Scientific Research Publishing

Phytochemical Profile and *In Vitro* Antioxidant Properties of Essential Oils from Powder Fractions of *Eucalyptus camaldulensis* Leaves

Venant Donald Noumi[1], Richard Marcel Nguimbou[2*], Marthe Valentine Tsague[3], Markusse Deli[2], Sandrine Rup-Jacques[4], Dicko Amadou[5], Elie Njantou Baudelaire[2], Selestin Sokeng[1], Nicolas Yanou Njintang[1,2]

[1]Department of Biological Sciences, Faculty of Sciences, University of Ngaoundere, Ngaoundere, Cameroon
[2]Department of Food Sciences and Nutrition, ENSAI, University of Ngaoundere, Ngaoundere, Cameroon
[3]Department of Biomedical Sciences, Faculty of Sciences, University of Ngaoundere, Ngaoundere, Cameroon
[4]Université de Lorraine, ICPM, Metz, France
[5]Université de Lorraine, UR-AFPA, Metz, France
Email: *marcelguri@yahoo.fr

Abstract

Dried leaves of *Eucalyptus camaldulensis* were finely grinded and fractionated by sieving into four granulometric classes (<100 μm, 100 - 200 μm, 200 - 355 μm and >355 μm). The obtained powder fractions were used for essential oil (EO) extraction by hydrodistillation and their phytochemical profile and *in vitro* antioxidant activities were evaluated. The mother powder (unsieved powder) was used for comparison. Particle size exerted a significant influence ($p < 0.05$) on the phytochemical composition and *in vitro* antioxidant properties of the EOs. Comparatively, the mother powder had the highest contents of α-pinene (55.6%), camphene (3.4%) and limonene (3.7%), while 1,8-cineole (26.6% and 22.4%), exo-fenchol (5.6% and 3.5%), α-campholenol (4.2% and 3.4%), L-trans-pinocarveol (5.5% and 2.7%), L-borneol (12.6% and 6.8%) and α-terpineol (16.4% and 7.6%) are the main compounds of EOs from the <100 μm and 100 - 200 μm fractions, respectively. The antioxidant activities of the EOs revealed higher radical-scavenging activities DPPH (90.62% and 70.46%) and ABTS (89.59% and 73.31%) for finer fractions (<100 μm and 100 - 200 μm, respectively). The best reducing power (36.15% and 34.27%) were also found in these finer powder fractions which improved by more than 2 times the value of mother powder (reducing power of 17.01%). These results suggest that grinding followed by sieve fractionation concentrates the majority of antioxidant phytochemicals in the EOs of the finer powder fractions of *E. camaldulensis* leaves. Finer powders could be used as functional ingredients in food formulations for the management of chronic diseases.

1. Introduction

In recent years, the interest for plant-derived bioactive products has increased
owing to their numerous health and nutritional benefits. Plants and their prod-
ucts are rich sources of phytochemicals including terpenes and polyphenols
having a wide variety of biological activities, such as anti-inflammatory, antimi-
crobial, and antioxidant activities [1] [2] [3]. With regard to antioxidant activity,
it is well known that the antioxidants could attenuate the oxidative damage of a
tissue indirectly by enhancing natural defenses of cell and/or directly by sca-
venging the free radical species. Because of that, growing attention has been paid
to phytochemicals characterized as antioxidant natural products.

Eucalyptus camaldulensis is a medicinal plant with a wide spectrum of appli-
cation in traditional medicine in several countries around the world. Numerous
studies conducted on its essential oil (EO) have demonstrated its high antioxi-
dant potential, mainly due to the great diversity of its phytochemicals. This is
how Salem *et al.* [4] attributed the strong antioxidant activity of this EO to a
high content of spathulenol. However, α-pinene has shown a strong antioxidant
effect on the DPPH radical, the hydroxyl radical and the superoxide anion [5]
[6] [7] [8]. Moreover, the high antioxidant effects of several terpenes like thy-
mol, carvacrol, borneol, 1,8-cineole and α-terpineol in *Eucalyptus camaldulensis*
leaves have been reported [9] [10] [11] [12]. Since EO phytochemicals can ac-
cumulate in various parts of plants, extraction represents a main process for
concentrating these active compounds. Hence, it ensures separation of these
compounds from plant cellular matrix and extraction conditions determine the
quality and/or quantity of extracted compounds [13] [14]. In this regard, plant
EOs are commonly obtained using hydrodistillation. In this process, water is
used for extraction of plant active compounds as it has been recognized as safe
solvent [15]. Unfortunately, this method usually requires long time with in-
creased risk of degradation of thermo-labile constituents and results in low
yields of extraction depending on the rigidity of plant material [16].

Grinding followed by particle size fractionation consists of producing powders
of the plant and spraying it on a column of sieves with decreasing mesh diame-
ters. Theoretically, the molecules are distributed according to the size of the par-
ticles, which makes it possible to concentrate on a fraction of a group of biomo-
lecules [17] [18] [19]. Using this technique, many authors demonstrated that
powder fractionation resulted in concentrating polyphenols in finer particle size
[20] [21] [22]. Otherwise, our previous study reported that finer fractions (<180
μm and 180 - 212 μm) of *Dichrostachys glomerata*, *Adansonia digitata*, *Boscia
senegalensis* and *Hibiscus sabdariffa* concentrated more polyphenols, micronu-

trients and antioxidant activities as ethanolic extract [23] [24] [25]. However, there is no study on the effect of powder fractionation on the EO phytochemicals in relation to their antioxidant activities. Yet, the application of this new approach which combines the advantages of sieve fractionation and hydrodistillation would allow efficient access to the phytochemicals of *E. camaldulensis* leaves. The high-antioxidant powders or their EOs could be useful as functional ingredients in nutraceutical formulations.

Therefore, this study aims to assess the effect of particle size fractionation by sieving on the concentration of phytochemicals and the antioxidant activities of the EO in powder fractions from *E. camaldulensis* leaves. To achieve this, the study focused firstly on assessing the mother powder granulometry and the EO yields from sieving fractionation. Secondly on comparing the phytochemical contents and the antioxidant activities (DPPH, ABTS, reducing power) of the EOs of different powder fractions. Mother powder was also analyzed and served as a control.

2. Materials and Methods

2.1. Plant Material

The fresh leaves of *E. camaldulensis* were used as plant material in the present study. This plant material was collected from Dang locality (Ngaoundere, Cameroon: latitude 7'42'46; longitude 13'55'59) in October 2017. Identification was made in comparison with the sample N˚4039 SRFT/am of the National Herbarium of Cameroon. After washing and cleaning, the leaves were dried in the shade of a hangar with air circulation at room temperature which varied from 14˚C to 38˚C. The leaves are spread on a clean sheet in thin layers and turned over frequently for seven days.

