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Geographical variation in total phenolics, flavonoids and antioxidant activities of *Eucalyptus camaldulensis* leaves in Burkina Faso

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This study aimed to elucidate the effect of sampling location of *Eucalyptus camaldulensis* leaves on antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC). Leaves of *E. camaldulensis* collected at three different locations in Burkina Faso (north, central, south-west) were subjected to hydrodistillation to obtain a water extract and subsequent ethanol extraction of the solid residue. The extracts were analysed for antioxidant activity using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging, TPC, TFC and high-performance thin layer chromatography (HPTLC) fingerprint. TPC were higher in water extracts and presented considerable variation from 129±9 to 266±4 milligram Gallic Acid Equivalent per gram of extract (mg GAE/g) with the highest value 266±4 mg GAE/g at the south-west site. This aqueous extract also showed high antioxidant activity with EC₅₀ 8.3 µg/mL. Furthermore, HPTLC fingerprint of the extracts showed both band intensity and size differences. For TFC, we found a slight differences within both water and ethanolic extracts with the highest value of 38.3 ±4.2 mg QE/g for the south-west ethanolic extract. The noticeable impact of climate on chemical variability of *E. camaldulensis* leaves needs to be taken into consideration for any further applications based on TPC biological activity of the extracts.

Key words: *Eucalyptus camaldulensis*, total phenolic content, total flavonoid content, antioxidant activity, sample location, chemical variation.

INTRODUCTION

Naturally occurring polyphenols have been paid increasing attention for their antioxidant activity and potential use in various applications, e.g. in the food,

pharmaceutical and agricultural industry (Pandey and Rizvi, 2009; Augustyniak et al., 2010; Choi et al., 2012; Siangu et al., 2019). These secondary metabolites are

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Table 1. Locations and characteristics of collection sites for *E. camaldulensis* leaves samples used in this study.

Sample code	GPS of collection site	Agro-ecological zone ¹	Rainfall ¹ (mm/year)	Rain days ¹ (days/year)	Annual temperature ¹ (°C)
BOD	11°11'44"N, 4°23'09"W	South-Sudanese (South-West)	900-1200	85-100	27
OUA	12°25'30"N, 1°40'44"W	North-Sudanese (Central)	700-900	50-70	28
DOR	14°04'22"N, 0°08'48"W	Sahelian (North)	200-500	<45	29

¹PANA (2007).

found throughout the plant kingdom in various plant parts, with over 8000 polyphenolic compounds identified from multiple plant species (Pandey and Rizvi, 2009). One of the most studied groups of polyphenols is the flavonoid group. They protect plants against biotic and abiotic stress (Panche et al., 2016) and possess a large variety of biological properties exploited in foodstuff, pharmacy and agriculture industries (Kumar and Pandey, 2013). It has been reported that the geographical location of a plant caused variation in its chemical composition including flavonoid content (Haytowitz et al., 2013; Bouterfas et al., 2016; Dolkar et al., 2017; Ismail et al., 2017; Rimkiene et al., 2017). When a plant is chosen to study its biological activities in correlation with chemical composition, it is necessary to evaluate its chemical variability in composition or concentration of secondary metabolites basing on their location.

Eucalyptus camaldulensis is the main *Eucalyptus* species in Burkina Faso. Its stem is highly used in constructions, making its leaves a cheap and available raw material which can be valorised. The essential oil from the *E. camaldulensis* leaves is used in various applications, such as pharmacy, cosmetic and numerous food product formulations. It has also been suggested as a biopesticide (Batish et al., 2008). Hydrodistillation generates residual water and solid residue, which contain recoverable bioactive substances (Santana-Méridas et al., 2014). High polyphenol content, as well as antioxidant, antimicrobial, and antitumor activity, have been reported for various *Eucalyptus* extracts (Boulekbache-Makhlouf et al., 2013; Ashraf et al., 2015; González et al., 2017). Bioactive compounds are known to be influenced by various parameters: location, climate, soil, plant varieties, tree age, sampling season and extraction methods (Viljevac Vuletić et al., 2017; Chiasson et al., 2001). In the case of essential oil from *E. camaldulensis*, both location and harvest time affected the obtained yield and the chemical composition of the oil (Moudachirou et al., 1999). This study aimed to investigate the effect of geographical location on the chemical composition of water extracts from hydrodistillation, and ethanol extracts carried out on the residual solid fraction. To achieve this objective, the variability of the contents in phenolics, flavonoids and antioxidant properties was evaluated. Importantly, we focused on the standardization of antifungal biopesticide formulations based on *E. camaldulensis* leaves.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used were analytical grades, including 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, quercetin dihydrate, Folin-Ciocalteu's phenol reagent, aluminium chloride hexahydrate (AlCl₃.6H₂O), from Sigma-Aldrich. All chemicals were used directly without further purification.

