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Phenolic Constituents of *Eucalyptus camaldulensis* Dehnh, with Potential Antioxidant and Cytotoxic Activities

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Abstract: A liquid chromatography-diode array detection-electrospray ionization mass spectrometric (HPLC–PDA–ESI/MS/MS) method was used for separation and characterization of the phytoconstituents of the aqueous acetone leaf extract of *Eucalyptus camaldulensis* Dehnh (Myrtaceae). The employed method was optimized for separation, identification and quantification of fifty six compounds including ellagitannins, flavonoids, phloroglucinol derivatives and galloyl esters. The antioxidant effect was determined *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\cdot}$), hydroxyl radical and super oxide anion radical scavenging assays. The cytotoxicity of the aqueous acetone extract was evaluated on MCF-7, Hep-2, HepG-2, HeLa, HCT-116 and Caco-2 cell lines. The results indicated that most of the fractions exhibited strong antioxidant activity. The aqueous acetone extract reduced the viability of all cell lines in a dose-dependent manner, and was more active on MCF-7 and HCT-116 cell lines.

Keywords: Myrtaceae; *Eucalyptus camaldulensis;* DPPH; 2-deoxy-2-ribose; HPLC–PDA–ESI/MS/MS; cytotoxicity.

1. Introduction

Eucalyptus camaldulensis Dehnh, also known as river red gum or Murray red gum, is a tree of the genus *Eucalyptus* and is native to Australia [1]. *E. camaldulensis* is probably the most widespread *Eucalyptus* species in Australia [2]. Several species of *Eucalyptus* are used in traditional medicine as antiseptics, and against upper respiratory tract infections, such as common cold, influenza and sinus

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congestion [3,4]. The essential oil obtained from these plants has a therapeutic application in treatment of pulmonary infections by inhalation [5,6]. Previous studies on the essential oil of the flowers of *E. camaldulensis* revealed the presence of 1,8-cineole, *β*-pinene and spathulenol as the most abundant constituents [7]. The essential oil of the leaves was found to contain *p*-cymene, *γ*-terpinene, *α*-pinene, 1,8-cineole, terpinen-4-ol, *α*-terpineol, carvacrol and thymol as the major components [8]. The major components of the essential oil of the fruits were aromadendrene, *α*-pinene, drimenol, and cubenol [9]. A pentacyclic triterpenoid, named camaldulin along with ursolic acid lactone acetate and ursolic acid lactone were isolated from *E. camaldulensis*, all exhibited spasmolytic action [10]. Later the same authors isolated another triterpenoid acid named eucalyptanoic acid which also exhibited a spasmolytic action [11]. Some flavonoid glycosides were isolated from the leaves of *E. camaldulensis* [12].

Numerous physiological processes in the body produce reactive oxygen species as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer and other degenerative diseases [13]. Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and hydroxyl radicals ($OH^{\cdot}$) are known to act in all phases of the carcinogenesis process, namely initiation, promotion and progression. ROS exert a mutagenic effect by oxidizing DNA bases, and also cause DNA strand breaks, eventually increasing the risk of cancer development [14]. Dietary polyphenols act as antioxidants and preventing injury caused by free radicals [14]. Polyphenols exhibit strong antioxidant properties and scavenge free radicals [14]. Moreover, polyphenolic compounds have been shown to inhibit the carcinogenic process through cell cycle arrest, regulation of cell death machineries, and arresting proliferation of cancer cells [14].

Chemoprevention is a rapidly growing practical approach that focuses on cancer prevention by the administration of one or more synthetic or naturally occurring agents to suppress or reverse the process of carcinogenesis. It is becoming increasingly clear that chemopreventive compounds present in diet offer great potential in the fight against cancer by inhibiting the process of carcinogenesis through regulation of cell-defensive and cell death machineries [15]. Dietary chemopreventive substances are regarded as being generally safe, inexpensive and they have been found to contain various phytochemicals which are antioxidant in nature [15]. In Japan, leaf extracts of *E. globulus* are used as food additive for the prevention of many chronic diseases [16]. The goal of the work described herein was to develop a simple and rapid method for the identification and quantification of the phenolic compounds of *E. camaldulensis* Dehnh using HPLC–PDA–ESI/MS/MS and to evaluate the use of its extract as an antioxidant and natural chemopreventive agent. This work represents the first study that utilizes the HPLC–PDA–ESI/MS/MS technique for in-depth identification and quantification of the phenolic composition of *E. camaldulensis* Dehnh. The employed method can be used to qualitatively describe the phytochemical composition of different plant extracts and /or herbal preparations.

