

Short Communication

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Acetone-water mixture is a competent solvent to extract phenolics and antioxidants from four organs of *Eucalyptus camaldulensis***Aseton-Su Karışımı, Dört Okalıptüs Camaldulensis Organından Fenolikleri ve Antioksidanları Çıkarmada Yetkili Bir Çözücü**<https://doi.org/10.1515/tjb-2018-0438>

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Abstract

Objective: *Eucalyptus camaldulensis* is recognized to be one of the major introduced eucalypts, the plant extracts possess a wide range of phytochemicals, which are accountable for its antioxidant and pharmacological activities. The extraction efficiency of such chemical compounds is influenced by the applied extraction conditions. To test the effect of solvent type on the yield of phenolics and antioxidants from *E. camaldulensis*, seeking for an optimization of the extraction process.

Method: Dry powders of four plant organs were extracted by some organic solvents besides water. The total phenolic content was detected in the crude extracts of leaf, bud, empty capsule and seed, and was further characterized by GC-MS.

Results: Acetone-water mixtures yielded high amounts of phenolic compounds with parallel antioxidant activities, correlation coefficients were significant (0.696–0.998). Bud and capsule are first accredited for their antioxidant potentials. The GC-MS analysis revealed the abundance of most detected phenolic constituents in the plant seed.

Discussion: Acetone-water is a successful and appropriate mixture for the extraction of phenolic compounds from *E. camaldulensis*. It could give better yields and enhance the biological activities of its corresponding extracts.

Conclusion: The accurate choice of an extraction solvent has a great influence on the yields of phenolics.

Keywords: *Eucalyptus camaldulensis*; Bud; Empty capsule; Phenolic compounds; Acetone; GC-MS analysis.

Öz

Amaç: Okalıptüs camaldulensis, tanıtılan en büyük okalıptüslerden biri olarak kabul edilir; Bitki özleri, antioksidan ve farmakolojik aktivitelerinden sorumlu olan çok çeşitli fitokimyasallara sahiptir. Bu tür kimyasal bileşiklerin ekstraksiyon etkinliği, uygulanan ekstraksiyon koşullarından etkilenir. Solvent tipinin, *E. camaldulensis*'ten elde edilen fenolik ve antioksidanların verimi üzerindeki etkisini test etmek, ekstraksiyon işleminin optimizasyonunu araştırmak.

Gereç ve Yöntem: Dört bitki organının kuru tozları, su dışında bazı organik çözücüler tarafından çıkarıldı, toplam fenolik içerik, yaprak, tomurcuk, boş kapsül ve tohumdaki ham özlerde tespit edildi ve ayrıca GC-MS ile karakterize edildi.

Tartışma: Aseton-su karışımları paralel antioksidan potansiyeli yüksek fenolik bileşikler üretti, korelasyon katsayıları anlamlıydı (0.696–0.998). Tomurcuk ve kapsül ilk önce antioksidan potansiyelleri için onaylıdır. GC-MS analizi, bitki tohumunda saptanan fenolik bileşenlerin bolluğunu ortaya koydu. Aseton-su, fenolik bileşiklerin *E. camaldulensis*'ten ekstraksiyonu için başarılı ve uygun

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bir karışımdır, çünkü daha iyi verim sağlar ve karşılık gelen ekstraktların biyolojik aktivitelerini artırır.

Sonuç: Bir ekstraksiyon çözücüsünün doğru seçimi fenoliklerin verimi üzerinde büyük bir etkiye sahiptir.

Anahtar Kelimeler: Okaliptüs *camaldulensis*; tomurcuk; boş kapsül; fenolik bileşikler; aseton; GC-MS analizi.

Introduction

Oxidation reactions are indispensable for producing energy to be consumed for biological pathways in all living cells. Still, the excess of oxidation rates is destructive since their over-produced free radicals may damage the cellular structures and as a result, many degenerative diseases are stimulated. Sometimes, DNA is also damaged which eventually leads to cancers [1]. Due to their safety and less environmental pollution, the natural antioxidants are recently privileged to be incorporated in both foods and medicines over than synthetic ones [2]. Phenolics and related compounds are the most frequent and natural plant-derived antioxidants [3]. Their redox properties allow them to behave through many mechanisms, such as reduction, hydrogen donation and singlet oxygen quenching [4]. Owing to their richness of phenolic compounds and noticeable antioxidant potentials, eucalypts have been already integrated to many clinical presentations to treat microbial and viral infections, in addition to various metabolic disorders [1, 5]. *Eucalyptus camaldulensis* Dehnh (Myrtaceae) is documented as one of the major introduced and cultivated eucalypts, its plantations occur in many countries [6]. The plant extracts are being used as anesthetic, anti-septic, astringent and as defensive barriers against some food-related infections [7]. Additionally, *E. camaldulensis* is lately recommended as a source of natural insecticides or herbicides in replace to synthetic chemicals [8]. A bulky range of active compounds, such as essential oils, phenolics, flavonoids and triterpenoids have been detected in its polar and non polar extracts [9, 10].

