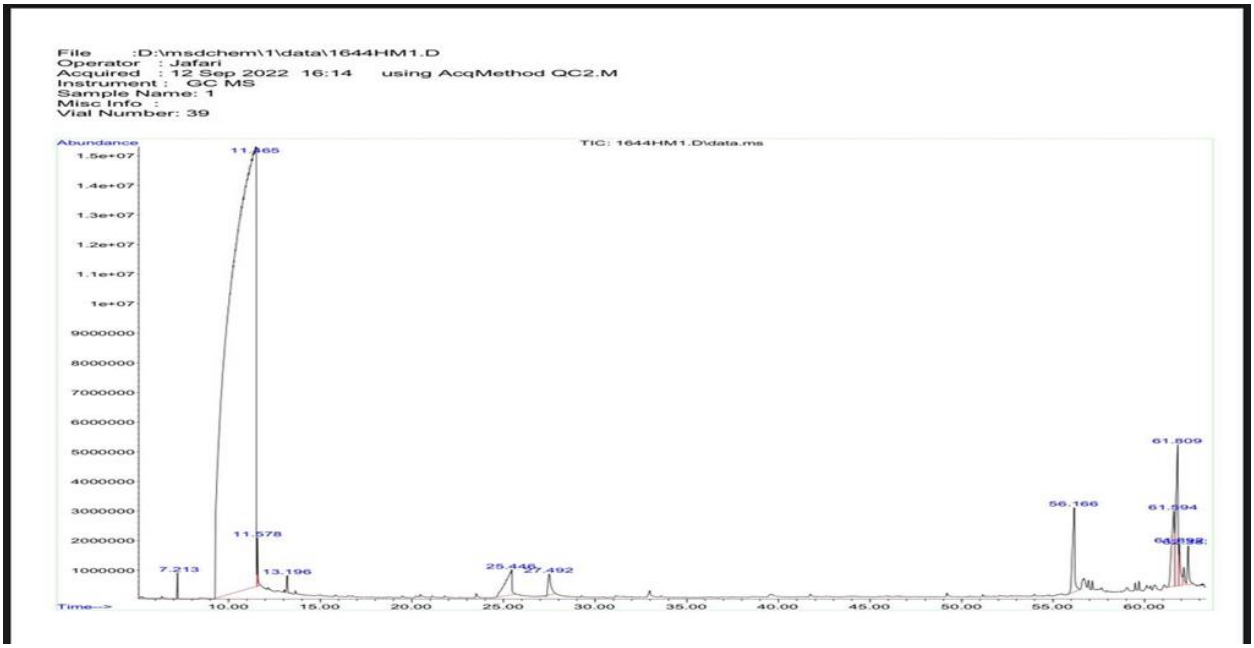
Table (1): The result of extraction soxhlet method

weight	color	case
14.5 gram	Dark green	semi-solid

3.1.2. GC-MS of methanolic extracts

In the leaves of the plant, compounds with different retention times can be seen in the chromatograms. According to Figure (1), the GC-MS chromatogram of the E. camaledonsis methanolic extract contains (11) distinct compounds. The bioactive compound found in the aerial portion of Eucalyptus camaledonsis can contribute to the plant's medicinal properties.

Figure (1): GC-MS of methanolic extract.



According to the curve's peak area percentage in Figure (1), the most prevalent phytochemicals 4 compounds in Camaledonsis methanolic extract are demonstrate as table (2)

Table 2: Important compounds found in methanolic extract

Compound	RT (min)	Corr. % max	Biological activity
Methane sulfinylbis	11.466	91.15 %	Antioxidant anti-inflammatory anticholinesterase activity, anti-histamine [23]
Benzoic acid	25.445	1.32%	Anti-fungal and antibacterial [24-25]
n-Hexadecanoic acid	56.165	1.48 %	Anti-inflammatory [26].
9,12-Octadecadienoic acid (Z,Z)-	61.595	1.69 %	antioxidant anticancer [27]

3.1.3. MTT Assay

The anticancer activity was studied by looking at the cytotoxic effect. Using the MTT assay for 24 hours, the methanolic extract of Eucalyptus camaledonsis leaves was effective against three types of cancer cells (MCF-7, HepG2, and PC3) and one type of normal cell (HdFn).

