RESEARCH ARTICLE

Isolation of Three Secondary Metabolites from the *Eucalyptus camaldulensis Dehnh.* Plant for the First Time

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ABSTRACT

Objectives: Phytochemical screening and identification of certain types of secondary metabolites that not separated yet from the *Eucalyptus camaldulensis* plant.

Background: *E. camaldulensis Dehnh*. (Family: Myrtaceae) is one of the most widely distributed species of eucalyptus trees and the original land of this variety is Australia. *E. camaldulensis Dehnh*. is the main species that distributed in various regions of Iraq. *E. camaldulensis* has active constituents rich in pharmacologically significant secondary metabolites with proven activities including anti-bacterial, anti-fungal, anti-viral, anti-oxidant, and others.

Methodology: Defatting step of leaves and fruits separately with n-hexane was preceding the extraction process by maceration with 50% acetone followed by subsequent fractionation process and isolation step achieved by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Results: The resulting three fractions had been undergone preliminary chemical tests suggesting the presence of steroids, terpenoids, and tannins. The isolation process had been given rise to the separation of three phytoconstituents identified by different chromatographic techniques resulting in the confirmation of the non-polar compounds isolated from the hexane fraction comprising the phytosterol stigmasterol and the possible diterpene turraeanin J. The polar secondary metabolite isolated from the ethyl acetate fraction is the dimer tannin oenothein B.

Conclusion: The Iraqi *E. camaldulensis* plant rich in various secondary metabolites presents diverse pharmacological activities. The first isolation might be around the world from the *Eucalyptus* genus, is the expected diterpene turraeanin J, and the first isolation from the species around the world are oenothein B and stigmasterol.

Keywords: *Eucalyptus camaldulensis*, Isolation, Phytoconstituents.

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INTRODUCTION

Between past and present, medicinal plants remain one of the major natural sources available as a part of Complementary and alternative medicine (CAM) consumed to manage different disorders.[1] In the past, thousands of years BC, many medicinal plants were described by Greek, Roman, and Arab physicians.[2,3] In Iraq, the historical utilization of different medicinal plants confirmed through a cave in the north of the country discovered in the early sixties of the last century showed a huge amount of plant remains from eight varieties, after investigating it, seven of which had been with medicinal significance and are used till now through phytomedicine.[3] Although "Us Food and Drug Administration (FDA)" not labeled herbal products as dugs.[4] However, the use of herbal medicines, as stated by the "World Health Organization" statistics, shows that about 80 percent of the total population utilizes these products.[5] In Iraq, a cross-sectional study in 2012 in Baghdad among healthcare providers revealed poor medical information regarding phytomedicine despite the great orientation to folk medicine and their good impression about its traditional use.[3] This elevation in the consumption of herbal products requires great awareness in the consumption of herbal medicines properly.

One of those medicinal plants is the *Eucalyptus camaldulensis Dehnh*. (Family: Myrtaceae) also commonly called and as mentioned in American Integrated Taxonomic Information System (ITIS) Murray River gum, Kaffour, and Kena. This species is considered one of the most widespread types of eucalyptus trees. Its planting area in various world's regions reaches around five million hectares.[6,7] The original

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land of this species is Australia, which spread in various country regions.[8] In Iraq, despite the introduction of specific varieties of the eucalyptus plant and researches indicating that there are more than 20 species of eucalyptus trees planted, *E. camaldulensis* is considered the main species cultivated in Iraq.[9]

Many pharmacological studies have been made on *E. camaldulensis,* which suggest significant biological activities of this species that attributed to the different phytoconstituents have been isolated from this species, among which essential oils, phytosterols, terpenoids, flavonoids, and tannins. The medical significance mentioned in literature reviews comprises anti-microbial activity against different bacterial kinds, strong anti-fungal activity attributed to the essential oil of the plant, proven anti-viral activity, distinct anti-oxidant activity, and spasmolytic, anti-inflammatory, and anti-tumor activities.[10-12] The direction of this article mainly was toward determining the existence (or absence) of a specific tannin that was previously isolated from another *Eucalyptus* species but has not been yet identified in the *E. camaldulensis Dehnh.* plant named oenothein B. Also, the analysis of the non-polar fractions and isolation of possible terpenoids and/or steroids was the study's main objective.