Plant material

Plant material consisting of leaves from *E. camaldulensis* was collected from three different geographical locations of Burkina Faso, namely, the South-Sudanese zone (Sudanese climate) with the collection site near the town of Bobo-Dioulasso (BOB), the North-Sudanese zone (Sudano-Sahelian climate) with the collection site near the city of Ouagadougou (OUA) and the Sahelian zone (Sahelian climate) with the collection site near the town of Dori (DOR). Table 1 contains pieces of information about annual rainfall, number of rain days and average temperatures in Burkina Faso. The collection was carried out within ten days at the end of March to the beginning of April 2018. This period corresponds to the dry season in Burkina Faso. At each sampling point, leaves from a minimum of three trees were collected, weighed and mixed in equal proportions. The study included only trees with a stem circumference of 20 to 50 cm (measured 1 m of the ground). GPS coordinates were logged using a GARMIN e-trex 30 GPS (satellite). The collected leaves were washed with distilled water, dried in the shade at room temperature for ten days, ground into a fine powder and stored in a closed container at room temperature until further processing.

Dr Zerbo Issouf made the identification of plant specimen and voucher specimens (N-ID17046, 6876) were kept at the Laboratory of Plant Biology and Ecology, University Joseph KI-ZERBO, Ouagadougou, Burkina Faso.

Moisture content

The moisture content of the leaves powder was measured as follows: 1.0 g of each powder was dried in an oven at 105°C for 3 h, left to cool in a desiccator before weighing. Samples were prepared and analysed in triplicates.

Total ash content

The ash content was determined as follows: 5.0 g of each powder

was calcinated in an oven at 550°C for 10 h, then left to cool for 10 h in the oven, followed by a 20 min drying step in a desiccator before weighing. The samples were prepared and analysed in duplicates. The ash content on dry basis was calculated in percentage (%) from the mass after calcination (m) and the moisture content (MC) according to:

$$\text{Ash (\%)} = (m / 5.0 \times (1 - MC)) \times 100$$

Extraction procedure

The hydrodistillation started with 100 g of plant powder in 1 L of distilled water (dH₂O) for 1 h using a Clevenger apparatus. The essential oil was collected but not analyzed further. The remaining crude was filtered using a fabric tissue to recover an aqueous solution and a solid residue. The aqueous volume extract was logged before being subjected to a second filtration using cotton. A sample of the final filtrate was frozen at -20°C overnight and dried through lyophilisation (LABCONCO FreeZone 2.5 Plus).

The remaining solid residue was dried at room temperature for 24 h and then subjected to ethanol extraction. The solid residue was mixed with 1 L of 70% ethanol, and the mixture continuously stirred for 24 h at room temperature before a two-step filtration, first using a fabric tissue and then filter paper. The ethanol was removed under vacuum and the sample re-dissolved in dH₂O, frozen at -20°C and dried through lyophilisation.

Flavonoids fingerprinting by high-performance thin layer chromatography (HPTLC)

The extracts were analysed by HPTLC using an automatic TLC sampling system (LAMAG). 0.1 mg of each extract was loaded onto a silica plate (Sigma Aldrich) and run in a mobile phase consisting of *n*-butanol-acetic acid-water (65:15:10). The plate was left to dry at room temperature, observed under UV-light at 254 nm, before the flavonoid specific NEU reagent (2-aminoethyl-diphenylborinate, 1% w/v in MeOH) was sprayed onto the plate followed by PEG-4000 (5% v/v in EtOH). The plate was then photographed under UV light at 365 nm.