2.2. Plant Grinding and Particle Size Analysis

The dried leaves of *E. camaldulensis* were ground in the Moulinex robot blender mill supplied with a sieve drilled with 1 mm trapezoid holes. The analytical method used by Nguimbou *et al.* [26] was applied for the analysis of particle size distribution of the obtained powder. The measurement was carried out using laser by Mastersizer 3000 (Malvern Instruments, Orsay, France) supplied with the Aero S wet dispersion unit. The chosen size estimator was the particle size in volume and classical granulometric parameters were determined: D10, D50 and D90 (where Dx means that x% of the volume of particles has a diameter inferior to Dx). The span, a common parameter related to the width of particle size distribution was calculated as follows:

$$\text{Span} = \frac{\text{D90} - \text{D10}}{\text{D50}} \tag{1}$$

2.3. Powder Sieving

The plant powder was separated into granulometric classes using a series of

three sieves of various apertures (100 μm, 200 μm and 355 μm) selected on the basis of the particle size analysis. The powder was sieved according to procedure used in previous studies [23] [27]. For that, 100 g of powder passed through sieve columns using an Analysette 3 Spartan apparatus (Fritsch, Idar-Oberstein, Germany) to obtain fraction powders. Sieve shaker vibration amplitude was set at 0.5 mm for 10 min. Thus, the fraction of the powder retained on each sieve is recovered and weighed for the calculation of the mass fraction of each granulometric fraction. The following powder fractions were obtained: <100 μm, 100 - 200 μm, 200 - 355 μm and >355 μm with a moisture content of about 10% - 12%. Non fractioned (mother powder) powder was taken as control. The powdered samples were stored at 10°C in polyethylene bags and placed at room temperature (25°C ± 2°C) until they were used.

2.4. Essential Oils Extraction

The EO were extracted from each powder fraction by hydrodistillation using an adapted device of the Clevenger type for 5 hours. The EO collected by decantation was filtered through a column of anhydrous sodium sulfate. The essences obtained were introduced into dark bottles and stored at 4°C protected from light. The essential oil yield was expressed as volume of the distillate (V) per kg of dry leaves (Ms) and calculated as follows:

$$\text{Yield}\left(\text{mL/kg}\right) = \frac{V}{Ms} \times 100 \qquad (2)$$

2.5. Chemical Composition Analysis

The analysis of the EO was carried out using a Varian CP-3380 type chromatograph equipped with a flame ionization detector and a capillary column (30 m × 0.25 mm) with a stationary apolar phase of methylsilicone type (DB5, film thickness 0.25 μm). The oven was programmed from 50°C to 200°C with a temperature gradient of 5°C/min. The injector temperature was 200°C and the detector set to 200°C. Nitrogen was used as the carrier gas with a flow rate of 1 mL/min. The retention indices of the constituents were determined relative to the retention times of a series of n-alkanes and their relative percentages calculated by electronic integration without taking into account their response factors. The coupling gas chromatography/mass spectrometry was carried out using an apparatus of the brand Hewlett-Packard HP 5970 A, equipped with an apolar capillary column (30 m × 0.25 mm) in fused silica of type HP -1 (film thickness 0.25 μm) and a quadrupole type detector (ionization energy 70 eV). The temperature of the injector was 220°C and that of the interface area was 210°C. The oven temperature was programmed from 70°C to 200°C with a gradient of 10°C/min. The carrier gas is helium with a flow rate of 0.6 mL/min. The acquisition was made in scan mode (35 - 300 amu) at 2.96 scan/s. The components were identified on the basis of their retention indices and their mass spectra by comparison with data from the literature.

2.6. *In Vitro* Antioxidant Activity Evaluation of EO

2.6.1. DPPH Radical Scavenging Capacity

The DPPH anti-free radical activity of the EO samples was evaluated in accordance with the method of Zhang and Hamauzu [28] with slights modifications. A volume of 0.5 mL of the methanolic solution of each EO extract at a concentration of 2 mg/mL was added to 1 mL of the DPPH solution (0.025 g/L). In parallel, a negative control was prepared by mixing 0.5 mL of methanol with 1 mL of the methanolic solution of DPPH. The optical density recorded at 517 nm using the methanolic solution for the blank tube and ascorbic acid as standard after 60 min of incubation in the dark and at laboratory temperature (25°C ± 2°C). The percentage of free radical scavenging effect was calculated as follows:

$$\text{DPPH scavenging effect}\left(\%\right) = \frac{\text{DO control} - \text{DO sample}}{\text{DO control}} \times 100 \qquad (3)$$

2.6.2. ABTS Radical Scavenging Capacity

The ABTS anti-free radical activity of the EO samples was determined according to the method described by Re *et al.* [29]. ABTS radical was produced with the mixture of 7 mM ABTS and 2.45 mM potassium persulfate and incubated at room temperature in the dark for 16 h before use. After incubation, the absorbance of the solution at 734 nm was adjusted to 0.70 ± 0.02 by dilution with 95% ethanol. Then in a test tube, 2 mL of this diluted ABTS solution and 150 μL of each ethanolic solution of the different EO extracts at the concentration of 2 mg/mL were introduced and well stirred, incubated at laboratory temperature (25°C ± 2°C) for 10 min and the absorbance recorded at 734 nm. Ascorbic acid solution (1 mg/mL) was used as a standard. The percentage of free radical scavenging effect was calculated as follows:

$$\text{ABTS scavenging effect}\left(\%\right) = \frac{\text{DO control} - \text{DO sample}}{\text{DO control}} \times 100 \qquad (4)$$

2.6.3. Total Reducing Power

The total reducing power of the methanolic/water extracts was investigated using the method developed by Oyaizu [30]. One milliliter of the extract of each sample was mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate [K3Fe (CN) 6] solution. The mixture was incubated in a water bath at 50°C for 30 min, cooled, mixed with 2.5 mL of 10% trichloroacetic acid solution and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was removed and mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (1.0%), allowed to react for 10 min at room temperature and the absorbance was measured at 700 nm. Ascorbic acid solution was used as standard. The reducing power is determined by referring to the calibration curve of ascorbic acid and expressed in terms of mg ascorbic acid equivalent per g of dry matter (mg AAE/g DM).

2.7. Statistical Analysis

Analyses were performed in triplicate and the results presented as mean ± SD.

Statistical significance of differences between sample means was determined using analysis of variance (ANOVA) followed by a Duncan's test at 95% confidence level using statgraphics 15.1. Principal component analysis (PCA) has been used to classify and correlate the factors that define powder fractions, phytochemical compounds and antioxidant activities and was realized by XL-STAT 2019.

3. Results and Discussion

3.1. Particle Size Characteristics of the Mother Powder

Table 1 presents the particle size characteristics of the mother powder of *E. camaldulensis* leaves studied. Particle size distribution of the mother powder of *E. camaldulensis* leaves was polymodal with a volume of fine particles around 34 μm (D10), a median volume of particles around 195 μm (D50) and a volume of large particles around 735 μm (D90). The major volume of *E. camaldulensis* mother powder corresponded to large particles, whereas fine particles were less numerous (10% of a total volume of the powder). The low span value of 2.3 reflects a low dispersion of the mother powder and confirmed the correct running of the grinding process. Indeed, a dispersion is said to be wide if its span is greater than 3 [22].