2. Materials and Methods

2.1. Plant Material

The leaves of *E. camaldulensis* Dehnh were collected in July 2007 from the zoo botanical garden, Giza, Egypt. The plant was botanically identified by the staff at the herbarium of the botanical garden of the zoo, Giza, Egypt. Voucher specimen was deposited at the herbarium of the faculty of pharmacy, Ain shams university, Cairo, Egypt (ASU-ECM2007).

2.2 Extraction and Isolation

Air-dried powdered leaves of *E. camaldulensis* (50 g) were extracted three times with 70% aqueous acetone (each 0.5 L). The total extract was evaporated under *vacuum* to remove the organic solvent, the remaining aqueous solution was freeze-dried to obtain a dry powder (5 g) representing the total aqueous acetone extract. Fractionation of 3 g of this extract on a column packed with Sephadex LH-20 (3×30 cm), eluted with H_2O followed by H_2O-MeOH mixtures of decreasing polarities (2 L each), yielded 4 major fractions (I-IV). Fraction I was eluted with water, fraction II was eluted with 30% MeOH, 60%MeOH was used for elution of fraction III. The last fraction was eluted with MeOH.

2.3 Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, quercetin, gallic acid and phenazine methosulfate (PMS) were obtained from Sigma-Aldrich GmbH, Germany; nitroblue tetrazolium (NBT) was obtained from BioChemika, Germany, nicotinamide adenine dinucleotide (NADH) was obtained from Merck, Sweden. RPMI-1640, penicillin, streptomycin, Sulphorhodamine-B, trypan blue, trypsin, acetic acid and trichloroacetic acid were obtained from Sigma Chemical Co., St. Louis, U.S.A. Fetal bovine serum (FBS) and doxorubicin were obtained from Sigma Chemical Co., St. Louis, U.S.A. Folin-Ciocalteu reagent was obtained from Sigma-Aldrich GmbH, Germany.

2.4 Sample Preparation for LC/PDA-ESI/MS/MS

Part of each fraction was dissolved in 20%MeOH (20 mg/mL) and the solution was filtered through 0.45 µm membranes.

2.5 Analysis of the Phenolic Composition by LC/PDA-ESI/MS/MS

LC-HRESIMS was performed on a Bruker Daltonics micrOTOF-Q (API) Time-of-Flight mass spectrometer (Bremen, Germany), coupled to 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a high performance autosampler, binary pump, and variable wavelength detector G 1314B, G 1314 C(SL). Chromatographic separation was performed on a Superspher 100 RP-18 (75 × 4 mm i.d.; 4 µm) column (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile (A) and 0.4 % formic acid (B). The elution profile was 0–3 min, 100% B (isocratic); 3–30 min, 0–30% A in B; 30–35 min, 30–70% A; 35–45, 70% A (isocratic) with constant flow rate 0.5 mL/min. The ionization technique was ion spray (pneumatically assisted electrospray). The mass spectrometer was operated in negative mode. Mass detection was performed in full scan mode in the range 50–2000 *m/z*. The following settings were applied to the instrument: capillary voltage, 4000 V; end plate offset, -500 V. Heated dry gas (N_2) flow rate was 10 L/min; the dry gas temperature was 200°C. The gas flow to the nebulizer was set at a pressure of 1.6 bar. For collision-induced dissociation (CID) MS/MS measurements, the voltage over the collision cell varied from 20 to 70 eV. Argon was used as collision gas. The mass spectrometer was operated in data-dependent mode to automatically select the 3 most abundant precursor ions. Data analysis software was used for data interpretation. Sodium formate was used for calibration at the end of the LC/MS run.