Total phenolic content evaluation (Folin-Ciocalteu method)

The total phenolic content (TPC) was determined using a modified version of the Folin-Ciocalteu method, adapted for microplate (Ainsworth and Gillespie, 2007). In short, 200 µL of Folin-Ciocalteu reagent (1:10 in dH₂O) was added to 100 µL of plant extract (0.5 mg/mL in dH₂O) and mixed thoroughly before the addition of 800 µL Na₂CO₃ (0.7 M in dH₂O). Samples were transferred to a microplate, 200 µL/well in triplicates, and incubated for 2 h at 28°C. The absorbance of the mixture was determined at 765 nm using pure reagents without extract as blank and reported to a standard curve ($y = 4.81x - 0.017$; $R^2 = 0.997$) made with gallic acid (0.2-0.025 mg/mL) used as standard compound. The TPC was expressed as milligram gallic acid equivalent per gram of extract (mg GAE/g). A multifunctional microplate reader (BMG Labtech SPECTRO star Nano) was used. All samples were prepared and analysed in triplicates.

Total flavonoid content (TFC) evaluation (AlCl₃-method)

TFC was evaluated using the AlCl₃-method (Pełkal and Pyrzyńska, 2013) adjusted for microplate. In short 0.5 mL of AlCl₃ (2% w/v in MeOH) was added to 0.5 mL of plant extract (1 mg/mL in MeOH), mixed and transferred to a 96 well microplate in triplicates, 200 µL/well. The microplate was incubated for 10 min at 28°C before

reading the absorbance at 420 nm (BMG Labtech SPECTROstar Nano) using the sample in pure MeOH as blank. The absorbance was compared to a standard curve ($y = 17.2x - 0.017$; $R^2 = 0.997$) prepared with quercetin (0.03-0.003 mg/mL) to give the TFC as milligram Quercetin Equivalent per gram of extract (mg QE/g). All samples were prepared and analysed in triplicates.

Antioxidant activity evaluation (DPPH-method)

The Antioxidant Activity (AA) of the extracts was determined using the DPPH-method (Cheng et al., 2006) adjusted for microplate. The samples in triplicates were serial diluted in the microplate, final concentrations 0.1-0.0016 mg/mL for aqueous extracts and 0.25-0.0078 mg/mL for ethanol extracts. In each well, 100 µL of MeOH or 100 µL of freshly prepared DPPH (0.2 mM in MeOH) were added to 100 µL of the sample. The microplate was incubated at 28°C for 20 min before reading the absorbance at 515 nm. The percentage of inhibition was plotted against sample concentration, and the EC₅₀-value in µg/mL was calculated using linear regression. Data points with inhibition >90% were excluded from the linear regression as it indicated saturation. The antioxidant activity of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was analysed as standard. All samples were prepared and analysed in triplicates. The percentage of DPPH inhibition was calculated for each sample concentration using the following formula:

$$\% \text{ DPPH inhibition} = \frac{(A_{\text{DPPH}} - A_{\text{MeOH}}) - (A_{\text{sample}} - \text{DPPH} - A_{\text{sample} + \text{MeOH}})}{A_{\text{DPPH}} - A_{\text{MeOH}}} \times 100$$

where A_x = absorbance measured for x.

Extract chemical stability

The chemical stability was performed for one collection point sample (OUA) using the antioxidant assessment. The water and ethanol extract solutions (1 mg/mL in MeOH) of this sample were stored at room temperature or +4°C for 6 weeks. The AA of the extracts in solution after storage was analysed and compared to the activity of freshly prepared samples.