EXPERIMENTAL DETAILS

Collection and Authentication of Plant Materials

Eucalyptus camaldulensis was harvested at the beginning of October 2020 from a local garden in eastern Baghdad. The collected parts (leaves & fruits) were detached from each other to be investigated separately and left to dry for two weeks in the shade at room temperature. The plant was authenticated as *E. camaldulensis Dehnh.* (Family: Myrtaceae).

Extraction of Plant Materials

Dried leaves of 140 gm & dried fruits of 20 gm were pulverized by a grinder and defatted respectively with 450 ml & 100ml n-hexane for 24 hours and filtered by a Whatman filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator (BÜCHI Rotavapor R-205 / Merck-German) and the obtained residue was kept for further investigation. About 70 gm of the defatted leaves and fruits were mixed and extracted simultaneously with 420 mL of 50% acetone (acetone: distilled water, 50:50 v/v) for 4 days. The total extract was filtered to get rid of the marc by Whatman filter paper (No.2) to remove any insoluble matters and fractionated with equal amounts of ethyl acetate (200 mL × 6). The resulting ethyl acetate fraction was dehydrated by anhydrous sodium sulfate and allowed to evaporate to dryness. The aqueous fraction was also dried, dissolved with methanol, and applied for the next analysis step.[13]

Qualitative Tests for Phytochemical Screening

Preliminary chemical tests were carried out to determine the presence or absence of steroids, terpenoids qualitatively, and tannins in Iraqi *E. camaldulensis* achieved for the defatted extraction of leaves and fruits and the ethyl acetate and aqueous fractions.

1- Detection of Steroids

Libermman-Burchard test was the phytochemical test for steroids. About 0.05 gram of ethanolic extract dissolved in 2 mL of chloroform followed by adding 1-mL acetic anhydride and 1-2 drops of concentrated sulfuric acid (added carefully to the wall of the tube).[14]

2- Detection of Terpenoids

A Qualitative test for terpenoids was Salkowski test. A few drops of chloroform were added to 1ml of the crude extract followed by the addition of about 0.5–1 mL concentrated sulfuric acid (added carefully to the tube wall) and shaked well.[15]

3- Detection of Tannins

- Braymer's test About 3 mL distilled water added to 2 mL of alcoholic extract followed by the addition of 3 drops of 10% methanolic ferric chloride $FeCl_3$.[16]
- NaOH test Alcoholic extract (0.4 mL) mixed with 10% NaOH and shaked well.[17]

Analysis of the Fractions

Analysis of the hexane fraction for leaves and fruits and the resulted two fractions achieved through thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Analysis by Thin-layer Chromatography

The main steps of this chromatographic analysis are the stationary phase and the mobile phase**.** The stationary phase is silica already available as an aluminum sheet (20 × 20 cm) coated with silica gel 60 GF_{254} with a thickness of 0.25 mm (Sigma-Aldrich, Germany) that activated by oven at temperature 110˚C for 10–15 minutes to get rid of any moisture absorbed from the atmosphere. The mobile phases consumed for the hexane fraction are hexane: methanol (70:30) and the mobile phase applied for the ethyl acetate and aqueous fractions is Toluene: ethyl acetate: formic acid: methanol (15:15:4:1). Sample and standards (Stigmasterol & Oenothein B) were prepared by dissolving in a few milliliters of methanol and applied in the diluted form on the base line of the plate developed in a glass TLC jar (22.5 cm x 22 cm x 7 cm) covered with a glass lid. The separated spots on the TLC plate can be visualized (if not colored) either by non-destructive way utilizing ultra-violet (UV) light (Desaga / Germany) with wavelengths of 254 nanometer (nm) and 365 nm or by a destructive method by specific chemical reagents[18] vanillin-H_2SO_4 reagent for steroids and terpenoids.