The fact that the major volume of *E. camaldulensis* powder corresponded to large particles can be explained by the expected non-sphericity of large particles, as ground fibers generally lead to large rod-shaped particles. So, fine particles can stick to larger particles and thus be retained by sieves of mesh size exceeding their diameter [27]. As the median distribution of the mother powder being located around 200 μm, the fractionation sieves were chosen as follows: a 200 μm sieve for studying the particles around the median; a 100 μm sieve for studying particles below the median and a 355 μm sieve for studying particles above the median.

3.2. Sieved Mass, Mass Fraction and EO Yield

The sieving fractionation was carried out on *E. camaldulensis* mother powder in order to check if the employed sieving procedure was efficient in producing powder fractions having well-different particle size (Table 2). It can be seen in Table 2 that the >355 μm fraction was the most represented in the mother powder of *E. camaldulensis* with a mass yield of 44.4% followed by the 100 - 200 μm, 200 - 355 μm and <100 μm fractions reaching 19.6%, 18.6% and 14.1%, respectively. The sieving procedure was effective in producing enough powder for further analyses in all granulometric classes. According to Becker *et al.* [20], a longer grinding time and/or a higher grinding speed could increase mass yields of *E. camaldulensis* powder fractions after sieving. Even more, using a grinding sieve of smaller mesh can also be considered to fulfill higher mass yields for finer fractions (<100 μm) of *E. camaldulensis* leaves powder. Based on these results, one would expect differences on EO extraction yields in these powder fractions.

Table 1. Particle size characteristics of the mother powder of *E. camaldulensis*.

Characteristics (μm)	Values
D10	33.5 ± 1.4
D50	194.5 ± 1.9
D90	734.3 ± 3.2
Span (-)	2.3 ± 0.1

Table 2. Sieved mass, mass fraction, and EO yield of the different granulometric classes and mother powder of *E. camaldulensis* leaves.

Granulometric classes	Sieved mass (g)	Mass fraction (%)	EO Yield (mL/kg)
<100 μm	84.6	14.1	0.40 ± 0.1[d]
100 - 200 μm	117.6	19.6	0.80 ± 0.1[b]
200 - 355 μm	111.6	18.6	0.68 ± 0.1[c]
>355 μm	266.4	44.4	0.32 ± 0.1[e]
Mother powder	600	100	1.60 ± 0.1[a]

Means ± standard in the EO column followed by different letters were statistically different ($p < 0.05$) according to Duncan's multiple range test (n = 3).

As shown in Table 2, the mother powder of *E. camaldulensis* has the best EO yield (1.6 mL/kg) compared to the powder fractions. These values are much lower than those obtained by El Hajji *et al.* [31] whose EO yields were comprised between 15 and 16 mL/kg for dried *E. camaldulensis* leaves. Observed differences in yield could be explained by the fact that after drying, the leaves were ground into a fine powder and then sieved, while our predecessors did so on dried and whole leaves. From this point of view, it could be admitted that, during the grinding and sieving processes of the leaves, there is loss of volatile compounds of low molecular weight. Indeed, EO are highly volatile liquid compounds synthesized in specialized histological structures often located on or near the plant structure [32]. The work of Cheftel *et al.* [33] demonstrated that the yields of EO are markedly reduced depending on the types of drying: in the shade, activated by ventilation, assisted by lamps or using a resistance. Even more, Akhihiéro *et al.* [34] demonstrated that there is progressive decrease of the EO yield from 8.4 to 5.1 mL/kg as the particle size of *Cymbopogon citratus* leaves decreases from 20 mm to 4 mm. Cryogenic grinding makes it possible to preserve sensitive active ingredients such as EO and vitamins [35], which has not been the case in this work. The leaves were crushed using an electric mill without a temperature stabilization device (thermogenic) which would further explain the great loss of volatile compounds. However, sieving fractionation allowed to concentrate more EO in the 100 - 200 μm fraction (0.80 mL/kg), which has the highest yield compared to the rest of the fractions: 200 - 355 μm (0.68 mL/kg), <100 μm (0.40 mL/kg) and >355 μm (0.32 mL/kg). This result could be explained by the fact that in Myrtaceae, EO are trapped in the secretory pockets of specialized cells [36]. From this point of view, we can admit that 100 - 200 μm fraction concentrates the maximum of secretory cells which remained unex-

ploded after the grinding operation; these EO were released later under the constraints of temperature and pressure during hydrodistillation.

3.3. Chemical Composition of the EO Samples

Table 3 displays the analytical results for phytochemicals of EO from all granulometric classes, as well as for the mother powder of *E. camaldulensis*. The variation in the retention time of the different phytochemicals is shown resumed in the chromatogram of Figure 1. The results show that the sieving procedure had a great influence on the chemical composition of studied samples. The α-pinene, 1,8-cineole and α-terpineol are the main compounds respectively in the EO of the mother powder (55.6%, 20.3%, 5.7%) and fractions > 355 μm (59.3%, 20.1%, 4.5%), 200 - 355 μm (51.2%, 23.9%, 5.8%), 100 - 200 μm (47%, 22.4%, 7.6%) and <100 μm (25.8%, 26.6%, 16.4%). These results are different from those obtained in EO of *E. camaldulensis* by Da Cruz Francisco *et al.* [37] whose main compounds were 1,8-cineole (59.5%), α-pinene (9.2%) and limonene (8.7%) and those identified by Nah *et al.* [38]: *p*-cymene (39.4%), 1,8 cineole (19.9%), limonene (15.4%) and α-pinene (5.4%) in malian *E. camaldulensis* leaves, as well as those obtained by Pagula *et al.* [39]: 1,8 cineole (40%), β-pinene (9.2%), terpinene (5.3%) and *p*-cymene (4.7%) in Mozambique *E. camaldulensis* leaves. As reported by the authors, the variation in the chemical composition of the EO samples as a function of the regions is due to edaphic and climatic factors [38] [40] [41]. Even more, the results show a higher content of L-borneol (12.6%), L-trans-pinocarveol (5.5%), α-campholenol (4.2%) and exo-fenchol (5.6%) in the <100 μm fraction. These values are higher than those of the other fractions and that of the mother powder. These results could be explained by the fact that terpenes react with oxygen (in the air) to form new compounds through the auto-oxidation mechanism [42]. In fact, in aerobiosis, oxygen promotes the oxygenation and hydroxylation reactions of the double carbon bond favoring the

Table 3. Chemical composition (%) of the different granulometric classes and mother powder of *E. camaldulensis* leaves.