2.6 Hydroxyl Radical Scavenging Activity (Deoxyribose Assay)

The assay was carried out as reported before [17]. The reaction mixture contained 2.8 mM 2-deoxy-2-ribose (dissolved in KH_2PO_4–K_2HPO_4 buffer, pH 7.4, 20 mM), 100 µM $FeCl_3$, 104 µM EDTA, 1.0 mM H_2O_2 and 100 µM ascorbic acid. Different concentrations of the tested samples were added. After an incubation period of 1 h. at 37 °C, the extent of deoxyribose degradation was measured by the reaction of formed malonaldehyde with thiobarbituric acid (TBA). Equal volumes of 1%TBA and 2.8% TCA were added to the reaction mixture and heated at 100 °C for 20 min. After

cooling the reaction mixture the absorbance was measured at 532 nm against a blank. The inhibition percentage of the radical scavenging activity was calculated using the equation: Inhibition (%) = 100 – 100 (A_S/ A_0) where A_0 is the absorbance of the blank and A_S is absorbance of the sample. All assays were conducted in triplicates. Quercetin was used as a positive control. The IC_{50} values were calculated using four parameter logistic curve (Hill equation) (GraphPad Prism 5.00) and data were statistically evaluated using Student's t-test (SigmaPlot 11.0).

2.7 DPPH Radical Scavenging Assay

The assay was done as reported before [18,19] with some modifications to be carried out in microtiter plate. Twenty µL of samples at different concentrations, standards (quercetin, and gallic acid) or solvent in case of blank was pipetted into each well of a 96-well plate, followed by 280 µL of 0.25 mM methanolic solution of DPPH. The mixture was incubated at room temperature in dark for 30 min, and the absorbance at 520 nm was measured with a Multiskan Ascent V1.24 microplate reader. All assays were conducted in triplicates. The inhibition percentage was calculated as in the deoxyribose assay. IC_{50} values were calculated from three independent experiments. Quercetin and gallic acid were used as positive controls.

2.8. Superoxide Anion Radical Scavenging Activity

Superoxide anion scavenging activity of all the tested samples was determined using the previous method [13] with some modifications. Superoxide radicals were generated in the PMS-NADH system and assayed by the reduction of NBT. Test solution (60 µL), 60 µL of 677 µM NADH solution, 60 µL of 144 µM NBT solution and 60 µL of 60 µM PMS solution in 0.1M phosphate buffer pH 7.4, were added to a microwell plate and incubated at room temperature for 5 min. The absorbance was read at 550 nm. IC_{50} values were calculated as before. L-ascorbic acid was used as a positive control. The assay was conducted in triplicates and repeated at least three times.

2.9. Determination of Total Phenols

Total phenolics were determined according to the previous method [20] with some modifications. Fifty µL of the methanolic solution of each sample was added to 100 µL of methanol and mixed with 100 µL of Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand at room temperature for 3 min before the addition of 500 µL of 20% Na_2CO_3. The solution was mixed thoroughly and the absorbance was measured at 730 nm after 2 h. Results were expressed as gallic acid equivalents per gram dry weight of each sample from a calibration curve of gallic acid (0-500 µg/mL).

2.10. Determination of Total Favonoid Content

Flavonoid content was determined according to a reported method [21]. Quercetin was used as a standard. An aliquot of either the methanolic solution of the samples or standard solution was mixed with an equal volume of $AlCl_3.6H_2O$ (0.2%). Absorbance was measured at 367 nm. Results were expressed as mg of quercetin equivalents per gram dry weight of each sample from a calibration curve of the standard (0-500 µg/mL).

2.11. Cell culture

Different tumor cell lines, including MCF-7 (breast adenocarcinoma), Hep-2 (human epithelial laryngeal carcinoma), HepG-2 (hepatocellular carcinoma), HeLa (human cervix adenocarcinoma), HCT-116 (colorectal adenocarcinoma) and Caco-2 (colon adenocarcinoma) were purchased from American Type Cell Culture (ATCC). All cell lines were maintained in RPMI-1640 supplemented with 2 mM L-glutamine, non-essential amino acids, 10 % fetal bovine serum (FBS), 2

mg/L streptomycin, and 100 IU/mL penicillin at 37 °C in a humidified atmosphere of 5% CO_2. Cell viability was estimated by trypan blue exclusion test.