Analysis by High-performance Liquid Chromatography

Qualitative examination by HPLC technique has been achieved for hexane fraction of fruits that compared to stigmasterol standard (Chengdu Biopurify-China / purity 98%) and the ethyl acetate fraction compared to oenothein B standard (Chengdu Biopurify-China / purity 98%) through different chromatographic conditions and via two different HPLC instruments that illustrated below. The sample is prepared for the next mentioned conditions by dissolving the dried extract

of the plant fractions (0.1-gm equal to 100 mg) in 25 mL for the hexane fractions and 15 mL for the ethyl acetate fraction in methanol grade and introduced to the HPLC column by filtration through a membrane filter (0.45 micrometer) preceding the injection. The matching between samples and standards had been performed by the retention time observed.

- Chromatographic conditions of hexane fraction analysis[19]
  Instrument model/ LC-2010 Shimadzu, autosampler
  Stationary Phase/ Column C18- ODS (25 cm* 4.6 mm)
  Mobile Phase/ Methanol only - HPLC grade
  Mode of Operation/ Isocratic elution
  Detector: UV-visible detector with wavelength 196 nm
  Flow Rate: 1-mL/min
  Injection Volume: 10 microliter
- Chromatographic conditions of ethyl acetate fraction analysis[20]
  Instrument model/ SYKAM, Germany
  Stationary Phase/ Column C18- ODS (25 cm* 4.6 mm)
  Mobile Phase/ Methanol: Distilled water: Formic acid (70:25:5 % v/v/v)
  Mode of Operation/ Isocratic elution
  Detector/ UV-visible detector (wavelength 230 nm)
  Flow Rate: 1.3 mL/min
  Injection Volume: 100 microliter

Isolation and Identification of Compounds C1, C2, and C3

Isolation of different natural products from Iraqi *E. camaldulensis Dehnh.* denoted by C1& C2 that isolated both from the hexane fraction and C3 isolated from the ethyl acetate fraction. The identification of the three isolated compounds was achieved by TLC, HPLC, high-performance thin-layer chromatography (HPTLC), Ultraviolet-visible (UV-vis) detector, Mass Spectrometry (MS), and Fourier-transform infrared spectroscopy (FT-IR).

Isolation and Identification of C1 Compound

Isolation of C1 compound by preparative liquid chromatography (PLC) had been achieved through ready-made glass plates (20*20 cm- San Point/China) covered with silica gel GF254 with a thickness of 1-mm that activated in the oven at 110°C for 10 minutes. The mobile phase used for the isolation from the hexane fraction of the leaves by application in the mobile phase hexane: methanol (70:30) consists of two non-miscible solvents, hexane, and methanol. The non-polar phase is consumed and placed in the jar and developed to separate the C1 compound. About 0.5 mg from the hexane fraction dissolved in 1.25 mL methanol and set on the plate and after entire dryness, detected under UV (wavelength: 365 nm & 254 nm) and enclitic by pencil and crushed by a spatula. The isolated compound has been interpreted then by TLC, high-performance Compact Mass Spectrometer (CMS) (ADVION®, USA), and FT-IR (Shimadzu-Japan / ATR Technique).

Isolation and Identification of C2 and C3 Compounds

The isolation of compounds C2 and C3 has been achieved through high-performance liquid chromatography. Chromatographic conditions applied for the separation of the C2 compound will be mentioned next. The chromatographic conditions through which compound C3 separated and the sample preparation are mentioned previously. The isolated phytoconstituents were collected through a fraction collector (model-FOXY R1).