The results of a test to see if a human breast adenocarcinoma cell line (MCF-7) was sensitive to cytotoxicity (Figure 2). When the concentration of the extraction was 50 micrograms/ml, the number of living cells dropped sharply to 85%. At a concentration of 250 micrograms/ml, there was only 54% viability, which was a pretty consistent pattern. The researchers were interested by the fact that the half maximal inhibitory concentration (IC50) was 102.3 micrograms/mL.

Figure 3 shows the results of a test for cytotoxicity done on a HepG2 cell line from a person with liver cancer. When the concentration of the extraction was 50 micrograms per milliliter, the number of living cells dropped sharply to 82%. At a

concentration of 250 micrograms/ml, the patterns of death were pretty consistent, with up to 42% of the cells still alive. The researchers were interested by the fact that the half maximal inhibitory concentration (IC50) was 32.17 micrograms/mL.

Figure 4 shows what happened when the human prostate cancer (PC3) cell line was tested for cytotoxicity. When the concentration of the extraction was 50 micrograms per mL, the number of living cells dropped sharply to 90%. At a concentration of 250 micrograms per ml, the patterns of death were pretty consistent, with up to 50% of the cells still alive. The IC50 value of 59.02 micrograms/mL (half maximal inhibitory concentration) was especially interesting.

Figure (2): Activity of methanolic extract against MCF7 Cell line.

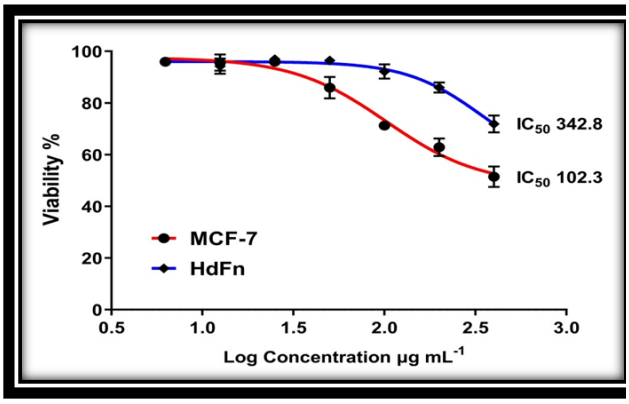


Figure (3): Activity of methanolic extract against HepG2Cell line.

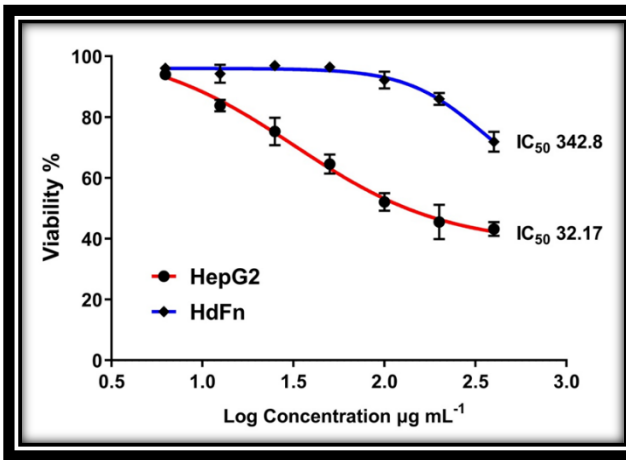
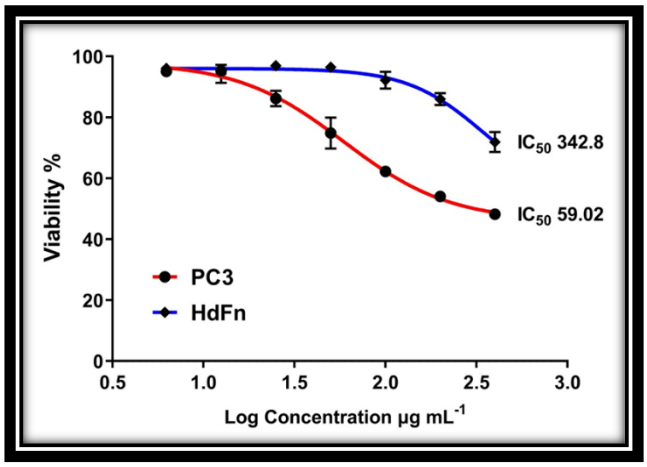