2.12. Cytotoxicity assay

Cytotoxicity was tested against cancer cell lines according to the procedure adopted by the National Cancer Institute (NCI, USA), that uses the protein-binding dye Sulphorhodamine-B (SRB) to assess cell growth [22]. The cell viability was compared to that of untreated controls. Briefly, a monolayer cell culture was trypsinized (0.025 % trypsin and 0.02% EDTA). Cells were harvested in 96-well microtiter plates (5000 cells/well). After a 24 h incubation to allow the cell to attach, different concentrations of the samples or the reference drug doxorubicin were added. The plates were then incubated at 37 °C for 2 days in a 5% CO_2 atmosphere. This was followed by treatment with 50 µL cold 50 % trichloroacetic acid (TCA) at 4°C for 1 hr. After washing with distilled water, the plates were stained for 30 min at room temperature with 50 µL 0.4 % SRB dissolved in 1 % acetic acid, and subsequently washed with 1% acetic acid to remove unbound stain, 10 mM tris base (pH: 10.5) was used to solubilize the dye. The plates were shaken vigorously, and the absorbance was measured using a Victor microplate reader (PerkinElmer Life Science) at 564 nm. The percentage of cell survival was calculated from the following formula: Surviving fraction (%) = (Absorbance of treated cells)/ (Absorbance of control cells) × 100 (%). The assay was conducted in 6 replicates for each cell line. The IC_{50} values were calculated from the dose-response curve (GraphPad Prism 5.00). Doxorubicin was used as a positive control.

3. Results and Discussion

In this study, HPLC–PDA–ESI/MS/MS was utilized to separate and identify the different phenolic compounds of *E. camaldulensis* extract. The subsequent fragmentation of the predominant negative ions in the MS/MS mode was used to obtain more information about the molecular masses of the different compounds. Using this method, fifty six compounds were tentatively identified and quantified. The fractionation process was conducted by fractionating 3 g. of the 70% aqueous acetone extract over a column packed with Sephadex LH-20. The elution started with water, followed by H_2O-MeOH mixtures of decreasing polarities to obtain 4 major fractions (I-IV). Part of each fraction was subjected to high performance liquid chromatography, coupled to photodiode-array and electrospray ionization mass spectrometric analysis (HPLC–PDA–ESI/MS/MS) in order to obtain a tentative identification of its components. The results are listed in Tables 1-4.

The major components of the first fraction (eluted with water) were identified as HHDP-glucopyranose, chlorogenic acid and phloroglucinol derivatives. The second fraction, which was eluted with 30% MeOH was found to contain different galloyl-HHDP-glucopyranose positional isomers and pedunculagin as major components. The fraction eluted with 60% MeOH was predominantly composed of digalloyl-HHDP-glucopyranose (tellimagrandin I) α and β anomers. The last fraction, eluted with MeOH, was composed of a mixture of ellagitannin dimers. The HPLC–PDA–ESI/MS/MS profiling of the obtained fractions indicated that ellagitannins were the most predominant components of fractions II, III and IV.

Most of the obtained fractions exhibited considerable inhibitory activity in 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$), hydroxyl radical and super oxide anion radical scavenging assays (Table 5). The most active fractions in the $DPPH^{\cdot}$ assay were found to be fraction III (60% MeOH) and fraction IV (MeOH fraction), with IC_{50} values of 13.4 µg/mL, which indicated that these fractions produced higher $DPPH^{\cdot}$ scavenging activity when compared with standard antioxidant compounds (quercetin and gallic acid). While in the deoxyribose assay, the 30% MeOH, MeOH fractions produced more hydroxyl radical scavenging (IC_{50}: 22.0 and 19.2 µg/mL respectively. The 30% MeOH and 60% MeOH fractions exhibited the highest superoxide anion radical scavenging activity, with IC_{50} values of 43.9 and 50.9 µg/mL respectively. The water fraction exhibited the least antioxidant activity in the three assays. These results were consistent with those obtained from the determination of the total phenol content and flavonoid content assays. The cytotoxicity of the total extract was evaluated on MCF-7, Hep-2, HepG-2, HeLa, HCT-116 and Caco-2 cell lines. The results indicated that the aqueous

acetone extract reduced viability of all cell lines in a dose-dependent manner. The cytotoxic effect of the total extract was greater on MCF-7 and HCT-116 cell lines, with IC_{50} values of 36.5, and 33.3 µg/mL respectively (Table 6).