The extraction of phenolics from source materials can be unpredictable, since it is influenced by many variables, counting species, habitat, seasonality, in addition to

sample preparation and the applied extraction techniques [11]. Thus, preparatory and extraction methods should be wholly investigated so as to attain the best combination for each particular group of active constituents [12]. Some earlier studies have been conducted on the use of dissimilar solvents to extract phenolic compounds from *Eucalyptus*, but mostly focused on the plant leaves [1, 6, 9]. A fewer number of reports have examined the influence of solvent choice on the bioactive constituents extracted from different plant organs [13].

In brief, the variable results obtained from different sources, make it very tricky and unreliable to decide the best solvent among them. Consistent circumstances should be employed to seek the solvent with enhanced extraction capabilities. So, the present study was proposed to compare among some extraction solvents and/or solvent mixtures in search of better yields of phenolics and antioxidants from *E. camaldulensis* leaf. Then, the best solvent would be further used with other plant organs and for the preparative extraction process prior to the GC-MS analysis.

Materials and methods

The contents of total phenolic compounds (TPC) and the antioxidant potentials via phosphomolybdenum assay (PMA), reducing power method (RPM) as well as hydrogen peroxide scavenging (HPS) were estimated; detailed experimental methods were provided in the supplementary file.

Results

Comparison TPC results

Eucalyptus leaf was initially extracted by a variety of organic solvents, solvent mixtures and pure water, independently. TPC was compared among them and the obtained results were represented in Table 1. In case of using solvents in pure state (100%), both acetone and

Table 1: Preliminary comparative phenolic contents (mg/g) dry leaves among different extraction solvents.

	Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol	Water
Solvent	9	6	17	30	24	30	17
Solvent-Water (1:1)	11	14	23	39	26	32	–

Values are rounded to the nearest integer.

methanol were equally effective with the highest TPC value (30 mg/g dry leaves). However, when organic solvents were mixed with water (1:1), acetone-water maintained the maximum TPC with a value of approximately 39 mg/g dry leaves and hence, this mixture has been subsequently used with different ratios.

Leaf

The highest phenolic content (TPC: 46.56 mg/g dry weight) was detected in the leaf of *E. camaldulensis* when extracted with 70% acetone, with the corresponding highest antioxidant potentials (PMA: 50.54 mg/g, RPM: 59.87 mg/g

and HPS: 89.28%) as well (Table 2, Figure 1). Since the results of both PMA and RPM were expressed in identical unit (mg/g dry plant weight), it is more rational to contrast between them and exclude the third protocol of HPS (expressed as %). Accordingly, RPM results were higher than PMA ones, a pattern which was also observed with the other three investigated organs of *E. camaldulensis*.

Bud

Similar to extraction tendencies found with leaf, the bud of *E. camaldulensis* was top extracted with 70% acetone-water and showed the maximum values of TPC:

Table 2: Content of total phenolic yields and antioxidant potentials (mg/g dry plant weight) in the acetone-aqueous extracts of *Eucalyptus* leaf, HPS expressed as %.

Leaf				
Acetone/water ratio	TPC	PMA	RPM	HPS (%)
15%	29.23 ± 1.21	33.56 ± 0.47	35.22 ± 1.41	81.95 ± 2.06
30%	37.43 ± 0.95	35.54 ± 0.05	41.08 ± 2.47	82.83 ± 2.55
50%	39.98 ± 0.13	38.98 ± 0.62	44.96 ± 0.34	86.66 ± 1.17
70%	46.56 ± 0.59	50.54 ± 0.48	59.87 ± 0.73	89.28 ± 1.05
90%	33.22 ± 0.26	50.36 ± 0.67	52.31 ± 1.85	89.02 ± 1.29
100%	30.20 ± 1.08	40.60 ± 0.15	41.40 ± 0.82	88.50 ± 3.51
F-value	0.01	4.93	3.07	0.00
p-Value	0.03	0.00	0.00	0.99

TPC, total phenolic compounds; PMA, phosphomolybdenum assay; RPM, reducing power method; HPS, hydrogen peroxide scavenging. p > 0.05, non significant; p ≤ 0.05, significant; p ≤ 0.01, highly significant.