RESULTS

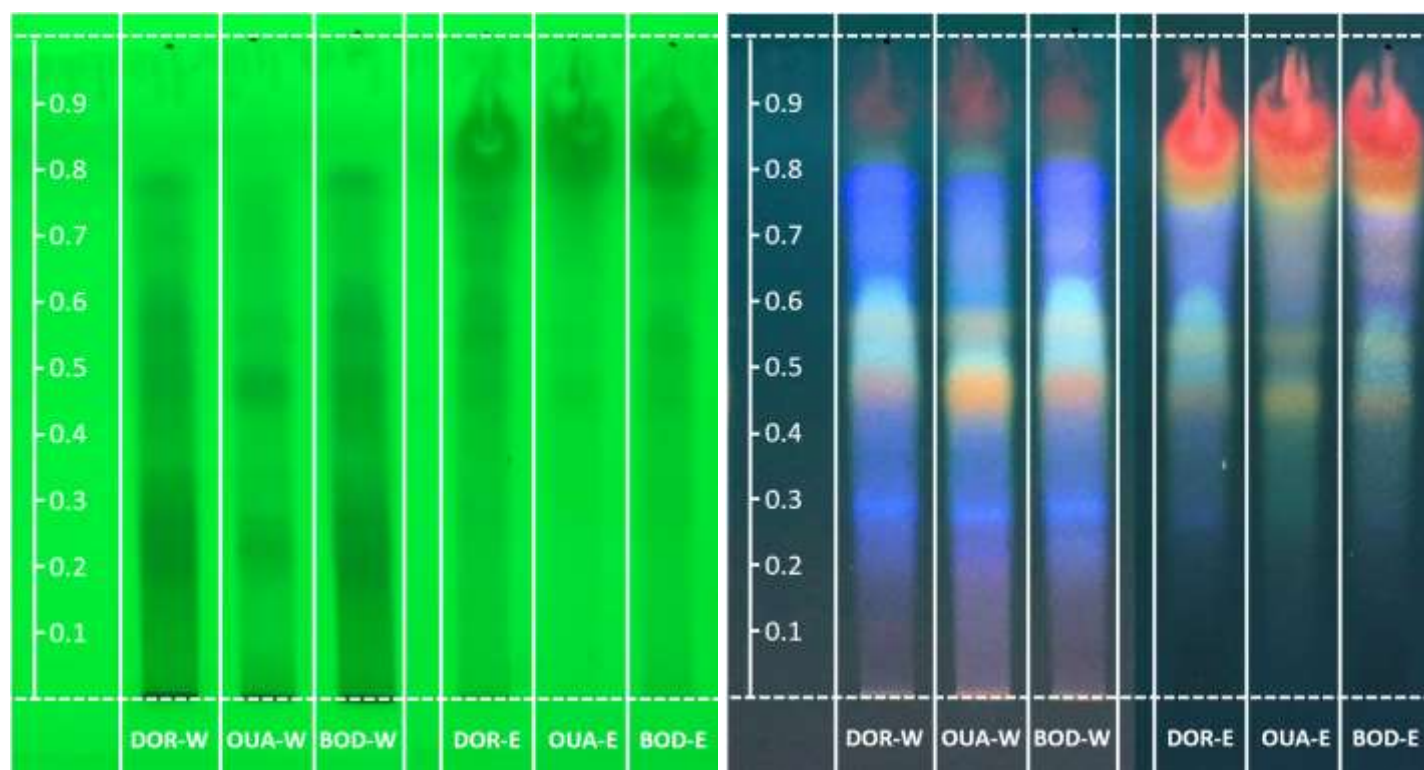
Table 2 summarises the dried powders of *E. camaldulensis* leaves' characteristics, including moisture content, total ash content and extraction yields.

Only minor differences in moisture content and total ash content could be observed between the powders. The moisture content was approximately 5%, and the total ash content was about 6%, for all three powders.

After drying, the aqueous phase from hydrodistillation yielded approximately 30 g dry extract per 100 g (dry base) of plant powder processed. Water extracts BOD-W and OUA-W gave slightly higher yields than DOR-W (33.6 and 31.0 g versus 26.4 g). Subsequent ethanol extraction of the solid residue remaining after hydrodistillation yielded approximately 10 g dry extract per 100 g (dry base) plant material processed. This value was slightly lower for OUA-E (8.5 g versus 10.0 g and 10.1 g for DOR-E and BOB-E, respectively). The sum of water and ethanol extracts for each sample was

Table 2. Characteristics, including moisture content (MC) and ash content (Ash) of dried powders and yields after hydrodistillation with following ethanol extraction (W =water extract; E = ethanol extract).

Sample code	MC (%)	Ash (%)	Extract code	Yield (g/100 g)
BOD	5.0 ±0.1	5.6 ±0.2	BOD-W	33.6
			BOD-E	10.1
OUA	4.9 ±0.3	5.8 ±0.0	OUA-W	31.1
			OUA-E	8.5
DOR	5.2 ±0.4	6.2 ±0.0	DOR-W	26.4
			DOR-E	10.0

**Figure 1.** HPTLC analysis of water (W) and ethanol (E) extracts from *E. camaldulensis* leaves collected at three different locations in Burkina Faso; mobile phase: butanol-acetic acid-water (65:15:10); visualised under UV-light 254 nm (left) or using the derivatising reagent (NEU reagent) and observed under UV light at 365 nm (right).

approximately equivalent to 40 g of extract per 100 g (dry base) of plant powder processed. The DOR sample has the lowest value, with 36.4 g and BOD present the highest value with 43.7 g.