Phytochemicals have been shown to be effective in preventing malignant transformation of cells in culture and experimentally induced tumorigenesis in various animal models. Mechanistically, chemoprevention with dietary phytochemicals could be achieved by stimulating inactivation of potential carcinogens, inhibition of abnormal cell proliferation, induction of apoptosis and delaying angiogenesis [23]. The present study was undertaken to investigate the potential of *Eucalyptus camaldulensis* Dehnh. extract as a chemopreventive agent by evaluating its effect on oxidation, viable cell number and by evaluating the sensitivity of different cancer cell lines to the extract. The results of the present study demonstrated that the aqueous acetone extract exhibited a dose-dependent growth inhibitory effect after a continuous exposure during a 48 h period. The cytotoxic effect was greater on MCF-7 and HCT-116 cell lines.

In conclusion, we have developed a method to separate, identify and estimate for the first time the amounts of different phenolic compounds of the aqueous acetone extract of *E. camaldulensis* Dehnh. Also we have shown that this extract and its associated antioxidants possess a strong potential to develop a chemopreventive agent against various human cancers, especially for breast and colon cancers which are considered to be among the most common cancers in the world. This finding is important from a nutritional point of view, because the extract may induce beneficial health effects due to its high antioxidant properties, and thus may be used as a dietary supplement for the prevention of cancer and other chronic diseases. Future studies to determine the mechanistic basis of the cytotoxic effects of *E. camaldulensis* extract, together with other studies on the bioavilability, toxicity and antitumor effects using animal models are now under investigation.

Table 1. LC-PDA-ESI/MS/MS Identification of the major constituents of *E. camaledulensis* fraction I (water fraction)

N	tR	DAD	$(M-H)^-$	Fragments	Tentative structural assignment	% Area
1	2.8	196.4, 213.0, 256.7 (sh)	481.06	300.99, 275.01, 257.01, 245.01	HHDP-glucopyranose	8.07
2	3.4	196.4, 213.0, 256.7 (sh)	481.06	301.00, 275.02	HHDP-glucopyranose	6.28
3	4.6	213.0, 271.0	331.06	169.01 , 125.02	galloylglucopyranose	4.11
4	6.2	213.8, 271.0	331.06	169.01 , 125.02	galloylglucopyranose	2.10
5	9.3	211.3, 268.9	343.06	191.05, 169.01, 125.02	galloyl quinic acid	2.83
6	10.4	211.0, 271.0	343.06	191.05, 169.01, 125.02	galloyl quinic acid	3.59
7	11.0	214.8, 273.0	325.05	169.01 , 125.02	galloyl shikimic acid	2.44
8	14.5	202.2, 254.9 sh, 273.3	539.09	237.83, 205.05	phloroglucinol derivative	3.41
9	13.6	208.9, 295.5	389.11	305.07	unidentified	3.18
10	15.4	211.3, 273.3, 302.0	495.08	191.05, 169.01, 125.02	phloroglucinol derivative	12.15
11	15.5	214.8, 250.0sh, 292.5sh, 322.6	353.08	191.03	chlorogenic acid	14.20
12	16.3	214.8, 271.0	483.08	169.01 , 125.02	digalloylglucopyranose	4.25
13	17.0	200.2, 275.7, 303.7 (sh)	389.11	209.80	unidentified	2.28
14	19.1	200.2, 275.7, 302.0 (sh)	523.11	371.10, 337.09	unidentified	3.24
15	21.3	202.2, 218.0, 25.41, 291.0 sd, 324.7	537.14	387.13, 375.09	cypellocarpin B	8.08
16	21.9	222.6, 273.3	421.11	313.05, 169.01 , 125.02	benzyl-galloylglucose	8.16
17	23.4	253.1, 351.8	477.55	301.03, 271.02, 255.03, 151.00	quercetin glucuronide	5.15
18	25.0	262.5, 338.6	461.34	285.08, 257.14, 229.05	kaempferol glucuronide	3.36
19	26.9	264.5, 333.8	497.17	169.01, 125.02	unidentified	2.86

Table 2. LC-PDA-ESI/MS/MS Identification of the major constituents of *E. camaledulensis* fraction II (30%MeOH)