- Chromatographic conditions for isolation of C2 compound[21]
  Instrument model/ SYKAM, Germany
  Stationary Phase/ Column C18- ODS (25 cm* 4.6 mm)
  Mobile Phase/Acetonitrile: Distilled water: acetic acid (27:25:5 % v/v/v)
  Mode of Operation/ Isocratic elution
  Detector/ UV-visible detector (wavelength 280 nm)
  Flow Rate: 1-mL/min
  Injection Volume: 200 microliter

Confirmation of compound C2 has been achieved through HPLC by comparison of the retention time with the standard, HPTLC (Eike Reich / CAMAG-Laboratory, Switzerland) in which the instrument utilizes a TLC plate (20.0 x 10.0 cm, 0.25 mm) with silica gel GF254 (Tklst/ China) consuming the solvent system hexane: methanol (70:30), and UV-Visible detector (UV-1900- Shimadzu / Japan).

Identification of compound C3 was by HPLC where the identification was made by injection of the isolated compound and the standard separately, then the retention time was observed. Another injection was accomplished include mixing both the standard and the isolated compound and observing the resultant peak (Spiking technique).[22] UV detector has been utilized to detect the absorbance of the isolated compound within the ultra-violet range. Further confirmation was made by comparing the absorbance of functional groups of isolated C3 compounds by FT-IR.

RESULTS AND DISCUSSION

Defatting Step and Extraction Process

Depending on the phytoconstituents of interest in this study, including non-polar compounds, the defatting process preceding the extraction step is usually performed by n-hexane to withdraw the less polar compounds and facilitate the fractionation process.

Extraction with 50% acetone as a specific method for complex polyphenolic compounds has been achieved according to the article[13] to extract only the desired compounds targeted, mainly the tannins.

Phytochemical Screening of Fractions

The primary analysis for the Iraqi *E. camladulensis Dehnh.* made with the chemical tests screening the existence or absence of the secondary metabolites that may be found in the plants (Table 1). The hexane fraction for both leaves and fruits part suggests the presence of steroids and terpenoids, and both ethyl acetate and aqueous fractions suggest a strong abundance of tannins in those fractions, especially with Braymer's test.

Analysis and Isolation of Compounds C1, C2, and C3

The analysis of the hexane fraction by TLC was achieved for the two plant parts (leaves & fruits) that were observed with

close spots and detected under UV and after spraying that displayed a close development between the hexane fraction of the two plant parts. The analysis of the hexane fraction done for the leaves shows next in Figure 1.

The analysis in Figure 1 shows distinct isolated spots with different colors observed in the wavelength 365 nm and quench in the 254 nm of the UV-light. The white arrow mentioned by the green fluorescent in the UV-365 nm and dark quench in UV-254 was of interest to be isolated and identified since its appearance is a clear spot. So, the next step was the isolation procedure from the leaves part made by glass plate of 1mm consuming the same solvent system hexane: methanol (70:30). Figure 2 referred to the isolation of C1 in which the separated band was indicated by an arrow.

Figure 1: Analysis of the hexane fraction for leaves / Detected under the UV-365 nm and 254 nm

Analysis of hexane fraction for both leaves and fruits achieved by TLC but no specific isolation of spots in both HF for fruits and leaves compared to the standard stigmasterol. Another analysis for the standard and hexane fraction has been achieved for fruits only by HPLC and illustrated in Figure 3. By comparing the retention time (RT) (x-axis) of the stigmasterol standard (blue peak at 9.9) is closely related to the retention time of the fraction (red peak at 9.9), which might suggest the presence of this phytosterol in this part from the plant. Compound C2 isolated as suspected stigmasterol. The isolation in the previous figure was accomplished through the chromatographic conditions mentioned previously through which the fraction was analyzed, where the peak at 9.90 occurred and collected (red peak) through a fraction collector.