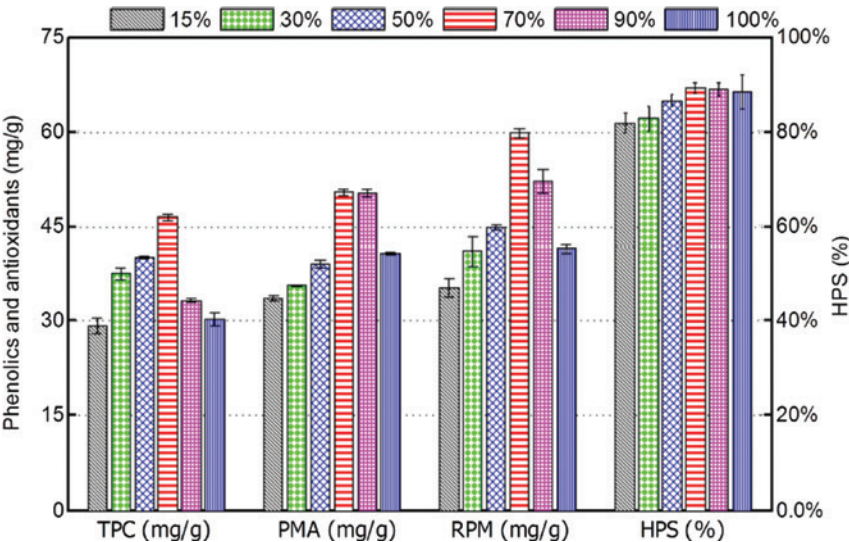


Figure 1: Total phenolic yields and antioxidant potentials in the acetone-aqueous extracts of *Eucalyptus* leaf (each column color represents particular ratio of acetone-water).

42.08 mg/g, PMA: 39.67 mg/g, RPM: 58.61 mg/g and HPS: 89.19%. Noticeably, there was a common and regular increment in the values of TPC and antioxidants with the increase of acetone fraction in the extraction mixture. They increased till peaked at 70% and then, their concentrations were reduced in case of 90% and absolute acetone (100%). Significant variations ($p \leq 0.01$) were detected within all assays barring HPS (Table 3, Figure 2).

Capsule

Empty capsule showed substantial contents of phenolics and antioxidants, they were comparable with those of leaf, bud and seed. Though, in some situations, they even

exceeded them. The values of TPC, PMA and HPS were the highest at 70% acetone (36.66 mg/g, 39.54 mg/g and 63.99%), in order. While the maximum RPM (40.40 mg/g) was recorded with 50% acetone (Table 4, Figure 3).

Seed

In Table 5 and Figure 4, total phenolic yields and antioxidant potentials of the seed acetone-aqueous extracts were represented. Over again, 70% acetone extracts exhibited the maximum values of TPC: 41.85 mg/g and PMA: 38.47 mg/g. But both RPM (58.89 mg/g) and HPS (81.25%) were better appraised in the 50% aqueous-acetone extract.

Table 3: Content of total phenolic yields and antioxidant potentials (mg/g dry plant weight) in the acetone-aqueous extracts of *Eucalyptus* bud, HPS expressed as %.

Bud				
Acetone/water ratio	TPC	PMA	RPM	HPS (%)
15%	35.35 ± 1.12	30.25 ± 1.14	34.44 ± 0.57	77.23 ± 1.04
30%	39.26 ± 0.16	34.94 ± 2.13	35.82 ± 1.05	81.89 ± 0.16
50%	39.19 ± 0.26	34.98 ± 0.07	53.55 ± 1.14	85.51 ± 0.25
70%	42.08 ± 0.71	39.67 ± 0.93	58.61 ± 1.21	89.19 ± 0.88
90%	38.66 ± 0.16	38.67 ± 1.61	55.12 ± 2.06	88.33 ± 0.33
100%	32.24 ± 2.11	37.41 ± 1.26	38.03 ± 1.21	85.46 ± 0.43
F-value	8.41	17.19	9.36	1.22
p-Value	0.01	0.00	0.00	0.99

TPC, total phenolic compounds; PMA, phosphomolybdenum assay; RPM, reducing power method; HPS, hydrogen peroxide scavenging. $p > 0.05$, non significant; $p \leq 0.05$, significant; $p \leq 0.01$, highly significant.