Compounds	Retention time (min)	Granulometric classes				
		<100 μm	100 - 200 μm	200 - 355 μm	>355 μm	Mother powder
α-pinene	6.42	25.8	47	51.2	59.3	55.6
camphene	6.74	1.8	3.6	3.5	3.4	3.4
limonene	8.16	1,5	3	3.5	4.1	3.7
1,8-cineole	8.24	26.6	22.4	23.9	20.1	20.3
exo-fenchol	9.72	5.6	3.5	2.9	2	2.5
α-campholenol	9.83	4.2	3.4	2.3	1.6	2.3
L-trans-pinocarveol	10.11	5.5	2.7	2.1	1.7	2.1
L-borneol	10.61	12.6	6.8	4.8	3.4	4.5
α-terpineol	10.94	16.4	7.6	5.8	4.5	5.7
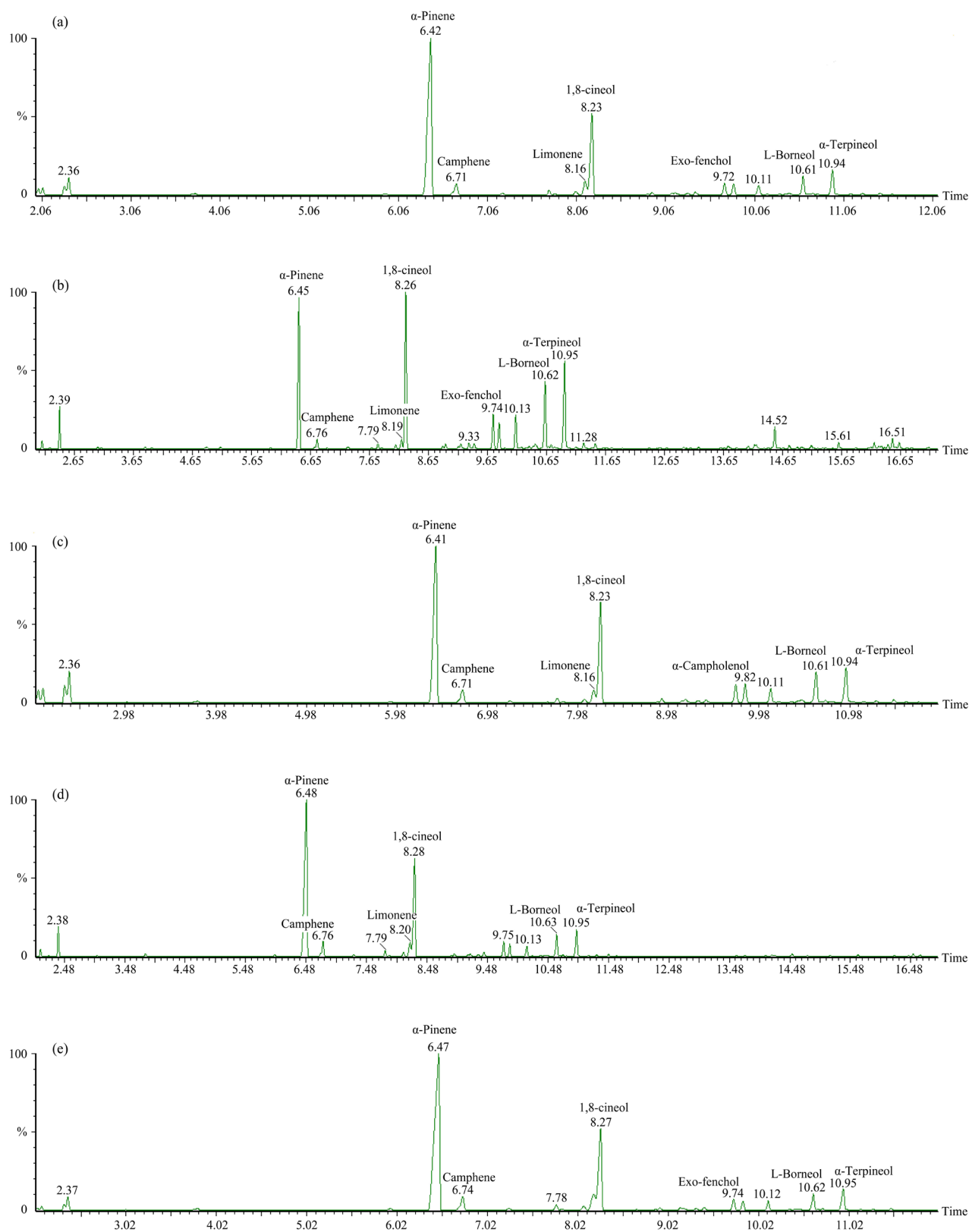
Figure 1. Gas chromatography/mass spectrometry profile of phytochemicals present in the EO from different granulometric classes and mother powder of *E. camaldulensis* leaves. (a) Mother powder, (b) ≤100 μm, (c) 100 - 200 μm, (d) 200 - 355 μm, (e) ≥355 μm.

transformation of hydrocarbon terpenes into oxygenated terpenes [43]. In this regard, we can admit that the reduction in the size of the particles by the grinding/sieving process, increases the contact surface with atmospheric oxygen. As a result, the EO self-oxidation process is more important in small particles compared to large particles. This justifies the reduction in the level of hydrocarbon terpenes, in particular α-pinene and limonene in the <100 μm and 100 - 200 μm fractions.

According to Neuenschwander *et al.* [44], the structure of α-pinene has four possible oxidation sites and its auto-oxidation depends on three factors: the ambient temperature, the oxygen flow and the exposure time. So, the auto-oxidation of α-pinene can lead to the formation of L-trans-pinocarveol [45]. Furthermore, the work of Rio [46] demonstrated that, atmospheric oxidants (hydroxyl radical OH, Nitrate NO_3, Ozone O_3) react with terpenes promoting the self-oxidation process. This is how limonene could react with the OH radical to form α-terpineol, as well as the oxidation of α-pinene in air which leads to the formation of derivative compounds [47]. However, the degradability of aerobic terpenes varies depending on the type of molecule and depends on the possible presence of a group. In this respect, Wilson and Hrutfiord [48] reported different rates of degradation mainly associated with molecular type. Alcohols are degraded to 99%, hydrocarbons to 75% and ketones to 12%. This aerobic metabolism can consist of several chemical reactions such as the hydroxylation of allylic carbons, the oxygenation of carbon-carbon double bonds, or the oxidation of alcohols to ketones as well as the breakdown of certain cyclic structures.

Otherwise, the transformation of limonene into α-terpineol by *Penicillium digitarum* is initiated by the epoxidation of the double bond in position 8, 9 and followed by the reductive rupture which forms α-terpineol [49].

3.4. Antioxidant Activity of EO Samples

The antioxidant activity of EO samples from *E. camaldulensis* leaves powders was measured in terms of radical scavenging ability and total reducing power. In Table 4 is reported the antioxidant activity of the EO assessed by different tests. The DPPH assay is known to provide reliable information concerning the antioxidant capacity of specific compounds or extracts across a short time scale. From the DPPH assay, the maximal antioxidant activity of EO was recorded for the <100 μm fraction (90.62% ± 0.13%) followed by the 100 - 200 μm fraction (73.31% ± 3.13%) whereas, the EO of mother powder had the lowest DPPH free radical scavenging activity (70.37% ± 21%). Compared to ascorbic acid (96.29% ± 0.28%) used as antioxidant reference, the fractions of *E. camaldulensis* powder were less active against the DPPH and ABTS radicals. Similarly, Barra *et al.* [50] demonstrated significant anti-radical activity with DPPH in EO of *E. camaldulensis* leaves collected in different localities of Sardinia in Italy. This author attributes the variation in anti-radical potential to the difference in phytochemical compounds in the EO. This suggests that, the particle size fractionation af-

fected the chemical composition of the EO of *E. camaldulensis* to different extents as mentioned above (Table 4). Thus, the highest DPPH radical scavenging activity of the EO in the <100 μm fraction would be due to its high concentration of oxygenated terpenes [51]. Earlier studies have indicated that oxygenated terpenes such as thymol, carvacol, borneol, 1,8-cineole, *a*-terpineol, have a significant antioxidant effect, mainly due to their redox properties [9] [11] [12]. Thus, the high contents in phytochemical compounds of EO of finer powder fractions are likely responsible for their DPPH scavenging capacity.