On the other hand, the analysis for the aqueous and ethyl acetate fractions compared to oenothein B standard have been achieved by TLC but was not accurate and did not separate compounds as spots to be compared with the standard, only appear like a continuous line. For this reason, the direction toward the examination and isolation by HPLC was done,

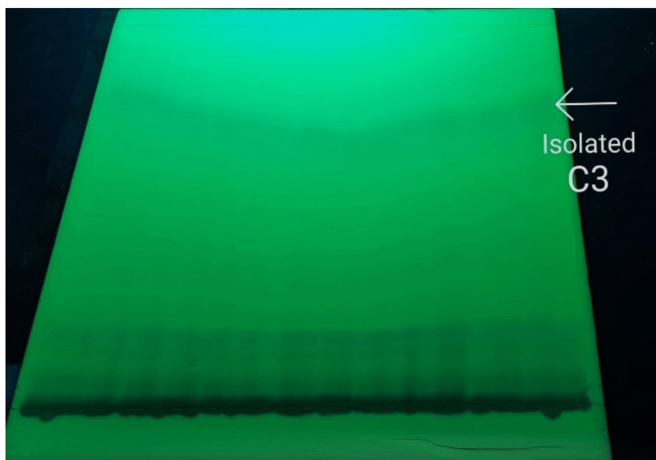
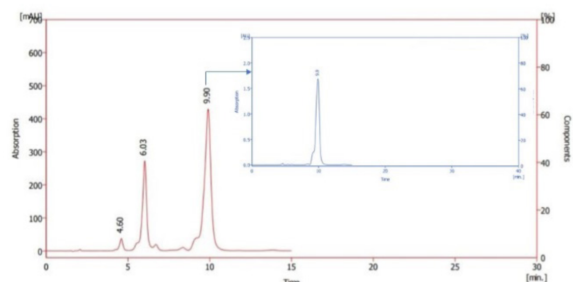


Figure 2: Isolation of compound C1 from the hexane fraction of leaves that developed on silica gel GF_{254}/ Detected by UV-254 nm



Result Table (Uncal - F:\sample - Detector 2)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	4.597	283.788	27.441	2.4	4.4	0.17	
2	6.030	3026.931	223.326	25.1	35.4	0.22	
3	9.903	8758.798	379.277	72.6	60.2	0.36	
	Total	12069.517	630.044	100.0	100.0		

Figure 3: HPLC chromatogram with peaks table for analysis and isolation of compound C2. The red peaks represent the sample while blue peak represent the stigmasterol standard

Table 1: Phytochemical screening of Iraqi *E. camaldulensis Dehnh*. plant

Test achieved	*Observation*	*HF L F*	*EF*	*AF*
Libermman-Burchard test	Dark green color	+ +	N/A	N/A
Salkowski test	Reddish ring between the two layers	+ +	N/A	N/A
Braymer's test	Dark blue color	N/A	+	+
NaOH test	Formation of white emulsion	N/A	+	+

(+): Present (N/A): Not achieved , HF (L): hexane fraction for leaves part, HF (F): hexane fraction for fruits part EF: ethyl acetate fraction, AF: aqueous fraction

as shown in Figure 4. The conditions applied for analyzing those fractions for leaves and fruits (mixed) are mentioned previously. The oenothein B standard retened at the time 8.25 (blue peak) and closest to that peak appeared in the retention time 8.06 (red peak) for the analyzed sample so that, the peak at 8.06 was collected to identify whether compound C2 is the phytoconstituent oenothein B or another secondary metabolite.

Identification of the Isolated Compounds

Identification of Isolated Compound C1

The isolation of this compound had been achieved with no idea about it and no standard to compare with. After analysis by TLC and spraying the plate with vanillin-H_2SO_4 reagent and heating at 100˚C, the isolated C1 compound shows a violet spot, indicating that this compound belongs to terpenes terpenoids. The direction was toward identifying this unknown compound by mass spectrometry (MS). Firstly, the analysis by TLC had been done to determine the purity of isolation to achieve a clear result(s) and detected by the UV- light with the short and long wavelengths and only one spot of the isolated C1 compound was observed.