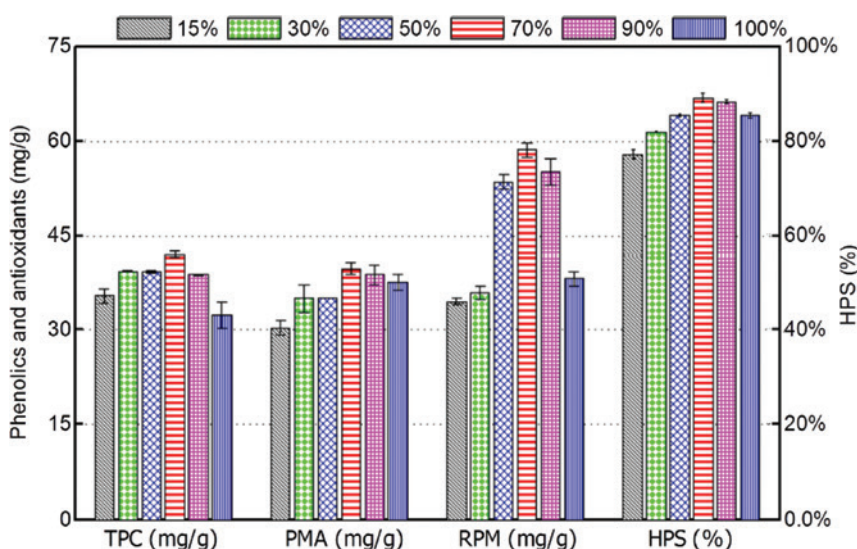
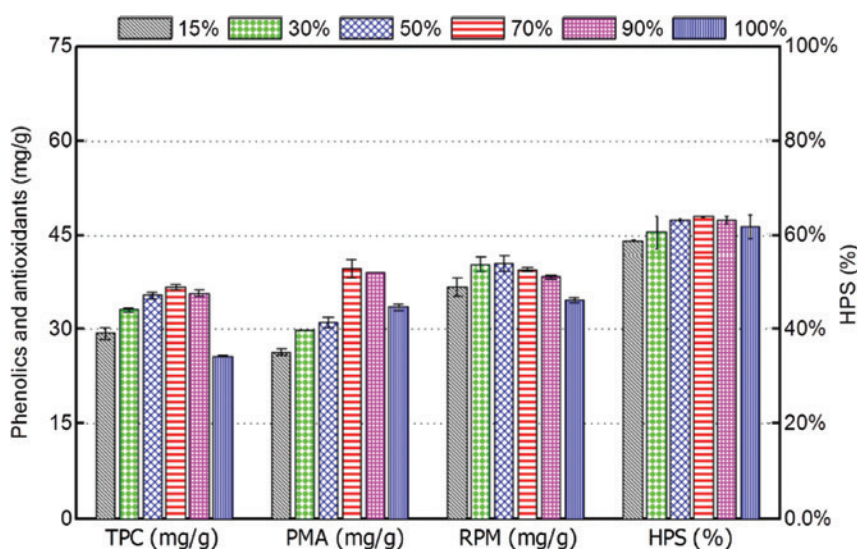


Figure 2: Total phenolic yields and antioxidant potentials in the acetone-aqueous extracts of *Eucalyptus* bud (each column color represents particular ratio of acetone-water).

Table 4: Content of total phenolic yields and antioxidant potentials (mg/g dry plant weight) in the acetone-aqueous extracts of *Eucalyptus* capsule, HPS expressed as %.

Empty capsule				
Acetone/water ratio	TPC	PMA	RPM	HPS (%)
15%	29.36±0.92	26.33±0.53	36.66±1.37	58.94±0.19
30%	33.05±0.36	29.80±0.08	40.25±1.15	60.78±3.44
50%	35.36±0.50	31.07±0.84	40.40±1.17	63.27±0.35
70%	36.66±0.38	39.54±1.44	39.45±0.26	63.99±0.18
90%	35.72±0.57	38.97±0.05	38.25±0.31	63.18±0.84
100%	25.71±0.11	33.47±0.47	34.60±0.38	61.97±2.55
F-value	12.17	23.32	26.4	7.11
p-Value	0.00	0.00	0.00	0.73

TPC, total phenolic compounds; PMA, phosphomolybdenum assay; RPM, reducing power method; HPS, hydrogen peroxide scavenging.
 $p > 0.05$, non significant; $p \leq 0.05$, significant; $p \leq 0.01$, highly significant.