The antioxidant activity of EO samples was also measured in terms of radical scavenging ability using the ABTS assay. Table 4 displays the ABTS radical scavenging activity of the EO in the different granulometric fractions and mother powder of *E. camaldulensis* leaves. Significant differences ($p < 0.05$) were denoted between the antioxidant activity of the different fractions and unsieved powder. As for the DPPH test, the maximal antioxidant activity of EO was recorded for the <100 μm fraction (89.59% ± 0.09%) followed by the 100 - 200 μm fraction (70.46% ± 2.83%). Compared to the mother powder, the 100 - 200 μm and <100 μm fractions were 1.5 times and 2 times more active against the ABTS radicals, respectively. For most of the samples tested, the effect obtained with the DPPH method is not correlated with that obtained by the ABTS method; the results differ by these two methods. These variations can be explained by the mechanisms involved in the antioxidant reactions of radicals. The EO samples were dissolved in ethanol for the ABTS test, while methanol was used for DPPH test. This suggests that, solubility of the EO in different solvents can influence their effect. Indeed, the antioxidant activity of the tested compounds depends on the agent used and insisting on the mechanism of action of the antioxidant [52]. Other factors, such as the stereo-selectivity of the radicals or the solubility of the compounds in the different test systems can also influence the capacity of each EO to react and to reduce the different radicals [53]. Antioxidant activity deduced by the ABTS experiment allowed sorting powders according to their antioxidant activity in the following decreasing order: <100 μm, 100 - 200 μm, 200 - 355 μm, >355 μm and the mother powder.

Table 4. Antioxidant activities for the different granulometric classes and mother powder of *E. camaldulensis* leaves.
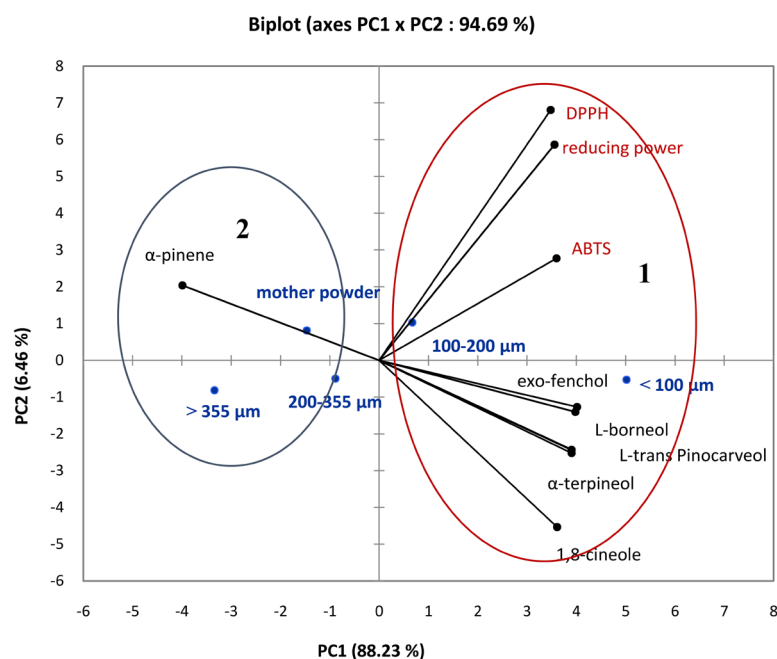
Granulometric classes	Antioxidant test		
	DPPH inhibition (%)	ABTS inhibition (%)	Reducing power (mg AAE/g DM)
<100 μm	90.62 ± 0.13[d]	89.59 ± 0.09[d]	36.15 ± 2.46[c]
100 - 200 μm	73.31 ± 3.13[c]	70.46 ± 2.83[c]	34.27 ± 1.97[c]
200 - 355 μm	47.77 ± 4.08[b]	68.03 ± 0.3[c]	13.48 ± 2.83[b]
>355 μm	28.11 ± 5.42[a]	18.00 ± 2.30[a]	7.19 ± 0.45[a]
Mother powder	70.37 ± 2.09[c]	50 ± 0.48[b]	17 ± 0.55[b]

AAE: ascorbic acid equivalent, DM: dry matter, Means ± standard in the EO column followed by different letters were statistically different ($p < 0.05$) according to Duncan's multiple range test (n = 3).

The reducing power of antioxidants is an important indicator of potential antioxidant activity. The antioxidant effect increases as a function of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power [30]. In Table 4 is presented the reducing power of studied EO samples. EO of the <100 µm fraction displayed the highest reducing power (36.15 ± 2.46 mg AA/g DM), while EO of the >355 µm fraction showed the lowest reducing power (7.19 ± 0.45 mg AA/g DM). These results of the reducing power test were shown to agree with those of the DPPH and ABTS free radical scavenging activities. This can be attributed to the fact that, DPPH, ABTS and reducing power tests are electron transfer reaction-based assays [54]. In fact, in the reducing power assay, the presence of reductants (antioxidants) terpenoids causes the reduction of the Fe^{3+} or ferricyanide complex to its ferrous form [55]. As stated by Malecky [36], oxygen that has a number of oxidation (II) easily gives electrons during oxido-reduction reactions, while molecular oxygen is the final electron acceptor, important in reduction reactions. Thus, terpenes which have a low number of functional groups (hydroxyl and carbonyl) have a reduced activity in the reactions [55]. However, the antioxidant activity of the majority compounds tested separately gives lower results compared to the activity of the total EO [56]. This suggests that, finer powder fractions richer in phytochemicals concentrate the various compounds of the EO, which act synergistically for much greater antioxidant activity [57].

3.5. Principal Component Analysis and Correlations

Principal component analysis (PCA) is one of the most widely used methods for multivariate analysis. It was performed to present an overview of the similarities and differences between the EO from powder fractions and to highlight the correlations between the terpene compounds and the antioxidant activities (Figure 2). This analysis makes it possible to identify more clearly the fractions having the EO with different contents of phytochemical compounds and therefore different antioxidant activities. The principal components PC1 and PC2 represented a total of 94.69% variation of powder properties. The distribution of powder samples on the PC1 × PC2 plot revealed a separation of powder samples into 2 groups of fractions made up for group 1 of <100 µm and 100 - 200 µm fractions and for group 2 of 200 - 355 µm, >355 µm fractions and the mother powder. Figure 1 shows that the powder fractions of group 1 whose contents of exo-fenchol, L-borneol, α-terpineol, L-trans-pinocarveol and 1,8-cineole have significantly increased ($p < 0.05$), were correlated to high anti-free radical effect and exhibit a high reducing power. On the other hand, these EO chemical compounds and antioxidant activities are lower in the fractions of group 2. We notice that most of the molecules which increased in the fractions of group 1 are essentially oxygenated terpenes. While group 2 recorded a high concentration of hydrocarbon terpenes.

