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Investigation and identification of active compounds present in the vegetative part and bark of eucalyptus tree (*Eucalyptus camaldulensis* Dhenh)

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Abstract

This study aimed to measure and identify the organic compounds present in the aqueous and alcoholic extracts of *Eucalyptus camaldulensis* vegetative parts and bark. This study sought to compare these extracts and discuss their therapeutic and industrial applications. The extraction was carried out by soaking for 72 hours using ethanol alcohol at a concentration of 70% and the aqueous extraction using distilled water. The presence of phytochemical compounds was detected, as the results showed the presence of tannins, flavonoids, saponins, glycosides, resins, saponins and coumarins. The importance of each of these compounds was clarified. The chemical compounds of the alcoholic and aqueous extracts of *E.camaldulensis* vegetative parts and bark were detected using GC-MS spectrometry. The numbers of chemical compounds differed from one extract to another, as the numbers of compounds in the cold aqueous extract of the green part and bark were more than (15) unique compounds, while the numbers of compounds in the alcoholic extract of the green part and bark ranged between (7) to (5) compounds.

Keywords: Eucalyptus, GC-MS, Phenols, Alcoholic Extract, Aquatic Extract.

تحديد المركبات الفعالة الموجودة في المستخلصات الكحولية والمائية للجزء الخضري وقلف شجرة اليوكالبتوس (Eucalyptus camaldulensis Dehnh)

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مستخلص:

هدفت هذه الدراسة الى قياس و تحديد المركبات العضوية الموجودة في المستخلصات المائية و الكحولية للأفرع الخضرية و قلف اليوكالبتوس. سعت هذه الدراسة الى مقارنة هذه المستخلصات ومناقشة التطبيقات العلاجية والصناعية لها. تم اجراء الاستخلاص بطريقة النقع لمدة 72 ساعة باستخدام كحول الايثانول بتركيز 70٪ والمائي باستخدام الماء المقطر . تم الكشف عن وجود المركبات الكيميائية النباتية في المستخلصات حيث أظهرت النتائج وجود العفص والفلافونويدات والصابونين والكومارين والكلايكوسيدات والراتنجات، تم توضيح اهمية كل مركب من هذه المركبات. تم الكشف عن المركبات الكيميائية للمستخلص الكحولي والمائي للأفرع الخضرية وقلف الكالبتوس باستخدام مطياف الكتلة. GC-MS كانت أعداد المركبات الكيميائية مختلقة من مستخلص لأخر حيث كانت أعداد المركبات المستخلص المائي البارد للجزء الخضري وقلف اليوكالبتوس (15) مركب فريد بينما كانت أعداد المركبات في المستخلص الكحولي للجزء الخضري وقلف اليوكالبتوس تراوحت بين (7) و (5) مركبات.

الكلمات المفتاحية: يوكالبتوس، كتلة كروماتوغرافيا الغاز، الفينولات، استخلاص الكحولي، استخلاص المائي .

Introduction

The *E. camaldulensis* plant was employed by the Indigenous population of Australia for medicinal applications, addressing a wide array of health conditions. The aforementioned conditions encompassed gastrointestinal problems such as colic, diarrhea, and dysentery, as well as respiratory ailments, in addition to severe colds, pharyngitis, bronchitis, pain relief from cuts and scrapes, and control of joint and muscle pain (Jaradat *et al*., 2023). Eucalyptus trees adapt to different environments, as they have the ability to withstand different temperatures, whether low or very high. They have the ability to adapt and live in different types of soil depending on the degree of PH. One of the most important features of the eucalyptus tree is its tolerance to high levels of organic matter in the soil, compared to trees that live in the same soil, which makes it desirable and widely spread in the world (Madejón *et al*., 2017). Eucalyptus tree is characterized by its hardwood, which is considered a source for the furniture industry and also the paper industry. Scientific experiments were conducted on eucalyptus extracts as an antimicrobial, antibacterial, and antifungal. The extracts of this tree have proven their inhibitory effectiveness due to their containing large quantities of volatile oils and chemical compounds that are effective against bacteria and fungi that infect different types of plants (Ghasemian *et al.*, 2019; Del Guacchio *et al*., 2019).