**Figure 3:** Total phenolic yields and antioxidant potentials in the acetone-aqueous extracts of *Eucalyptus* capsule (each column color represents particular ratio of acetone-water).**Table 5:** Content of total phenolic yields and antioxidant potentials (mg/g dry plant weight) in the acetone-aqueous extracts of *Eucalyptus* seed, HPS expressed as %.

Seed				
Acetone/water ratio	TPC	PMA	RPM	HPS (%)
15%	33.32±0.42	30.23±0.03	47.78±0.36	71.19±5.03
30%	41.6±0.062	35.21±0.92	57.03±0.81	75.96±2.34
50%	41.36±1.67	36.62±0.69	58.89±0.27	81.25±2.77
70%	41.85±1.41	38.47±0.02	43.23±1.67	80.50±2.94
90%	39.04±0.16	35.75±0.18	38.45±4.94	76.64±4.40
100%	36.25±1.21	37.34±0.19	34.68±0.08	68.93±5.08
F-value	54.95	19.09	42.63	1.12
p-Value	0.00	0.01	0.00	0.99

TPC, total phenolic compounds; PMA, phosphomolybdenum assay; RPM, reducing power method; HPS, hydrogen peroxide scavenging.
 $p > 0.05$, non significant; $p \leq 0.05$, significant; $p \leq 0.01$, highly significant.

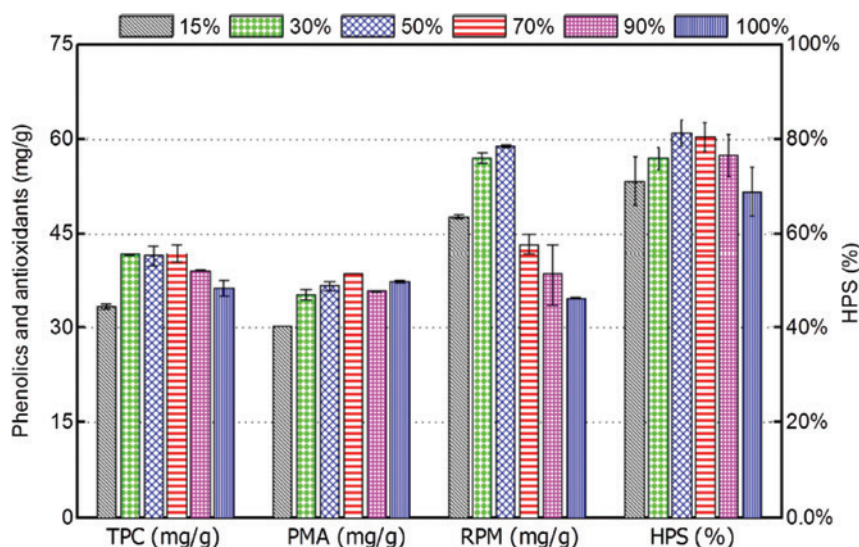


Figure 4: Total phenolic yields and antioxidant potentials in the acetone-aqueous extracts of *Eucalyptus* seed (each column color represents particular ratio of acetone-water).

Correlation coefficients

Correlation coefficients were examined in order to interpret the relationships between total phenolics and the antioxidant results of *E. camaldulensis*. A correlation value up to 0.998 was established between TPC and the antioxidant activity assayed by PMA. Comparatively, the least correlation (0.696) was between TPC and HPS outcomes (Table 6).

GC-MS results

A number of main peaks were detected for some compounds on GC-MS chromatogram. They represented gallic acid, ellagic acid, catechin and tricetin with a maximum presence in the seed of *E. camaldulensis*. Gallic acid recorded the highest relative abundance (1898.8 %), followed by catechin (1360.76%) and then, ellagic acid (1025.2%). Interestingly, a major detection of tricetin was recorded for the four plant organs (118–1323%). Also, epicatechin

and taxifolin had comparable contents of 154.45% and 154.29% in the plant seed, respectively (Table 7). Some of the abundant compounds were highlighted on the generated GC-MS chromatograms (TICs) and shown in Figures (S1, S2, S3 and S4).