Figure 1 illustrates the extracts' HPTLC fingerprints, before the derivatisation under UV-light at 254 nm and after derivatisation with NEU reagent under UV-light at 365 nm. The chromatogram of each extract contained several bands with a pink-yellow fluorescence. These bands indicated the presence of flavonoids in the extracts (Kinghorn, 1997). Some differences appeared between

the extracts from the different collection sites. A notable difference was the orange band with a retention factor (R_f) at around 0.45. In both the water and ethanol extracts, a green smear spot around $R_f \approx 0.6$ was visible in the DOR and BOB extracts but less in the OUA extract. In general, the ethanol extracts showed a similar profile as the water extracts, but with lower intensity of bands. The most significant difference was the yellow-orange bands migrating with the eluent front ($R_f \approx 0.8$). Table 3 summaries the TPC, TFC and AA values from the extracts.

Table 3. Chemical characteristics of *E. camaldulensis* leaves extracts from three different collection sites in Burkina Faso: Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity (AA) measured as DPPH radical scavenging.

Collection site	Extract	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (EC ₅₀ , µg/mL)
BOD	BOD-W	266 ±40	11.4 ±1.20	8.30
	BOD-E	129 ±90	38.3 ±4.20	26.40
OUA	OUA-W	171 ±12	11.1 ±1.10	27.80
	OUA-E	68 ±30	37.4 ±2.10	60.30
DOR	DOR-W	251 ±13	8.2 ±1.80	9.30
	DOR-E	123 ±70	30.1 ±1.30	20.00
Trolox				6.30