Eucalyptus camaldulensis contains large amounts of essential oils and many secondary metabolites that were explained by (Aleksic Sabo & Knezevic, 2019). The most important of which are terpenoids, which played a role in inhibiting Staphylococcus aureus bacteria at different concentrations. An experiment was also conducted by (Gakuubi *et al*., 2017) showing the effect of the essential oil on the growth of five test of *Fusarium species: F. oxysporum, F. solani, F.verticillioides, F. proliferatum, and F. subglutinans*. Traditionally, *E.camaldulensis* has been used to treat various conditions. Eucalyptus oil is popular in aromatherapy and is a common ingredient in cough syrups and inhalants for respiratory issues, helping to clear mucus (Salvatori *et al*., 2023; Horváth & Ács, 2015)which are commonly used nowadays in cosmetics ,health care,

traditional medicine and food industry, could be one of the promising solutions for this worldwide problem .EOs have a complex mode of action due to their multiple composition .Respiratory tract diseases) RTDs.

Since the *E.camaldulensis* is considered one of the most important trees it has been used for many therapeutic purposes, the study aimed to identify the most important phytochemical compounds found in the *E.camaldulensis* and the defining the importance of these compounds.

Materials and Methods

Sample Collection: Samples of green parts and bark were collected from *E. camaldulesis* from Baghdad Governorate. The samples were washed with tap water to remove suspended residues and dried at 50°C for 24 h. Each sample was ground using an electric mixer and placed in paper bags, labeled, and kept until use.

Preparation of alcoholic plant extract: 100 g of vegetative branches/ bark powder was placed in 1000 ml Erlenmeyer flasks containing 1000 ml of 70% ethanol. The mixture was shaken for 72 h then filtered by Whatman No4 filter paper to obtain a clear filtrate.finally, the plant extracts were stored in tightly sealed tubes in the refrigerator at 4°C until used (Hameed *et al.,* 2020).

Preparation of cold aquatic plant extracts: 100 g of vegetative branches/ bark powder was placed in 1000 ml Erlenmeyer flasks containing 1000 ml of sterile distilled water. The mixture was shaken for 72 h, filtered by Whatman No4 filter paper to obtain a clear filtrate, and then stored in tightly sealed tubes in the refrigerator at 4°C until used (Hameed *et al.,* 2020).

Detection of active compounds and groups: Phytochemical examinations of the extracts were conducted following the standard procedures that were recommended by the Biology Department, College of Science, Al-Mustansiriyah University. The extraction process typically involves grinding the plant material, followed by solvent extraction, filtration, and concentration.

Alkaloids: A 0.5 mg amount of the methanol extract was obtained and treated with drops of 1% HCl, followed by the addition of 6 drops of Dragendorff's reagent. The observation of a reddish-brown solid indicates the existence of alkaloids (Raal *et al.,* 2020).

Saponins: A volume of 5 ml of distilled water was measured and com-

bined with 200 mg of plant extract. Next, a volume of 0.5 ml of the filtrate was taken out in a test tube and then mixed with 5 ml of distilled water. The resulting mixture was intensely shaken for a duration of 2 minutes. The presence of saponins was shown by the production of stable foam (Yadav & Agarwala, 2011).

Flavonoids: To detect flavonoids, 4 ml of the extract are mixed with 1.5 ml of methanol. The mixture was heated with the addition of mineral magnesium. After that, 4-5 drops of HCl was added. The formation of yellow color indicating the presence of flavonoids (Yadav & Agarwala, 2011).

Phenoles: A volume of 2 ml of the extract are taken in a test tube. A few drops of ferric chloride solution are added. The appearance of a bluish dark color indicates the presence of phenols (Yadav & Agarwala, 2011).

Glycoside: Few drops of ferric chloride solution to concentrated sulfuric acid, then the mixture was added to 2 ml of the extract. The appearance of two clear layers, brown color layer located at the bottom and the blue-green color layer at the top, indicating the presence of the glycoside (Yadav & Agarwala, 2011).

Terpenoids and steroid: A volume of 2 ml of alcoholic extract was carefully added to 2 ml of chloroform and 3 ml of concentrated sulphuric acid. The observation of reddish brown color indicate the existence of terpenoids and steroids (Yadav & Agarwala, 2011).

Tannins : 200 mg of the extract was immersed in 10 ml of distilled water, boiled and filtered. A few drops of $FeCl_3$ were added to the filtered solution. A dark blue precipitate appeared, that indicate the presence of tannins (Yadav & Agarwala, 2011).

