INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 21, No. 6, p. 348-358, 2022

RESEARCH PAPER OPEN ACCESS

Phenols compounds and antioxidant activity of five medicinal plants acclimated in Burkina Faso

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Key words: Phenolic compounds, Antioxidant activity, Medicinal plants, *Eucalyptus camaldulensis*, Burkina Faso

http://dx.doi.org/10.12692/ijb/21.6.348-358 Article published on December 07, 2022

Abstract

The objective of this study was to contribute to a better knowledge of the medicinal flora by evaluating the antioxidant potential of extracts of five medicinal plants acclimatized in Burkina Faso. A phytochemical screening was performed by using the tests tube and thin chromatography methods. The content of phenolic compounds was evaluated by techniques using the Folin ciocalteu reagent. The antioxidant activity of extracts was assessed using 2, 2-azinobis-3-ethylbenzothiaz oline-6-sulfonic acid (ABTS), Ferric Reducing Antioxidant Power (FRAP) and 2-2-Diphenyl-1-Picrylhydrazyl (DPPH) methods. The results showed that chemical compounds such as tannins, flavonoids and saponosides were present in most methanolic extracts. The determination of phenolic compounds showed that the methanol extract of *E. camaldulensis* contains the highest content (68.62 mg EAG/g extract). The antioxidant activity of the studied plants varies from one extract to another depending on the method used. The methanol extract of *H. suaveolens* showed the highest antioxidant content using the FRAP method (54.41 TEQ/g extract), while the methanol extract of *E. camaldulensis* recorded the highest antioxidant contents by ABTS and DPPH methods (28.99 ± 0.17 and 30.74 mg TE/g extract respectively). In addition, the methanol extract of *E. camaldulensis* exhibited very high antioxidant activity with an antioxidant activity index of 45.97. These plants are potential sources of natural antioxidant. In perspectives, the compounds responsible for the antioxidant activity will be isolated and their structure elucidated.

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Introduction

Free radicals produced by chemical, photochemical or biochemical pathways are highly reactive and potentially damaging chemical species. They are implicated in the etiology of various human chronic diseases that are considered public health issues (Pajares *et al.*, 2018). Free radicals generated by the human organism are eliminated or trapped by molecules with antioxidant properties (Hermes-Lima, 2005). However, the use of synthetic antioxidant molecules, in various fields, as a solution to this situation, has been widely criticized in recent years. Indeed, due to the potential health risks and toxicity they are capable of causing, the available synthetic antioxidant molecules are currently being questioned (Kicel *et al.*, 2016; Liu and Yang, 2018).

Moreover, medicinal plants contain chemical compounds with a wide range of physicochemical properties, which exhibit a wide range of biological activities such as antitumor, antiviral, antiparasitic, antibacterial, antifungal, antioxidant, insecticidal, anti-inflammatory, activities etc. (Bruneton, 2006). This makes the search for natural substances with antioxidant activity from plants a major scientific topic. Hence the interest of this study, which aims to identify the phytochemical contents and evaluate the antioxidant properties of extracts of five medicinal plants, acclimated in Burkina Faso.

Materials and methods

Materials

The plant material used in this study is comprised of five aromatic and medicinal plants from the flora of Burkina Faso: *Cymbopogon nardus, Eucalyptus camaldulensis, Hyptis suaveolens, Lantana camara* collected in the botanical garden of the Research Institute of Applied Sciences and Technology in Ouagadougou (N 12°25'28.2"; W 1°29'15.06") and *Lippia multiflora* collected in Zorgho (N 12°11.211'; W 000°43.454') during the month of October 2018. This plant material was identified and authenticated by KOURA S. Paulin, botanist at the herbarium of the Centre National de la Recherche Scientifique et Technologique (CNRST). Reference specimens have been deposited at the CNRST herbarium.

The reagents Folin-ciocalteu, gallic acid, quercetin, trolox, NaOH, $AlCl_3$, ABTS, DPPH were provided by SIGMA-Aldrich (France). All other solvents were of analytical grade.

Methods

Preparation of extracts

The plant material, previously cleaned, was dried in the laboratory at room temperature and protected from the sun, then ground into powder and stored in clean bags. Extraction was performed by maceration with solvents of increasing polarity. Thus, 40 grams of the plant material were successively exhausted with 400 mL of dichloromethane (DCM) and methanol (MeOH) under stirring for 24 hours. After filtration and concentration, the extraction yield was determined and the extracts were stored in a refrigerator at 4°C until use (Landoulsi, 2018).

Phytochemical screening

Phytochemical screening by solution reactions in test tubes and by Thin Layer Chromatography was performed according to the classical methods of Ciulei (1982) and Pachaly (1997).