Figure 2. Distribution of the chemical compounds and antioxidant parameters of investigated EO from *E. camaldulensis* powders (granulometric classes and mother powder) on the PC1 × PC2 axes system.

In this regard, it was observed a strong correlation between the exo-fenchol variable and the anti-free radical test with DPPH (r = 0.82, $p < 0.05$) as well as the total reducing power (r = 0.85, $p < 0.05$). Concerning the anti-free radical test with ABTS, the strongest correlation (r = 0.87, $p < 0.05$) was observed with 1,8-cineole. Thus, exo-fenchol and 1,8-cineole appear to be the terpenes with higher antioxidant potential. Pearson's correlation between terpenes and the different antioxidant tests makes it possible to note that the different variables, namely 1,8-cineole, exo-fenchol, L-trans-pinocarveol, L-borneol and *α*-terpineol are positively correlated, in particular with regard to the inhibition of DPPH, ABTS radicals and reducing power. These strong correlations (r > 0.5) would be due to the fact that the presence of the oxygen molecule as well as the carbon-carbon double bond in the structure of terpenes makes them more willing to redox reactions which can therefore reduce free radicals in solutions [54].

4. Conclusion

This study permitted to investigate the effects of size reduction technology on the phytochemical compounds, and antioxidant properties of EO from *E. camaldulensis* leaves. EO of *E. camaldulensis* leaves is mainly composed of *α*-pinene, 1,8-cineole and *α*-terpineol. The particle size fractionation by sieving made it possible to significantly concentrate the *α*-terpineol, L-borneol, L-trans-pinocarveol and exo-fenchol in the EO of the <100 μm and 100 - 200 μm fractions of the leaves of *E. camaldulensis*. However, it is important to control grinding speed, particle size and hydrodistillation conditions to avoid phyto-

chemicals degradation by heat and oxidation. The distribution of molecules as a function of particle size revealed the EO of the <100 μm and 100 - 200 μm fractions as the most effective in reducing the concentration of DPPH and ABTS in solution. Ultimately, the results of this work show that particle size fractionation by sieving concentrates the phytochemical compounds in finer fractions of the powders of *E. camaldulensis* leaves, while enhancing the antioxidant potential of their EOs. Overall, these finer powder fractions offer high potential in the management of oxidative reactions associated with human and plant pathologists.

Acknowledgements

Article Highlights

- Here we have chosen a new approach which combines the advantages of sieve fractionation and hydrodistillation to access phytochemicals in *E. camaldulensis* leaves;
- The higher antioxidant activities were associated with essential oils from finer powder fractions which concentrated the majority of phytochemicals;
- The essential oils from finer powder fractions of *E. camaldulensis* leaves have shown a huge antioxidant potential useful in the management of oxidative reactions associated with human and plant pathologists.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Kuate, D., Etoundi, B.C., Judith, L.N., Wan, A.M.B. and Julius, E.O. (2013) Anti-Inflammatory, Anthropometric and Lipomodulatory Effects of *Dyglomera* (Aqueous Extract of *Dichrostachys glomerata*) in Obese Patients with Metabolic Syndrome. *Functional Foods in Health and Disease*, **11**, 416-427. https://doi.org/10.31989/ffhd.v3i11.35

[2] Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I. and Heinrich, M. (2014) *Hibiscus sabdariffa* L.—A Phytochemical and Pharmacological Review. *Food Chemistry*, **165**, 424-443. https://doi.org/10.1016/j.foodchem.2014.05.002

[3] Jafarian, A., Zolfaghari, B. and Shirani, K. (2014) Cytotoxicity of Different Extracts of Arial Parts of *Ziziphus spina-christi* on Hela and MDA-MB-468 Tumor Cells. *Advanced Biomedical Research*, **3**, 38. https://doi.org/10.4103/2277-9175.125727

[4] Salem, M.Z., Ashmawy, N.A., Elansary, H.O. and El-Settawy, A.A. (2015) Chemotyping of Various Eucalyptus Species Grown in Egypt and Antioxidant and Antibacterial Activities of its Respective Essential Oils. *Natural Product Research*, **7**, 681-685. https://doi.org/10.1080/14786419.2014.981539

[5] Wei, A. and Shibamoto, T. (2007) Antioxidant Activities and Volatile Constituents

of Various Essential Oils. *Journal of Agricultural and Food Chemistry*, **55**, 1737-1742. https://doi.org/10.1021/jf062959x

[6]   Wang, W., Wu, N., Zu, Y.G. and Fu, Y.J. (2008) Antioxidative Activity of *Rosmarinus officinalis*. *Food Chemistry*, **108**, 1019-1022. https://doi.org/10.1016/j.foodchem.2007.11.046

[7]   Singh, H.P., Mittal, S., Kaur, S., Batish, D.R. and Kohli, R.K. (2009) Characterization and Antioxidant Activity of Essential Oils from Fresh and Decaying Leaves of *Eucalyptus tereticornis*. *Journal of Agricultural and Food Chemistry*, **57**, 6962-6966. https://doi.org/10.1021/jf9012407

[8]   Ho, C.L. and Su, Y.C. (2012) Composition, Antioxidant and Antimicrobial Activities of the Essential Oil Leaf of *Machilus japonica* from Taiwan. *Natural Product Communications*, **7**, 109-112. https://doi.org/10.1177/1934578X1200700136

[9]   Miguel, M.G. (2010) Antioxidant Activity of Medicinal and Aromatic Plants. A Review. *Flavour and Fragrance Journal*, **25**, 291-312. https://doi.org/10.1002/ffj.1961

[10] Bicas, J.L., Neri-Numa, I.A., Ruiz, A.L.T.G., De Carvalho, J.E. and Pastore, G.M. (2011) Evaluation of the Antioxidant and Antiproliferative Potential of Bioflavors. *Food and Chemical Toxicology*, **49**, 1610-1615. https://doi.org/10.1016/j.fct.2011.04.012

[11] Dar, M.Y., Shah, W.A., Rather, M.A., Qurishi, Y., Hamid, A. and Qurishi, M.A. (2011) Chemical Composition, *in Vitro* Cytotoxic and Antioxidant Activities of the Essential Oil and Major Constituents of *Cymbopogon jawarancusa* (Kashmir). *Food Chemistry*, **129**, 1606-1611. https://doi.org/10.1016/j.foodchem.2011.06.016

[12] Moon, H.K., Kang, P., Lee, H.S., Min, S.S. and Seol, G.H. (2014) Effects of 1,8-Cineole on Chronic Exposure to Nicotine in Rats. *Journal of Pharmacy and Pharmacology*, **66**, 688-693. https://doi.org/10.1111/jphp.12195

[13] Golmakani, M.T. and Rezaei, K. (2008) Comparison of Microwave-Assisted Hydrodistillation with the Traditional Hydrodistillation Method in the Extraction of Essential Oils from *Thymus vulgaris* L. *Food Chemistry*, **109**, 925-930. https://doi.org/10.1016/j.foodchem.2007.12.084