Hence, mass spectrometry was the next step. In the MS analysis, the vertical axis represents the intensity of the fragments, and the horizontal axis is m/z (mass to charge ratio). So, the m/z mean the molecular mass of the fragments related to the molecular weight, and this is the main target to get the molecular weight which can lead to the chemical formula and then to the chemical structure of the unknown compounds. According to the general analysis of mass spectrometer, there are two significant peaks, the molecular ion peak (commonly named parent peak and/or M+) and the base peak. The base peak represents the most stable fragment and appears in the spectrum with the highest intensity and the molecular ion peak is marked by the highest value of m/z ratio and with no specific intensity. However, it usually presents with somewhat low intensity.[23] This interpretation lead to the resulted base peak of the unknown compound is that with m/z 152.2 and the molecular ion peak of the isolated C1 compound observed with m/z 338.9.

This analysis is made by the ionization mode called atmospheric pressure chemical ionization, abbreviated as APCI. From the name, this type of analysis includes ionization and the addition of proton to the chemical compound before fragmentation. For this reason, the molecular ion peak 338.9 might represent the molecular weight of the unknown C1 compound subtracted from it the atomic mass of the ionized proton to be 337.9. The next step is to expect the molecular formula of this molecular weight. After long investigation, several molecular formulas are expected, including the chemical formula $C_{20}H_{33}O_4$ that belongs to a certain group of diterpenes named labdane diterpenes. Unfortunately, this possible labdane diterpene which named turraeanin J presents with little available online data and isolated only in one research that identified this compound by high resolution-mass spectrometry and results in m/z 337.2 with no details on the other possible fragments.[24]

Another analysis for the isolated C1 compound has been done by FT-IR that reveals several absorptive peaks among which 3375 cm^{-1} could be related to the O-H stretching, 3088 cm^{-1} might be for the O-H stretching of carboxylic acid, and 1691 cm^{-1} the distinctive area of C=O stretching. It is worthy to note that the research mentioned this possible diterpene describing its nature as yellow oil (droplets) and observed after isolation and dryness of the isolated C1 compound.

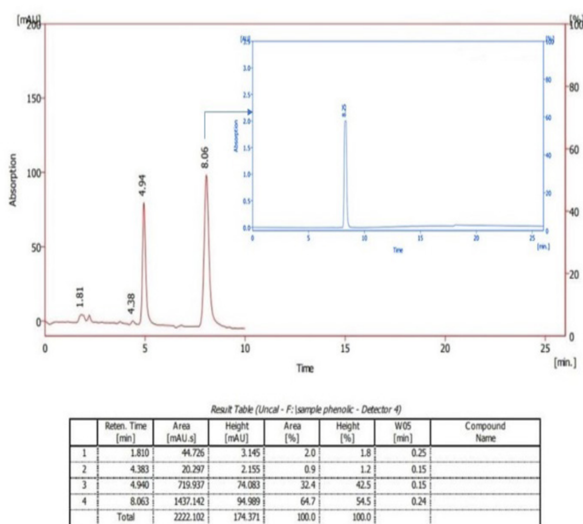
Identification of Isolated Compound C2

Based on the analysis by HPLC and the comparison of the retention time for the analyzed hexane fraction and stigmasterol standard, the isolation of the C2 compound was achieved. The run of the isolated sample with the standard through the HPLC technique, attained first to identify the compound and its purity which shows a close retention time of the isolated C2 retend at 9.85 compared to the standard observed in a retention time 9.9minute.

Another verification was made for the isolated C2 by using HPTLC to compare the maximum retardation factor (Max. R_f) of the stigmasterol standard and C2 compound. From max R_f value detected in the wavelength of 210 nm, There is an approximate matching between the standard (max R_f value is 0.70) and the isolated C2 compound (max R_f 0.73).