Emodins : A solution of 2 ml of NH_4OH and 3 ml of Benzene was added to the plant extract. The mixture was then vigorously shaken, followed by a 10-minute waiting until a red color was observed. The manifestation of a red hue signifies the existence of emodin residues (Yadav & Agarwala, 2011).

Fatty acid: A volume of 0.5 ml of the extract was mixed with 5 ml of ether. The mixture was heated until evaporation, then filtered. The filter paper was dried. The appearance of a transparent layer on the filter paper indicates the presence of fatty acids (Yadav & Agarwala, 2011).

Iodine test : A mixture of 2 ml of plant extract and 2 ml of iodine solu-

tion was slowly agitated. A dark blue to purple color was observed that indicate the existence carbohydrate (Yadav & Agarwala, 2011).

Determine the main components of the samples: An investigation was conducted separately at the Ministry of Technology and Science, Environment and Water Directorate to examine the basic compositions and dynamic formations of alcoholic and cold fluid concentrates utilizing GC-MS.

Results and Discussion

Table 1 shows the phytochemical properties of four extracts of *E. camaldulensis*; aqueous and alcoholic extracts of the vegetative part and bark. The results revealed the presence of medically active compounds in the four extracts. Noted that carbohydrates, tannins, flavonoids, and coumarins appeared in all extracts, while saponin appeared in the aqueous extract only of the vegetative part and bark. Terpenes appeared in the aqueous and alcoholic extracts of the bark and only in the aqueous extract of the vegetative part, while resins appeared in the aqueous extract of the vegetative branches and the alcoholic extract of the bark, while alkaloids, proteins, and steroids appeared in all extracts, and glycosides were absent only in the aqueous extract of the bark.

Tannins are a group of polyphenolic compounds with molecular weight ranging from 500 to 30.000 Dalton (Serrano *et al*., 2009). They were recognized for their astringent effects as well as possible health advantages such as antioxidants, antimicrobials, and anti-inflammatory agents. Tannins have been shown to inhibit the growth of bacteria, fungi, and viruses, making them useful in treating infections and preserving food. Tannins scavenge free radicals, thereby protecting cells from oxidative stress and reducing the risk of chronic diseases such as cancer and heart disease, and it can reduce inflammation by inhibiting the production of pro-inflammatory mediators, which is beneficial in treating inflammatory conditions (Jyske *et al.*, 2023).

Table 1. Active groups and components in plant extracts

Plant extract components	Cold aqueous plant extract		Alcoholic plant extract	
	Branches and leaves	Bark	Branches and leaves	Bark
Tannins	+	+	+	+
Carbohydrate	+	+	+	+
Glycosides	+	-	+	+
Phenols	-	-	-	-
Resins	+	-	-	+
Flavonoids	+	+	+	+
Saponin	+	+	-	-
Alkaloid	-	-	-	-
Protein	-	-	-	-
Coumarins	+	+	+	+
Terpenes	+	+	-	+
Steroides	-	-	-	-

Carbohydrates are an important and essential coumpounds that play an effective role in the nutritional and therapeutic properties of the plant. The research conducted by (Deans & Svoboda, 1990) revealed that eucalyptus leaves contain different forms of carbohydrates represented by glucose, fructose and sucrose, which are necessary for storing energy. In addition, a study of (Suryawanshi, 2014) revealed that the aqueous extract of eucalyptus bark contains large amounts of polysaccharides that play a role in enhancing immunity and antioxidants.

Glycosides are essential compounds which contain a sugar part linked to a non-sugar component. These compounds have many medical benefits, as anti-inflammatory, antimicrobial, and antioxidants (Yulvianti & Zidorn, 2021). EI-Sawi, 2010 explained that the bark of the eucalyptus tree contains large amounts of glycosides, which show effective effect against different types of bacteria and fungi. This supports the use of eucalyptus extract as an anti-inflammatory, as it supports its use as medical and therapeutic materials.

Eucalyptus extracts are rich in phenolic compounds, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. Isolating

pure phenols from plant extracts can be problematic because of the variety of compounds present. (Nasr *et al.*, 2019).

Resins are complex mixtures of organic compounds usually secreted by plants. Eucalyptus trees produce various types of resins that contain a range of bioactive compounds. These resins are renowned for their antimicrobial, anti-inflammatory, and medicinal properties. The primary components of Eucalyptus resins include terpenoids, phenolic compounds, and essential oils (Barbosa *et al*., 2016).