[14] Akram, A., Younis, A., Akhtar, G., Ameer, K., Farooq, A., Hanif, M.A., Saeed, M. and Lim, K.B. (2017) Comparative Efficacy of Various Essential Oil Extraction Techniques on Oil Yield and Quality of *Jasminum sambac* L. *Science International*, **5**, 84-95. https://doi.org/10.17311/sciintl.2017.84.95

[15] Monroy, Y.M., Rodrigues, R.A.F., Sartoratto, A. and Cabral, F.A. (2016) Extraction of Bioactive Compounds from Cob and Pericarp of Purple Corn (*Zea mays* L.) by Sequential Extraction in Fixed Bed Extractor Using Supercritical CO_2, Ethanol, and Water as Solvents. *Journal of Supercritical Fluids*, **107**, 250-259. https://doi.org/10.1016/j.supflu.2015.09.020

[16] Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahena, F., Jahurul, M.H.A., Ghafoor, K., Norulini, N.A.N. and Omar, A.K.M. (2013) Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. *Journal of Food Engineering*, **117**, 426-436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

[17] Baudelaire, E. (2013) Comminution and Controlled Differential Screening Method for the Dry Extraction of Natural Active Principles. Google Patent WO2013057379A1.

[18] Malela, K.E., Petit, J., Nzikou, J.M. and Scher, J. (2016) Physicochemical Characterization and Phytochemical Study of *Grewia coriacea* Mast Powders: Focus on Polyphenols. *International Journal of Innovative Science*, *Engineering & Technology*, **3**, 512-525.

[19] Nguang, S.L., Yeong, L.Y., Pang, F.S. and Gimbun, J. (2017) Ultrasonic Assisted Extraction on Phenolic and Flavonoid Content from *Phyllanthus niruri* Plant. *Indian Journal of Science and Technology*, **10**, 1-5. https://doi.org/10.17485/ijst/2017/v10i2/110391

[20] Becker, L., Zaiter, A., Petit, J., Zimmer, D., Karam, M.C., Baudelaire, E., Scher, J. and Dicko, A. (2016) Improvement of Antioxidant Activity and Polyphenol Content of *Hypericum perforatum* and *Achillea millefolium* Powders Using Successive Grinding and Sieving. *Industrial Crops and Products*, **87**, 116-123. https://doi.org/10.1016/j.indcrop.2016.04.036

[21] Becker, L., Zaiter, A., Petit, J., Karam, M.C., Sudol, M., Baudelaire, E., Scher, J. and Dicko, A. (2017) How Do Grinding and Sieving Impact on Physicochemical Properties, Polyphenol Content, and Antioxidant Activity of *Hieracium pilosella* L. Powders. *Journal of Functional Foods*, **35**, 666-672. https://doi.org/10.1016/j.jff.2017.06.043

[22] Zaiter, A., Becker, L., Karam, M.C. and Dicko, A. (2016) Effect of Particle Size on Antioxidant Activity and Catechin Content of Green Tea Powders. *Journal of Food Science and Technology*, **53**, 2025-2032. https://doi.org/10.1007/s13197-016-2201-4

[23] Deli, M., Djantou, E.B., Ngatchic, M.J.T., Petit, J., Njintang, Y.N. and Scher, J. (2019a) Successive Grinding and Sieving as a New Tool to Fractionate Polyphenols and Antioxidants of Plants Powders: Application to *Boscia senegalensis* Seeds, *Dichrostachys glomerata* Fruits, and *Hibiscus sabdariffa* Calyx Powders. *Food Science and Nutrition*, **7**, 1795-1806. https://doi.org/10.1002/fsn3.1022

[24] Deli, M., Baudelaire, E., Nguimbou, R.M., Njintang, Y.N. and Scher, J. (2020) Micronutrients and *In Vivo* Antioxidant Properties of Powder Fractions and Ethanolic Extract of *Dichrostachys glomerata* Forssk. *Food Science and Nutrition*, **8**, 3287-3297. https://doi.org/10.1002/fsn3.1606

[25] Deli, M., Nguimbou, R.M., Baudelaire, E.N., Njintang, Y.N., Scher, J. and Mbofung, C.M. (2020) Effect of Controlled Differential Sieving Processing on Micronutrient Contents and *in Vivo* Antioxidant Activities of *Hibiscus sabdariffa* L. Calyxes Powder. *Food Science and Technology*. https://doi.org/10.1007/s10068-020-00828-1

[26] Nguimbou, R.M., Fomekong, G.C., Deli, M., Tsague, M.V., Baudelaire, E.N. and Njintang, Y.N. (2020) Enhancing the Quality of Overripe Plantain Powder by Adding Superfne Fractions of *Adansonia digitata* L. Pulp and *Hibiscus sabdarifa* L. Calyces: Characterization and Antioxidant Activity Assessment. *SN Applied Sciences*, **2**, 1832. https://doi.org/10.1007/s42452-020-03638-6

[27] Deli, M., Petit, J., Nguimbou, R.M., Djantou, E.B., Njintang, Y.N. and Scher, J. (2019b) Effect of Sieved Fractionation on the Physical, Flow and Hydration Properties of *Boscia senegalensis* Lam., *Dichostachys glomerata* Forssk. and *Hibiscus sabdariffa* L. Powders. *Food Science and Technology*, **28**, 1375-1389. https://doi.org/10.1007/s10068-019-00597-6

[28] Zhang, D. and Hamauzu, Y. (2004) Phenolics Ascorbic Acid Carotenoids and Antioxidant Activity of Broccoli and Their Changes during Conventional and Microwave. *Food Chemistry*, **88**, 503-509. https://doi.org/10.1016/j.foodchem.2004.01.065

[29] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999) Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radical Biology and Medicine*, **26**, 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

[30] Oyaizu, M. (1986) Studies on Products of Browning Reaction: Antioxidative Activities of Products of Browning Reaction Prepared from Glucosamine. *Japanese Jour-*

nal of Nutrition and Dietetics, **44**, 307-315.
https://doi.org/10.5264/eiyogakuzashi.44.307

[31] Ndiaye, E.H.B., Gueye, M.T., Ndiaye, I., Diop, S.M., Diop, M.B., Fauconnier, M.L. and Lognay, G. (2017) Eucalyptus Species from Senegal: *Eucalyptus alba* Renv, *Eucalyptus camaldulensis* Dehnh and *Eucalyptus tereticornis* Hook. *American Journal of Essential Oils and Natural Products*, **5**, 1-7.

[32] Bruneton, J. (1987) Elements of Phytochemistry and Pharmacognosy. Lavoisier, Paris, 585 p.

[33] Cheftel, J.C., Cheftel, H. and Besançon, P. (1983) Introduction to Biochemistry and Food Technology. Volume 2. Techniques and Documentation, 4th Edition. Lavoisier. Paris, 420 p.

[34] Akhihiero, E.T., Ayodele, B.V. and Akpojotor, G.E. (2013) Effect of Particle Size and Temperature Variation on the Yield of Essential Oil from Lemon Grass Using Steam Distillation. *African Journal of Physics*, **6**, 105-112.