Final identification of this secondary metabolite was achieved by the ultra-violet spectrophotometer (UV-visible) to detect the maximum absorption at a certain wavelength (λ_{max}) and compare it with the standard, as illustrated in Figure 5.

As clarified in the previous figure, two λ_{max} observed for both the standard and the separated compound, first one at the wavelength of 268 nm and the second at 217 nm. From the clear matching in the peaks of absorption and as known that phytosterols absorbance occurs at short wavelengths.[25,26] all together with the identification by HPLC and HPTLC, suggesting that C2 compound is the phytosterol stigmasterol.



Result Table (Uncal - F:\sample phenolic - Detector 4)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	1.810	44.726	3.145	2.0	1.8	0.25	
2	4.383	20.297	2.155	0.9	1.2	0.15	
3	4.940	719.937	74.083	32.4	42.5	0.15	
4	8.063	1437.142	94.989	64.7	54.5	0.24	
	Total	2222.102	174.371	100.0	100.0		

Figure 4: HPLC chromatogram with peaks table for analysis and isolation of compound C3 .The red peaks represent the fraction while blue peak represents the oenothein B standard

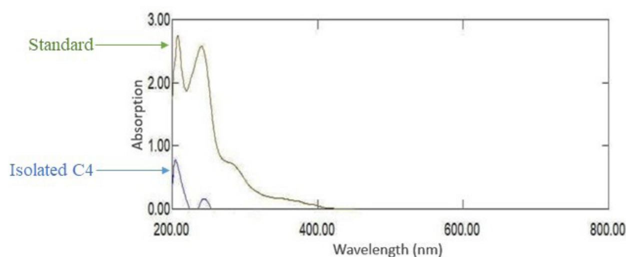
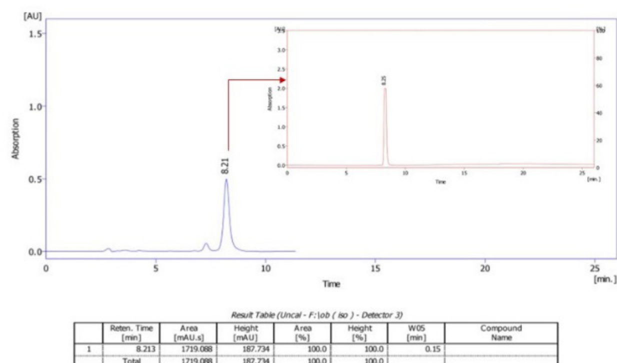
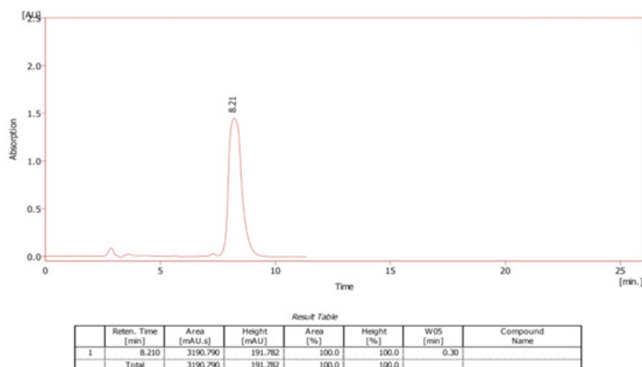


Figure 5: UV-visible spectrum for both isolated C2 (blue) and stigmasterol standard (green)



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8.213	1719.088	187.734	100.0	100.0	0.15	
	Total	1719.088	187.734	100.0	100.0		

Figure 6: HPLC chromatogram of the isolated C3 compound (spectrum in blue color) with its relative peak table and oenothein B standard (spectrum in red color)



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8.210	3190.790	191.782	100.0	100.0	0.30	
	Total	3190.790	191.782	100.0	100.0		

Figure 7: HPLC chromatogram of the standard oenothein B and isolated C3 compound (Spiking)

While the identification of this phytosterol was achieved from the genus of *Eucalyptus,*[27] its separation in the species *E. camaldulensis Dehnh.* not performed previously. For this reason, the isolation of stigmasterol from the fruits part of the Iraqi plant might be the first recording for this phytosterol.