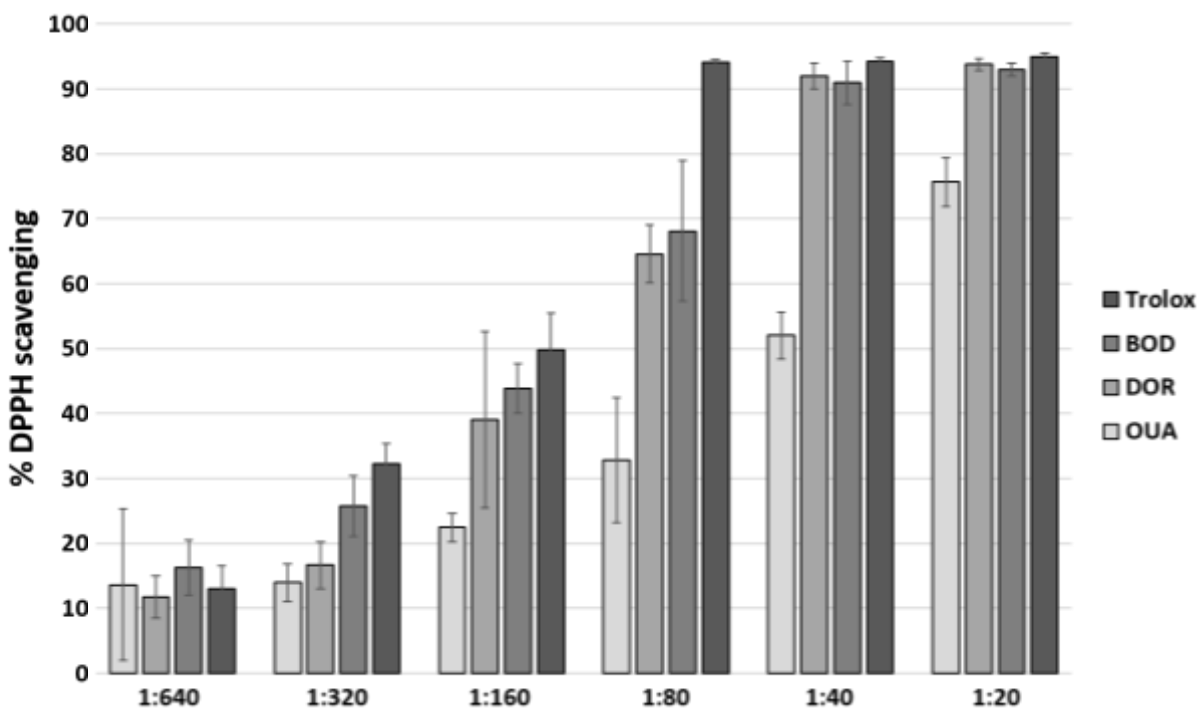


Figure 2. Histogram of Antioxidant Activity (AA) in the form of % DPPH-radical scavenging. Data for various concentrations of aqueous extracts from *E. camaldulensis* and Trolox (standard). Data shown was used for calculation of EC₅₀ values through linear regression.

TPC was generally higher in the aqueous extracts than in the ethanol extracts. For the aqueous extracts, the obtained values according to collection locations were 171 ±12 mg GAE/g for OUA, 266 ±4 mg GAE/g for BOD and 251 ±13 mg GAE/g for DOR extract. The ethanol extracts were 68 ±3, 129 ±9 and 123 ±7 mg GAE/g, respectively for OUA, BOD and DOR extracts.

For TFC, the ethanol extracts generally showed a higher content compared to the aqueous extracts (Table). When we compared the different collection points, the

DOR extracts had lower amounts of flavonoids, 8.2 ±1.8 mg QE/g for DOR-W versus 11.1 ±1.1 and 11.4 ±1.2 mg QE/g for OUA-W and BOD-W, respectively. For the ethanol extracts, the obtained values were 30.1 ±1.3 mg QE/g for DOR-E versus 37.4 ±2.1 and 38.3 ±4.3 mg QE/g, respectively for OUA-E and BOD-E.

The highest AA was observed for BOD-W, followed by DOR-W extracts (Table 3 and Figure 2), with EC₅₀-values of 8.3 and 9.3 µg/mL, respectively. These EC₅₀ values were comparable to the standard antioxidant Trolox (EC₅₀

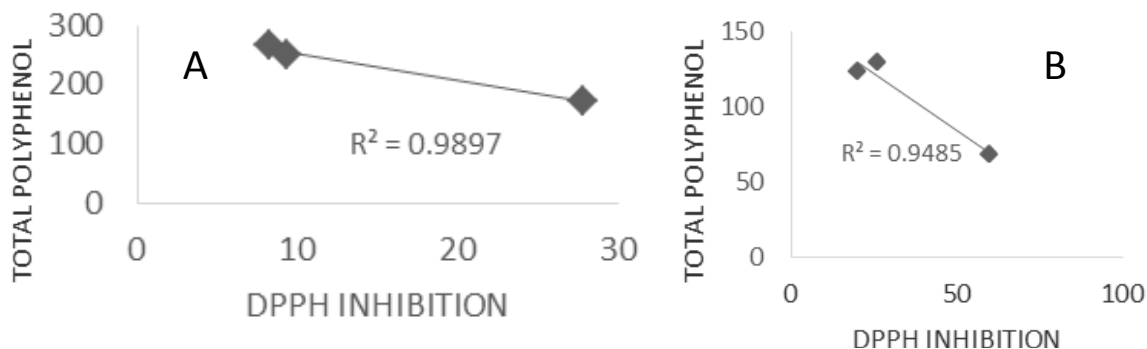


Figure 3. (A) Correlation between AA and TPC for water extracts; Correlation coefficient $r = 0.9948$. (B) Correlation between AA and TPC for ethanol extracts; Correlation coefficient $r = 0.9739$.

Table 4. Stability of water and ethanol extracts from *E. camaldulensis* after storage for 6 weeks in MeOH, either at +4°C or room temperature (r.t.). Antioxidant Activity (AA) given as EC₅₀ concentration and % DPPH inhibition by 0.025 mg/mL water extracts or 0.0625 mg/mL ethanol extracts.