Flavonoids are essential compounds found in many vegetables and fruits. Eucalyptus also contains these compounds, as Table 1 shows that eucalyptus contains flavonoids in all extracts. Flavonoids have many and varied properties, the most important of which is that they are one of the most important anti-inflammatory compounds. A study conducted by (Safe *et al*., 2021) revealed that eucalyptus contains large amounts of flavonoids and has multiple medical effects and benefits. A recent study conducted on eucalyptus leaves showed that they contain the essential flavonoids epicatechin, 200-O-galloylhyperin, isoquercitrin, isorhapontin, quercitrin, and quercetin-3-O-glucuronid, which have proven their effectiveness as antioxidants (Safe *et al*., 2021; Park *et al*., 2023)catechin, flavonoids, and others. To optimize its antioxidative phenolic contents, E. globulus was extracted under various solvent conditions using 0, 10, 30, 50, 70, 90, and 100% ethanol. The 50% ethanol extract possessed the highest content of total phenolics with 497.7 mg GAE (gallic acid equivalent.

Saponins are compounds found in eucalyptus trees, and these compounds have many benefits, the most important of which is the pharmaceutical industry. Saponins contribute to reducing blood fat levels and reducing the risk of cancer. Also, it used as expectorants and cough suppressants (Timilsena *et al*., 2023). Table 1 shows the presence of saponins in the aqueous extract of eucalyptus bark and leaves.

Alkaloids are bioactive molecules present in various varieties of the Eucalyptus tree, and are recognised as fundamental constituents responsible for the plant's therapeutic attributes (Salvatori *et al*., 2023). The current investigation of the *E. camaldulensis* tree reveals the absence of alkaloids in both the alcoholic and aqueous extracts. Nevertheless, they can manifest as al-

kaloid fractions, specifically quinoline and pyridine, as illustrated in Tables 2, 3, and 4.

Proteins and steroids do not appear in both alcoholic and aqueous extracts, but they may appear as fraction components in GC-MS spectroscopy.

Coumarins are compounds classified as natural compounds, their main component is benzopyrones, found in about 150 species of plants, characterized by their ability to inhibit different types of microbes. The eucalyptus tree contains different types of coumarins such as umbelliferone and esculetin, which are characterized by their therapeutic properties and which increase the ability of eucalyptus extracts to inhibition (Deryabin *et al.*, 2021).

Terpenes are widely distributed metabolic compounds in various plants. The eucalyptus tree, which is distinguished by its unique fragrance, has many biological and medical activities as its content of terpenes which considered one of the most important compounds that enhance the activity of the eucalyptus tree (Arooj *et al.*, 2023).

Gas chromatography assay of plant extracts: Chromatographic evaluate the composite structure of the plant extracts. Results showed that the cold aqueous extract of eucalyptus leaves showed (15) unique chemical fractions (table 2). It contains a large percentage of coumarin (benzopyrone) (Mohammed Ali, 2021) and tannins (acetyl) compounds (Adamczyk et al., 2017), which are one of the important essential oils in eucalyptus that gives it its distinctive scent. The alcoholic extract of bark showed (7) different chemical fractions (table 3), while the cold aqueous extract of bark showed (15) unique chemical fractions (table 4). It contains valeric acid which is propyl compound that belong to the flavonoid group (Goldberg & Stefan Rokem, 2019) organic acids are used mostly as food acidulants and as building blocks for other useful chemicals of low and high molecular weight (polymers,and has various pharmaceutical applications, the most important of which is its use as an anti-epileptic. The alcoholic extract of eucalyptus branches and leaves (table 5) showed (5) unique chemical fractions.