N	tR	DAD	$(M-H)^-$	Fragments	Tentative structural assignment	% Area
1	4.3	220.5, 262.5	633.07	301.00, 275.01, 249.03, 169.01	galloyl-HHDP-glucopyranose	3.33
2	4.8	218.6, 262.5	633.07	301.00, 275.01, 249.04, 169.01	galloyl-HHDP-glucopyranose	13.38
3	5.5	218.6, 262.5	633.07	301.00, 275.01, 249.04, 169.01	galloyl-HHDP-glucopyranose	9.88
4	8.3	214.8, 273.0 (sh)	933.56	631.05, 301.00	vescalagin	6.10
5	8.7	218.6, 262.5	633.07	301.00, 275.01, 249.03, 169.01	galloyl-HHDP-glucopyranose	15.09
6	9.6	220.5, 273.0 (sh)	783.06	481.06, 301.0, 275.02	pedunculagin isomer	9.40
7	9.9	214.8, 273.0 (sh)	933.06	631.05, 301.00	castalagin	1.10
8	11.1	214.8, 271.0	483.08	313.06, 169.01	digalloylglucopyranose	1.76
9	11.7	205.6, 264.5	633.07	301.00, 275.01, 249.03, 169.01	galloyl-HHDP-glucopyranose	6.58
10	12.2	220.5, 273 (sh)	783.06	481.06, 301.0, 275.02	pedunculagin isomer	8.63
11	13.4	211.3 , 263.0	951.07	907.08, 301.00, 169.01	valoneoyl-HHDP-glucopyranose	1.40
12	13.9	214.8, 271.0	483.08	313.06, 169.01	digalloylglucopyranose	2.28
13	14.8	205.8, 264.8 (sh)	1067.63	935.05, 633.08, 301.00, 169.01, 125.02	pterocarinin A	0.56
14	15.5	214.8, 264.5	633.07	301.00, 275.01, 249.03, 169.01	galloyl-HHDP-glucopyranose	2.06
15	16.4	205.6, 264.5	633.07	301.00, 275.01, 249.03, 169.01	galloyl-HHDP-glucopyranose	1.70
16	17.0	214.8 ,263.0	951.07	907.08, 301.00, 169.01	valoneoyl-HHDP-glucopyranose	4.17
17	18.3	211.3, 251.4, 363.7	469.00	425.01, 301.00, 169.01	valoneic acid dilactone	1.65
18	18.6	213.0, 271.0	679.14	527.13, 473.83, 375.09	unidentified	1.14
19	21.7	210.0, 255.0, 273.3	689.21	537.19	galloyl Cypellocarpin B	2.59
20	22.7	200.2, 256.7, 346.3	433.04	301.01, 271.02, 255.03	quercetin pentoside	2.64
21	24.1	200.2, 253.1, 357.5	635.20	447.10, 300.99, 315.01	ellagic acid derivative	2.08

Table 3. LC-PDA-ESI/MS/MS Identification of the major constituents of *E. camaledulensis* fraction III (60%MeOH)

N	tR	DAD	$(M-H)^-$	$(M-2H)^{2-}$	Fragments	Tentative structural assignment	% Area
1	9.5	218.6, 273 (sh)	783.06		481.06, 301.00, 275.02	pedunculagin isomer	6.18
2	10.2	214.8, 262.5	1417.16	708.08	785.08, 765.05, 633.07, 301.00, 169.02	ellagitannin dimer	6.75
3	12.2	214.6, 262.5	1417.16	708.08	785.08, 765.05, 633.07, 301.00, 169.02	ellagitannin dimer	1.62
4	12.7	218.6, 273 (sh)	783.07		481.06, 301.00, 275.03	pedunculagin isomer	5.25
5	13.6	214.8, 262.4	1569.17	784.08	935.07, 785.08, 765.05, 633.07, 301.00, 169.02	sanguiin H10-like ellagitannin dimer	9.79
6	14.9	217.0, 268.8	785.08		301.00, 275.02, 169.01	digalloyl-HHDP- glucopyranose (tellimagrandin I)	27.94
7	15.6	214.8, 264.4	935.07		633.07, 301.00, 169.02	galloyl-bis-HHDP-glucopyranose isomer	1.02
8	16.4	216.6, 268.8	785.08		301.00, 275.02, 169.01	digalloyl-HHDP- glucopyranose (tellimagrandin I)	31.97
9	16.8	214.8, 260.5	953.08		635.09, 301.00, 169.01	valoneoyl-digalloyl-glucopyranose	1.45
10	17.0	214.8, 266.6	1720.16	859.08	935.06, 633.07, 301.00, 169.02	ellagitannin dimer	1.59
11	18.2	213.0, 254.9, 360.6	469.00		425.01, 301.00, 169.01	valoneic acid dilactone	2.70
12	19.4	211.3, 268.8	953.09		301.00, 169.01	valoneoyl-digalloyl-glucopyranose	0.24
13	21.1	213.0 , 273.3	787.09		465.07, 313.05, 169.01	tetragalloylglucopyranose	1.79