[35] Tischer, B., Vendruscolo, R.G., Wagner, R., Menezes, C.R. Barin, C.S., Giacomelli, S.R., Budel, J.M. and Barin, J.S. (2017) Effect of Grinding Method on the Analysis of Essential Oil from *Baccharis articulata* (Lam.) Pers. *Chemical Papers*, **71**, 753-761. https://doi.org/10.1007/s11696-016-0052-0

[36] Malecky, M. (2008) The Metabolism of Terpenoids in Caprins. Life Sciences [q-bio]. AgroParisTech, 201 p. https://pastel.archives-ouvertes.fr/pastel-00004406

[37] Francisco, J.D.C., Järvenpää, E.P., Huopalahti, R. and Sivik, B. (2001) Comparison of *Eucalyptus camaldulensis* Dehn. Oils from Mozambique Obtained by Hydrodistillation and Supercritical Carbon Dioxide Extraction. *Journal of Agricultural and Food Chemistry*, **49**, 2339-2342. https://doi.org/10.1021/jf0013611

[38] Nah-Traore, Bouaré, S., Sidibe, L., Somboro, A.A., Fofana, B., Tangara, O., Diallo, D., Chalchat, J.C. and Figuiredo, G. (2014) Antimicrobial Activity of Essential Oils of *Eucalyptus camaldulensis* from Mali. *Asian Journal of Plant Science and Research*, **4**, 69-73.

[39] Pagula, F.P., Kurkcuoglu, M. and Baser, C.K.H. (2000) Essential Oil Composition of *Eucalyptus camaldulensis* Dehn. from Mozambique. *Journal of Essential Oil Research*, **12**, 333-335. https://doi.org/10.1080/10412905.2000.9699530

[40] Tchoumbougnang, F., Jazet, D.P.M., Sameza, M.L., Nkouaya, M.E.G., Tiako, F.G.B., Amvam Zollo, P.H. and Menut, C. (2009) Larvicide Activity on *Anopheles gambiae* Giles and Chemical Composition of Essential Oils Extracted from Four Plants Grown in Cameroon. *Biotechnology Agronomy Society and Environment*, **13**, 77-84.

[41] Jouault, S. (2012) The Quality of the Essential Oils and Its Influence on Their Efficiency and Toxicity. Thesis, Pharmacological Faculty, University of Lorraine, Nancy, 137 p.

[42] Collet, E. (2002) Progress in Dermatology. John Libbey Eurotext, France, Dijon, Vol. 8, 271 p.

[43] Misra, G., Pavlostathis, S.G., Perdue, E.M. and Araujo, R. (1996) Aerobic Biodegradation of Selected Monoterpenes. *Applied Microbiology and Biotechnology*, **45**, 831-838. https://doi.org/10.1007/s002530050770

[44] Neuenschwander, U., Guignard, F. and Hermans, I. (2010) Mechanism of the Aerobic Oxidation of α-Pinene. *ChemSusChem*, **3**, 75-84. https://doi.org/10.1002/cssc.200900228

[45] Krings, U., Lehnert, N., Fraatz, M.A., Hardebusch, B., Zorn, H. and Berger, R.

(2009) Autoxidation versus Biotransformation of α-Pinene and Stereoselective Dehydrogenation of Verbenol. *Journal of Agricultural and Food Chemistry*, **57**, 9944-9950. https://doi.org/10.1021/jf901442q

[46] Rio, C. (2009) Study of the Mechanisms of Oxidation of Terpene Compounds by the OH Radical. Thesis, Doctoral School of Chemical Sciences, Bordeaux I University, Bordeaux, 143 p.

[47] Baser, K.H.C. and Buchbauer, G. (2010) Handbook of Essential Oils: Science, Technology and Applications. CRC Press, Boca Raton/London/New York. https://doi.org/10.1201/9781420063165

[48] Wilson, D. and Hrutfiord B. (1975) The Fate of Turpentine in Aerated Lagoons. *Pulp Paper Canada*, **76**, 195-197.

[49] Demyttenaere, J.C.R., belleghem, K.V. and Kimpe, N. (2001) Biotransformation of (R)-(+)- and (S)-Limonene by Fungi and Use of Solid Phase Microextraction for Screening. *Phytochemistry*, **57**, 199-208. https://doi.org/10.1016/S0031-9422(00)00499-4

[50] Barra, A., Coroneo, V., Dessi, S., Cabras, P. and Angioni, A. (2010) Chemical Variability, Antifungal and Antioxidant Activity of *Eucalyptus camaldulensis* Essential Oil from Sardinia. *Natural Product Communications*, **5**, 329-335. https://doi.org/10.1177/1934578X1000500232

[51] Engonga, O.L.C., Koudou, J., Chalchat, J.C., Bassolé, I., Edou, P., Ouattara, A.S. and Traore, A. (2007) Antioxidant and Antimicrobial Activities of *Canarium schweifurthii* Engl. Essential Oil from Centrafrican Republic. *Scientific Research and Essays*, **6**, 2319-2323. https://doi.org/10.5897/AJB2007.000-2363

[52] Soares, D.G., Andreazza, A.C. and Salvador, M. (2003) Sequestering Ability of Butylated Hydroxytoluene, Propyl Gallate, Resveratrol, and Vitamins C and E against ABTS, DPPH, and Hydroxyl Free Radicals in Chemical and Biological Systems. *Journal of Agricultural and Food Chemistry*, **51**, 1077-1080.

[53] Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J. and Qian, M. (2002) Free Radical Scavenging Properties of Wheat Extracts. *Journal of Agricultural and Food Chemistry*, **50**, 1619-1624. https://doi.org/10.1021/jf010964p

[54] Dontha, S. (2016) A Review on Antioxidant Methods. *Asian Journal of Pharmaceutical and Clinical Research*, **9**, 14-32. https://doi.org/10.22159/ajpcr.2016.v9s2.13092

[55] Dhami, A., Singh, A., Palariya, D., Kumar, R., Prakash, O., Rawat, D.S. and Pant, A.K. (2019) α-Pinene Rich Bark Essential Oils of *Zanthoxylum armatum* DC. from Three Different Altitudes of Uttarakhand, India and Their Antioxidant, *in Vitro* Anti-Inflammatory and Antibacterial Activity. *Journal of Essential Oil-Bearing Plants*, **3**, 660-674. https://doi.org/10.1080/0972060X.2019.1630015

[56] Safaei-Ghomi, J., Ebrahimabadi, A.H., Djafari-Bidgoli, Z. and Batooli, H. (2009) GC/MS Analysis and *in Vitro* Antioxidant Activity of Essential Oil and Methanol Extracts of *Thymus caramanicus* Jalas and Its Main Constituent Carvacrol. *Food Chemistry*, **115**, 1524-1528. https://doi.org/10.1016/j.foodchem.2009.01.051

[57] Benyoucef, F., El Amine, D.M., Arrar, Z., Costa, J. and Muselli, A. (2018) Synergistic Antioxidant Activity and Chemical Composition of Essential Oils from *Thymus fontanesii*, *Artemisia herbaalba* and *Rosmarinus offcinalis*. *Journal of Applied Biotechnology Reports*, **4**, 151-156. https://doi.org/10.29252/JABR.05.04.03