Table 2: GC-MS of *E.camaldulensis* dehen leaves cold aqueous extract

No	Rotation Time	%Area	Chemical Structure	M W g/mole	Compounds
1	2.115	3.94	C6H12O3	132	Isopropyl lactate-
2	2.917	50.19	C6H14O2	118	Hexylene Glycol
3	9.239	0.77	C8H18O2	146	,Peroxide
4	9.369	0.14	C7H13ClO	148	1-Hexen-4-ol
5	9.487	0.58	C3H9NO2	91	2-Amino-1,3-propanediol
6	14.036	1.16	C12H10O4	156.2	-8-acetyl-7-hydroxy-4-methyl
7	14.759	1.67	C11H12O2	176	1,4-Methanonaphthalene-5,8-diol
8	15.045	0.59	C5H10O2	102	3-Hydroxy-3-methyl-2-butanone
9	15.548	1.26	C12H24	168	5-Dodecene
10	19.292	13.86	C13H26	182	5-Tridecene
11	21.120	19.13	C8H19N	129	N,1-Dimethylhexylamine
12	21.301	2.14	C12H24	168	,4-Dodecene
13	23.915	1.38	C8H15NO2	157	2-Propenoic acid,2-methyl,2(dimethylamino)ethyl ester
14	24.454	0.71	C6H11N	97	-4-methyl
15	26.307	2.48	C24H46O2	366	9-Octadecenoic acid(Z)-, hexyl ester

Table 3: GC-MS of *E. camaldulensis* dehen bark alcoholic extraction

No	Rotation Time	%Area	Chemical Structure	M W g/mole	Compounds
1	2.042	26.23	C3H4O6	136	-dihydroxy
2	6.679	15.15	C6H13NO2	131	-HexaneHexane,1-nitro
3	13.243	17.32	C7H14O2	130	-(Oxirane, (butoxymethyl
4	14.044	4.69	C7H13NO	127	-Acetamide, N-cyclopentyl
5	15.052	15.052	C12H16N4O2	248	Triazolo[2,3-a]pyrimidine-2-car--[1,2,4] boxylic acid
6	20.740	18.53	C9H20O	144	1-Pentanol
7	27.509	12.99	C17H14O6	314	Cyclopenta[c]furo[3',2':4,5]furo[2,3-h] [1]benzopyran-1,11-dione

Table 4: GC-MASS of *E. camaldulensis* dehen bark aquatic extraction

No	Rotation Time	%Area	Chemical Structure	M W g/mole	Compounds
1	2.037	2.05	C4H9NO	87	Ethanimidic acid, ethyl ester
2	3.550	0.32	C6H11N	97	-Pyridine,1,2,3,6-tetrahydro-1-methyl
3	3.866	0.14	C3H6F2O	96	1,3-Difluoro-2-propanol
4	8.794	5.53	C5H11Cl	106	-Butane, 2-chloro-3-methyl
5	10.267	40.53	C5H6O2	98	Methylenecyclopropanecarboxylic acid
6	11.555	0.59	C6H14	86	-Pentane, 3-methyl
7	12.603	0.49	C7H7NO4	169	1-Oxo-2-propenyl)oxy]-2,5-pyrro-)]1-lidinedione
8	12.999	5.82	C10H10O2S2	226	Bis(2-furfuryl)disulfide
9	14.302	25.01	C6H12O2	116	-Pentanoic acid, 3-methyl
10	15.617	25.01	C2H5NO	59	Acetaldoxime
11	19.199	3.34	C24H49N3O2	411	2-Butylamino-N1,N4-diheptyl-N1,N4-dimethylsuccinamide
12	20.577	0.69	C12H15NO4	237	Carbonic acid, monoamide, N-(2,4-dimethoxyphenyl)-, allyl ester
13	20.971	1.68	C10H16O4	200	Oxalic acid, butyl cyclobutyl ester
14	24.891	2.18	C17H17NO5	315	2,3,5,7-Tetramethoxy-10H-acridin-9-one
15	25.968	4.19	C19H13NO	271	-3-Acridinol, 9-phenyl

Table 5: GC-MS *E. camaldulensis* dehen leaves alcoholic extract

No	Rotation Time	%Area	Chemical Structure	M W g/mole	Compounds
1	2.042	51.24	C3H4O6	136	-Propanedioic acid,dihydroxy
2	2.895	23.82	C5H13NO	103	-Ethanamine, 2-propoxy
3	15.067	5.48	C4H10O2	90	DL-2,3-Butanediol
4	19.270	9.02	C12H16O	176	Cyclooct-1-enyl)-furan)2-
5	24.588	10.45	CH4N2O	60	Urea

Conclusion

This study successfully identified and quantified the main chemical compounds present in the alcoholic and aqueous extracts of *E. camaldulesis* vegetative parts and bark. The study confirmed the presence of a wide

chemical diversity in eucalyptus and the presence of many phytochemical compounds represented by tannins, flavonoids, saponins, glycosides and coumarins. Also the types of chemical compounds present in the extracts were determined by GC-MS spectrometry.

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