Sample code	Storage conditions	DPPH (%)	DPPH (EC ₅₀ , µg/mL)
OUA-W	Day 1	52 ± 4	27.8
	6 weeks, +4°C	57 ± 4	26.3
	6 weeks, r.t.	60 ± 6	23.6
OUA-E	Day 1	55 ± 3	60.3
	6 weeks, +4°C	41 ± 5	85.5
	6 weeks, r.t.	32 ± 6	120.7

6.3 µg/mL). The less active was OUA-W with EC₅₀ 27.8 µg/mL. The ethanol extracts were also less active. The DOR-E and BOD-E showed a higher AA than OUA-E, with EC₅₀ 20.0 and 26.4 µg/mL, respectively, compared to EC₅₀ 60.3 µg/mL for OUA-E. Noteworthy, a higher antioxidant ability corresponds to a lower EC₅₀ value.

Correlation between TFC and TPC values with antioxidant properties

To correlate the results between AA with TPC and TFC for each type of extract (water and ethanol), a regression analysis was performed, and the correlation coefficient r was determined. Figure 3 (A and B) shows the results of DPPH inhibition and TPC correlations for water and ethanol extracts. Significant correlations were found between DPPH inhibition and TPC with an r 0.9948 for water extracts and 0.9739 for ethanol extracts. In contrast, the correlations of DPPH inhibition and TFC were weak, with an r 0.5336 for ethanol extracts and 0.3830 for water extracts.

Chemical stability study

The storage conditions and duration were considered for

chemical stability study, and the AA was the parameter. The AA of the water extract did not decrease significantly after 6 weeks in the prepared solution stored at 4°C or room temperature (Table 4), suggesting adequate stability under the test conditions. However, the ethanol extracts' EC₅₀ values increased, suggesting a loss of activity. The loss was high for the extract stored at room temperature (approximately 25°C) compared to the one stored at 4°C. The EC₅₀ values were 120.7 and 85.5 µg/mL, respectively, compared to 60.3 µg/mL for the first day (original sample).

DISCUSSION

The presence of water is generally favourable for the development of germs and also hydrolysis reactions in plant material. It is therefore essential to know its content in plant powders before further processing and conservation. The moisture content levels (4.9 - 5.2%) were less than 10% (Table 2), and according to the European Pharmacopoeia (Pharmacopée, 2007), these powders were storable over a long period without the growth of molds or yeasts. The ash content in plant materials is an indicator of the content of inorganic elements (minerals). The ash contents of the powders

from the three geographical locations were similar (from 5.6 - 6.2%). This study therefore expected that the TPC, TFC and AA would be affected similarly by the minerals.

The total extraction yield of 40% was higher or similar to the one obtained for *Eucalyptus* species using various organic solvent-based extraction methods (3-46 %) (El-Ghorab et al., 2003; Safaei-Ghomi et al., 2010; Singa et al., 2011; Boulekbache-Makhlouf et al., 2013). But the sample DOR, collected in a dry environment gave low quantities of extractives compounds. In contrast, the humid areas' sample (BOD) gave higher amounts. The observed variation suggested a systematic effect to take into account for future use after the chemical analysis of the extract.

By HPTLC fingerprint, a high amount of active substances is effectively present in the aqueous phase after hydrodistillation and the ethanol extract. Some slight qualitative differences were observed in the six extracts. Still, the quantification of chemicals can confirm the chemical variation in TPC, TFC and AA of *E. camaldulensis* leaf extracts from the three different agro-ecological zones in Burkina Faso.

TPC was higher in the aqueous extracts than in the ethanol extracts, whereas for TFC, the ethanol extracts showed higher values compared to the aqueous ones. Two possible explanations for the observed reversal: (i) the extraction method used and (ii) the higher solubility of flavonoids in ethanol. First, the water extraction happened at high temperature, and most of the phenolic compounds soluble in water will be extracted, including phenolic acids, tannins, glycosides, etc. The overall yield of extraction by the high-temperature method was higher compared to a cold extraction (Siddesha et al., 2011). Secondly, flavonoids are more soluble in alcohol than water. Thus, a higher fraction of flavonoids compounds would remain in the solid residue and recovered only by the aqueous ethanol used in this study. Aqueous ethanol is generally the best solvent for flavonoids extraction. For example, optimisation of solvent extraction showed that TFC increased when the ethanol concentration and temperature increases and the optimum concentration lies between 50 and 60% ethanol (Gullon et al., 2017; Echeverry et al., 2018). These ethanol concentrations are closed to 70% used in the present study.