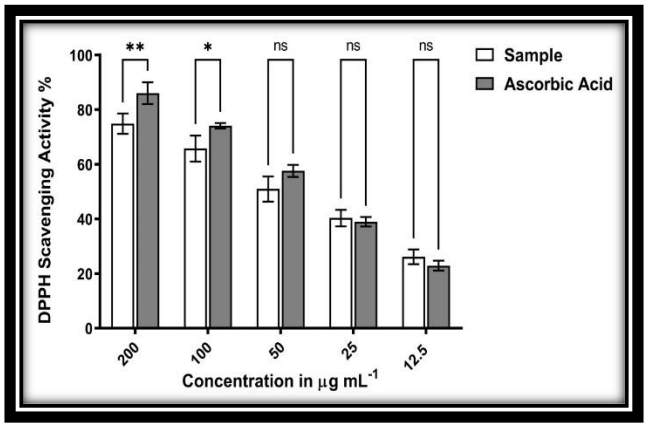
Figure (4): Activity of methanolic extract against PC3Cell line.



3.1.4 Scavenging activity DPPH

At a concentration of 100 micrograms/mL, E. camaledonsis leaf methanolic extract scavenged DPPH with 65% efficiency. A dose-response relationship was observed, with the percentage of inhibition increasing slightly as plant extract concentration increased (Figure 5).

Figure (5) Scavenging Activity DPPH.



3.2. Discussion

The GC-MS analysis of the E. camaledonsis extract revealed that different compounds (methane, sulfinylbis, benzoic acid, n-Hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z). have distinct anticancer and antioxidant activity. According to MTT results, E. camaledonsis leaf methanolic extract strongly

inhibited HepG2 cell proliferation and was more effective in killing HepG2 cells than MCF7 and PC3 cells. Many pathways for tumor cell inhibition by extract have been proposed. The cytotoxicity assay and DPPH scavenging activity were used to investigate these activities. The activity was variable and varied according to the concentrations and the 50% maximal inhibitory concentration (IC50) value. The phytochemical compounds of E. Camaledonsis greatly contributed to anticancer and antioxidant activity in present study. E. camaledonsis leaf methanol extract was cytotoxic. IC50 102.3 µg/ml against MCF-7 cell line ,IC50 32.17 µg/ml against HepG2 cell line and IC50 59.02 µg/ml against PC3 cell .this activity show more effect against HepG2 cell line and less activity against MCF7 and PC3 cell line depending on IC50 degree . On the other hand, methanol extract of E. camaledonsis leaf in present study exhibited moderate antioxidant activity. It is clear that different levels of anticancer and antioxidant properties were found in the methanol extract of E. camaledonsis, which is important for the development of new therapeutic agents. More research is needed in the future to link the specific compound to its biological property.

4. Conclusion

Different essential and fixed oils have been isolated from the fresh leaves of Eucalyptus camaledonsis plant located in maysan city-Iraq. The obtained oils were characterized using GC-MS analysis. The main constituents were (Methane sulfinylbis Benzoic acid , n-Hexadecanoic acid ,9,12-Octadecadienoic acid (Z,Z)- and 9-Octadecenoic acid, (E)-).The isolated oils showed remarkable anticancer activity and antioxidant activity. The antioxidant activity exhibited the potential of this plant and may suggest it as cheap and new antioxidant source. Thus, the use of naturally available compounds from plant origin against cancer cells and as antioxidant agents could be considered a suitable alternative to the synthetic medicinal products.

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