Identification of Isolated Compound C3

From the analysis by HPLC, isolation of C3 compound was done and linked to the standard oenothein B. To confirm this isolation and detect its purity, the isolated C3 compound and the standard were analyzed by HPLC and the retention time compared. Figure 6 will show the close retention time of the standard (8.25) to the isolated (8.21) with the detection of only one peak. This gives a primary idea for the isolated C3 compound and its purity.

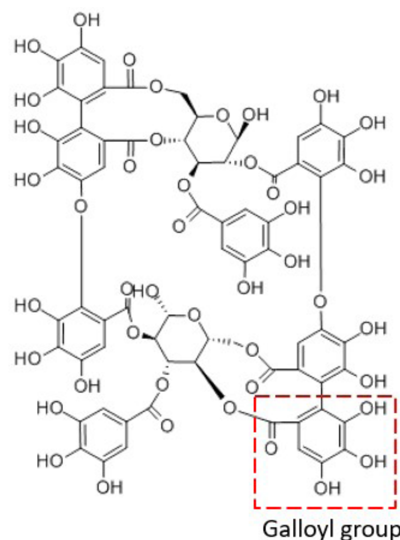


Figure 8: Chemical structure of the tannin oenothein B

HPLC also achieved another verification of this compound through mixing the standard with the sample (spiking technique) and detect the peak recorded with its retention time (figure 7) with noticing the increment in the percentage area in the spiking peak (3190) compared to the %area of the isolated C3 alone (1719) which suggests that the standard and the isolated C3 compound is compatible.

Another identification for the isolated C3 compound was done by the UV-visible spetrometic technique, which records two λ_{max} at 297nm and 313nm. The wavelength of 297nm might relate to the carbonyl group absorbed by the UV as chromophore.[28] The second λ_{max} at 313 nm might related to the galloyl moiety,[29,30] the basic backbone for the macrocyclic dimer oenothein B (Figure 8).

Last confirmation was with the infrared spectra that observed with the number of absorptive peaks among which is 3618.48 cm^{-1} and 3298.28 cm^{-1} might be related to the two hydroxyl groups of suspected oenothein B to the carbonyl group occur in the region of 1662 cm^{-1}. Which is related to the C=O group occurs in the range of 1660-1800cm^{-1},[31-33] as well as the symmetric C-H stretching may be correlated to methylene group (-CH_2) observed at 2831.50 cm^{-1} and aromatic C-H group detected at 2939.52 cm^{-1}.[34] All these identifications suggest that the isolated C3 compound is the dimer tannin oenothein B. Since the literature reviews not detected the isolation of this macrocyclic tannin from the leaves of the species *E. camaldulensis Dehnh.* yet, this is the first record for oenothein B around the world.

CONCLUSION

Based on the results of this study, here are the outlines summarize the outcomes of this research.

- The phytosterol, stigmasterol isolated from the fruits, and the tannin Oenothein B isolated from the leaves have

not yet been isolated from the species *E. camaldulensis Dehnh*., despite their separation from the *Eucalyptus* genus. This proposes to be the first time these secondary metabolites are found in the species worldwide confirming the distribution of phytoconstituents in various species that belong to the same genus.

- A possible terpenoid compound named turraeanin J belongs to the group labdane diterpenes has been isolated from the leaves of Iraqi *E. camaldulensis Dehnh.* with no isolation recorded in the *Eucalyptus* genus and only one observed in international researches. This diterpene needs further identifications and more pharmacological studies in the future alone and detects its rule as there is no available data recommending its medical importance.

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