Discussion

Phenolic compounds are extracted from plant tissues by various solvents, including methanol, ethanol, acetone or water [14]. In the current study, some preliminary solvents and/or solvent combinations including hexane, chloroform, ethyl acetate, acetone, ethanol and methanol were first examined to extract total phenolics from *E. camaldulensis* leaves via single-solvent extraction. The solvents were selected from the literature reports pertaining to chemical investigations of *E. camaldulensis*. After the preliminary screening, acetone was found the most powerful (either in pure state or mixed with water) and hence, it was presented here. The efficiency of acetone-aqueous mixture as an extraction solvent has been established. In general, 70% acetone extract was the best extraction mixture for TPC, PMA, RPM and HPS. Considering the plant organs, the leaf of *E. camaldulensis* recorded the highest total contents of phenolic compounds with the maximum antioxidant activities (PMA) and reducing powers (RPM) as well, followed by the plant bud. Previously, the antimicrobial activities of acetone crude extracts from leaf, bud, capsule and seed of *E. camaldulensis* were evaluated against some

Table 6: Correlation coefficient values between the phenolic contents and the antioxidant potentials of *E. camaldulensis*.

	TPC	PMA	RPM	HPS
TPC	1.0	0.998 ^a	0.985 ^a	0.696 ^b

^aCorrelation probability <0.01, ^bcorrelation probability <0.05, PMA, phosphomolybdenum assay; RPM, reducing power ability; HPS, hydrogen peroxide scavenging.

Table 7: Relative presence (%) of some chemical constituents in the leaf, bud, capsule and seed of *E. camaldulensis* as detected by GC-MS.

Compound	RT (min)	Relative abundance (%)			
		Leaf	Bud	Empty capsule	Seed
Hydroquinone	12.05	4.03	4.74	6.70	25.48
Syringic acid	12.13	7.02	8.54	8.35	11.32
Vanillic acid	12.89	1.30	2.21	1.95	2.25
P-coumaric acid	13.26	1.02	0.966	0.213	0.28
Luteolin	13.55	0.11	0.12	0.08	0.08
5-hydroxy-7,4'-dimethoxyflavone	13.68	0.94	0.56	0.00	0.12
Ursolic acid	14.11	0.68	14.09	11.71	14.16
Myricetin	14.25	0.89	1.052	1.02	1.13
Ferulic acid	14.31	23.44	33.24	11.96	19.85
Quercetin 3-glucoside	16.13	0.32	0.12	0.11	0.12
Hesperitin	16.70	1.17	0.00	0.27	0.00
Pyrogallol	16.74	3.19	1.27	1.97	10.84
Cinnamic acid	16.98	1.27	0.68	0.213	0.00
Epicatechin	18.77	26.35	77.48	16.33	154.45
P-hydroxybenzoic acid	19.04	0.62	0.94	1.39	9.18
Resorcinol	19.29	3.46	0.75	0.90	0.00
Chlorogenic acid	19.41	144.37	0.00	0.00	5.91
Kaempferol	19.94	4.42	9.64	7.88	8.23
Quercetin-3-O- β -D-glucuronide	20.40	1.79	0.36	0.09	0.15
Methyl cinnamate	23.86	0.40	0.46	0.85	0.00
Protocatechuic acid	23.96	1.95	0.00	2.17	21.88
Ellagic acid	24.56	124.21	98.64	86.00	1025.2
Kaempferol-3-O- β -D-glucuronide	25.01	2.03	1.96	0.99	1.72
Galloyl quinic acid	25.14	0.00	0.21	0.77	0.00
Taxifolin	25.55	0.00	12.11	8.84	154.29
Gallic acid	27.39	161.07	130.65	191.08	1898.8
Epigallocatechin-3-gallate	28.85	0.00	0.88	1.72	0.00
Naringenin	41.13	0.90	0.22	1.23	0.04
Phloretin	41.71	0.00	0.47	0.00	7.90
Neohesperidin	43.03	0.00	27.96	5.81	11.25
(+)-Catechin	43.74	14.71	112.69	151.33	1360.76
Tricetin	47.73	118.14	122.29	247.87	1323.37

RT, retention time, compounds were identified in reference to known standards; MS spectra were compared through Fiehn or NIST database.