Table 4. LC-PDA-ESI/MS/MS Identification of the major constituents of *E. camaledulensis* fraction IV (MeOH)

N	tR	DAD	$(M-H)^-$	$(M-2H)^{2-}$	Fragments	Tentative structural assignment	% Area
1	12.2	216.6, 268.8	1567.17	783.08	935.06, 633.07, 301.00, 169.02	sanguiin H10-like ellagitannin dimer	0.47
2	14.9	218.6, 266.6	1569.17	784.08	935.09, 785.09, 633.07, 301.00, 169.02	ellagitannin dimer	15.22
3	16.6	218.6, 264.5	1569.17	784.08	935.08, 785.08, 765.05, 633.08, 301.00, 169.02	ellagitannin dimer	18.22
4	17.3	214.8, 262.5	1567.17	783.07	935.08, 633.07, 301.00, 169.02	sanguiin H10-like ellagitannin dimer	17.63
5	18.5	218.8, 266.6	1569.18	784.10	935.09, 765.05, 633.07, 301.00, 169.02	ellagitannin dimer	39.80
6	19.5	216.6, 268.8	1569.18	784.10	935.08, 785.08, 765.05, 633.07, 301.00, 169.02	ellagitannin dimer	1.35
7	20.6	218.6, 271.0	937.10	468.05	785.09, 301.00, 169.01	trigalloyl-HHDP-glucopyranose	2.69
8	21.9	218.6, 271.0	1721.18	860.08	935.06, 633.07, 301.00, 169.02	ellagitannin dimer	4.50

Table 5. Antioxidant activity of *E. camaledulensis* fractions using DPPH, deoxyribose and super oxide anion radical scavenging assays.

	DPPH·	Deoxyribose	Super oxide anion	Phenol content (mg GAE g−1)	Flavonoid content (mg QE g−1)
Total extract	14.0 ± 0.2	ND	106.6 ± 0.8	364.1 ± 8.2	80.5 ± 0.9
Column fractions:					
Water Fr I	47.8 ± 0.7	97.0 ± 1.3	491.2 ± 11.4	110.1 ± 18.1	53.0 ± 0.6
30% MeOH Fr II	14.0 ± 0.6	22.0 ± 1.1	43.9 ± 0.9	653.5 ± 21.5	129.5 ± 1.1
60% MeOH Fr III	13.4 ± 0.3	37.4 ± 1.1	50.9 ± 4.2	729.1 ± 8.9	91.9 ± 0.8
MeOH Fr IV	13.4 ± 0.1	19.2 ± 1.1	58.5 ± 1.9	701.1± 16.7	64.8 ± 0.3
Quercetin	19.7 ± 1.0	3.9 ± 1.2			
Gallic acid	17.9 ± 0.9				
L-Ascorbic acid			56.7 ± 3.9		

Means of three IC_{50} replicates ± S.E (µg/mL); mg GAE g−1: milligram gallic acid equivalent per gram of the dry sample; mg QE g−1: milligram quercetin equivalent per gram dry sample; ND: not determined

Table 6. Cytotoxicity of *E. camaledulensis* aqueous acetone extract on MCF-7, Hep-2, HepG-2, HeLa, HCT-116 and Caco-2 cell lines.

	IC_{50} (µg/mL)					
	MCF-7	Hep-2	HepG-2	HeLa	HCT-116	Caco-2
Total extract	36.5	57.7	38.7	49.0	33.3	38.3
Doxorubicin	4.8	3.6	4.2	4.8	4.5	3.4

The cell viability of different cell lines was determined with respect to the control by SRB assay, after 48 h treatment with *E. camaledulensis* aqueous acetone extract and doxorubicin. The concentration range was 0 to 200 µg/mL for the aqueous acetone extract and 0-50 µg/mL for doxorubicin. Values represent the mean of six measurements ± SE.

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