The highest TPC, TFC and AA corresponded to the sample from the south-west (BOD), which also presented a high extraction yields. The obtained values for TPC and TFC were respectively 266 ±4 mg GAE/g for water extract and 38.3 ±4.3 mg QE/g for ethanol extract. Regarding the AA, the EC₅₀-value was 8.3 µg/mL for the water extract, closed to the standard Trolox (6.3 µg/mL). These results stemmed from the fact that this region of Burkina Faso with a Sudanese like climate presented the lowest temperature (average temperature is 27°C) and the highest pluviometry, compared to the others sites, with rainfall of 900 to 1200 mm/year (Table 1).

E. camaldulensis growing at Sudanese areas were, therefore, more bioactive than those in dry areas with

high water stress (Zohra et al., 2014). As mentioned earlier, the highest AA was found for BOD-W with EC₅₀ value of 8.3 µg/mL, followed by DOR-W (EC₅₀-value 9.3 µg/mL). The less active one was observed for OUA-E (Central) with an EC₅₀-value 60 µg/mL. The sample from the central part (OUA) also had the lowest TPC with 68 ±3 mg GAE/g for ethanol extract. But the lowest TFC was obtained in the North (DOR) part with 8.2 ±1.8 mg QE/g for water extract.

The AA of the extracts (EC₅₀ = 8-60 µg/mL) was in line, with previous reports for other eucalyptus extracts using the same DPPH radical scavenging-assay, EC₅₀ in the range of 17 to 480 µg/mL (Amakura et al., 2009; Safaei-Ghomi et al., 2010; Singa et al., 2011).

The AA correlated well with polyphenol content, both with water and ethanol extracts, with a correlation coefficient r 0.9948 for water extracts and 0.9739 for ethanol extracts (Figure 3A and B), suggesting that the AA of the extracts was due to polyphenols other than flavonoids (Dudonné et al., 2009). Previous studies showed that *Eucalyptus globulus* contains some phenolic acids (gallic acid and ellagic acid) which possess a high antioxidant ability. Other high molecular weight polyphenols were also identified but they did not contribute to the antioxidant activity (Amakura et al., 2002). Gallic acid and ellagic acid were the prevailing antioxidants in the ethanol extract from *E. camaldulensis* collected in Egypt (El-Ghorab et al., 2003).

As the TFC was similar in extracts from different sites, it appeared that phenolic acids in water extracts explained the considerable variation in TPC in the water extracts of samples from the three sites. The TPC measurement was easy and in correlation with AA. It was proposed that correlation as an indicator for the chemical diversity of *E. camaldulensis* leaves.

Depending on the targeted biological activity for plant extract valorisation, one can choose the ethanol extract if flavonoids are in focus or the water extract if phenolic acids are the interesting ones as bioactive molecules. Generally, extracts rich in phenolic compounds have good AA. In this study, the collection took place for ten days in the middle of the peak dry season in Burkina Faso. Day time temperatures during this period can reach above 40°C, with no rainfall for weeks before collection. In the north, this temperature was the highest; so, the sample DOR possessed the highest total ash content when the moisture contents were similar. This means that the leaves from DOR possessed more minerals and a bit less organic material molecules to extract. Secondary metabolites of plants play an essential role to face biotic and abiotic stress as dust, high temperature, and disease (Imelouane et al., 2011).

Conclusion

Water and ethanol extracts from hydrodistillation process of *E. camaldulensis* leaves collected at three different

sites in Burkina Faso showed different TPC, TFC and AA. Among the sites, the South-Sudanese zone sample extract (south-west of Burkina Faso) showed superior antioxidant properties, TPC and TFC. It also exhibited higher extractives than the sample from the Sahelian area (North of Burkina Faso). The geographic variation was more evident in water-soluble compounds (water extracts) rich in phenolic acids than the ethanol extracts rich in flavonoids. A significant correlation between antioxidant properties and TPC indicated that phenolic compounds were the major contributors to the antioxidant properties of *E. camaldulensis* leaves extracts. Regarding the differences between the extracts, we concluded that the collection sites of samples affected the phytochemicals of the plant.

For any future application as an antifungal biopesticide in organic agriculture, the collection site needs to be included for the standardisation of the formulations. The management of the geographical differences are essential to any successful industrial or agricultural application.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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