bacterial and fungal pathogens and exhibited good anti-bacterial and antifungal actions [13]. This, in turn, may further substantiate our present findings because whenever phenolic compounds are abundant, they exert powerful antioxidant and antimicrobial activities, which have been also approved in numerous reports. Our results are also in harmony with Singab et al. [15] who reported about the therapeutically implication of *E. camaldulensis* aqueous-acetone extract, which may be able to develop preventive factors against some cancers. Before, Ashraf et al. [1] found that leaf-methanol extract of *E. camaldulensis* had maximum amounts of total phenolic contents and antioxidant actions when compared with both hexane and chloroform. However, acetone was not investigated in their study. Besides, the current work included more solvents which were initially tested for the best extraction

power and then, acetone-aqueous mixture was further applied with four organs of *E. camaldulensis*, two of them (bud and capsule) are being investigated for the first time.

Herein, significant variations were recorded in most experimental cases ($p \leq 0.05$, or $p \leq 0.01$). The manner of extraction influences the extract yields and the resultant antioxidant actions of the plant materials [16]. This is possibly attributed to the different antioxidant compounds with varied polarities and chemical properties affecting their solubility in picky solvents [9]. Otherwise, this is most likely due to the dissimilar responses to different radical or oxidant compounds [16]. The relative compatibilities between the solvent and the physicochemical properties of the bioactive compounds might contribute to such outcome differences. So, it is imperative to optimize the extraction conditions to guarantee the entire

capture of potential bioactive compounds [12]. Furthermore, El-Ghorab et al. [17] suggested that different polarities of extraction media affect the phenolic composition. These extracts do not particularly modify the nutritional or medicinal values of the derived products, but rather improve their yield characteristics [18].

In this study, antioxidant potentials were assayed via a range of protocols. Accordingly, high correlation (0.998) was spotted between the obtained data of TPC and those of antioxidant activities examined via PMA. Also, another strong and positive association (0.985) was highlighted between TPC and the reducing powers (RPM) of extracts. Earlier, positive and considerable correlation values were also recorded between the phenolic compounds and the antioxidant activities of some *Eucalyptus* species [1, 9].

Interestingly, from the applied GC-MS analysis, gallic acid, ellagic acid, catechin and tricetin represented the maximum presence percentages in the seed of *E. camaldulensis*. While the highest total phenolic contents were ascribed to the plant leaf and bud. However, the plant seed recorded comparable values of total phenolics and antioxidant contents. Earlier, both gallic and ellagic acids were found the main antioxidant compounds in the leaf extract of *E. camaldulensis* with substantial antioxidant activities [17]. It may be considered that further compounds, other than phenolics, might have contributed to the increased antioxidant potentials of leaf and bud. Moreover, the four investigated plant organs of *E. camaldulensis* were found to be rich in tricetin and taxifolin. In a study carried out by Hsu et al. [19], tricetin was proved to maintain anticancer properties on human breast adenocarcinoma MCF-7 cells, while taxifolin has been manifested to restrain the growth of ovarian cancer cell [20]. It may be concluded that acetone-water is a successful and appropriate mixture for the extraction of phenolic compounds from different four organs of *E. camaldulensis*. This extraction mixture could give better yields and enhance the biological activities of its corresponding extracts. This allows the practically managing of the plant tissues for pharmaceutical targets, as well as using it as a supplying resource of effective natural antioxidants, which can be safely integrated into food additives. Previously, Eucalypts extracts have been approved as natural food additives because of their antioxidant properties and included in the List of Existing Food Additives in Japan [2].

The current work simply represents a consistent comparison system to verify the efficiency of some solvents to pull out active compounds from *E. camaldulensis*, highlighting its yields of main phenolics and antioxidants. We think that such onset optimization in the extraction process is more fitting to the subsequent GC-MS analysis,

especially for the plant seed. To the best of authors' knowledge, the antioxidant importance of two organs (bud and capsule), are reported here for the first time. Also, the analysis of non-volatile compounds (phenolics) of the plant seed via GC-MS has not been reported elsewhere. We presented preliminary findings which may be considered as a spotlight on the phenolic composition and the antioxidant potential of *E. camaldulensis*. However, to give a better insight, additional analyses, such as LC-MS, are still required to be carried out for the exact quantification of individual phenolic compounds in diverse extracts. Furthermore, the antioxidant activity of individual compounds should be independently examined in order to verify the relation between their abundance and the antioxidant potentials in different organs of *E. camaldulensis*.

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Conflict of interests statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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