Dosage of total phenolic compounds

Dosage of total phenolic compounds (TPC) in the extracts was performed by Folin-Ciocalteu Reagent (FCR) according to the method described by Turkmen *et al.* (2007). Stock solutions were prepared at a concentration of 5 mg/mL in DMSO for DCM extracts and in MeOH for methanolic extracts. A calibration curve was established by preparing a range of gallic acid solution (used as a standard) with a concentration varying from 0.01 to 1 mg/mL (cascade dilution of the order of 1/2). Then 60 µL of RFC was added to the gallic acid solution at different concentrations. The mixture was kept at room temperature for 8 minutes, and then 120 µL of 7.5% Na_2CO_3 solution was added to neutralize the residual reagent. Absorbances were read at 760 nm against a blank using an MP96 SAFAS spectrophotometer after incubation for 30 minutes at 37°C. The extracts' TPC content was determined by relating the absorbances read, following the same procedure of the standard, to the standard curve established with gallic acid (Fig. 1).

Results are expressed as mg gallic acid equivalent per gram of extract (mg GAE/g extract) as mean ± standard deviation.

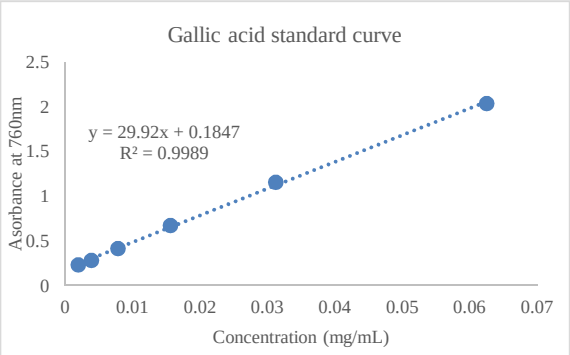

Fig. 1. Gallic acid standard curve.

Dosage of total flavonoids (TF)

The method of the determination of flavonoids is based on the ability of these compounds to form chromogenic complexes with aluminum chloride. The principle of the method is based on the oxidation of flavonoids by aluminum trichloride ($AlCl_3$) and sodium hydroxide (NaOH). The total flavonoid (TF) content was evaluated by the aluminum trichloride method using quercetin as standard (Zhishen *et al.*, 1999). A calibration curve was established by preparing a range of quercetin solution with concentration ranging from 0.01 to 1 mg/mL (1/2 fold dilution). At t = 0 s, 0.3 mL of 5% NaNO2 was added to each solution; 5 minutes later, 0.3 mL of a 10% AlCl3 solution was added. At time t = 6 minutes, 2 mL of NaOH (1 M) was added; then the mixture was immediately diluted and homogenized with 2.4 mL of bi-distilled water.

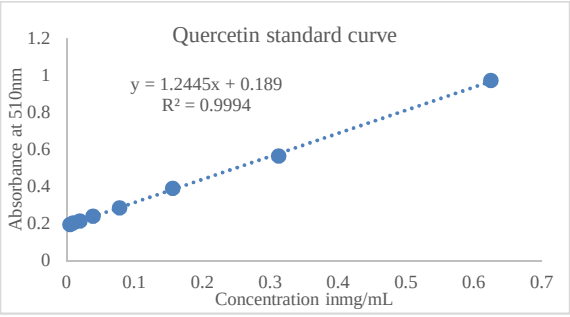

Fig. 2. quercetin standard curve.

The absorbance were read at 510 nm against a blank using a SAFAS MP96 spectrophotometer. The TF content of the extracts was determined by relating the absorbances read, following the same procedure of the standard, to the standard curve established with quercetin (Fig. 2). The results are expressed asmg quercetin equivalent per gram of extract (mg QE/g extract) as mean ± standard deviation.

In vitro antioxidant activity

ABTS radical scavenging activity

A volume of 200 µL of ABTS reagent was added to 50 µL of extract at different concentrations ranging from 0.01 to 1 mg/mL (cascade dilution of the order of 1/2) and the absorbance of the intense blue-green coloration was read 10 minutes later at 734nm against a blank using a SAFAS MP96 spectrophotometer (Miller *et al.*, 1993). The antioxidant content of each extract was assessed by relating the absorbances read to the standard curve established with Trolox (Fig. 3).

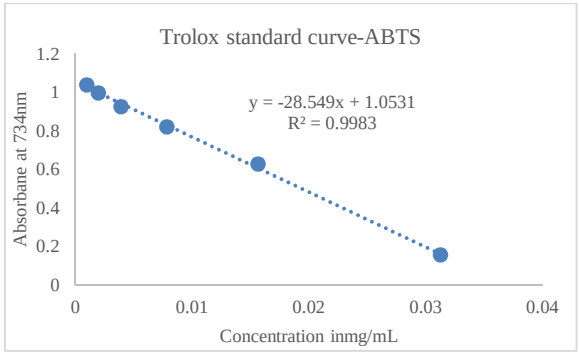

Fig. 3. Trolox standard curve.

Ferric Reducing Antioxidant Power

A volume of 30 µL of distilled water was mixed with 20 µL of extract at different concentrations ranging from 0.01 to 1 mg/mL (1/2 fold cascade dilution). To this mixture, a volume of 200 µL of FRAP reagent was added. The absorbance of the intense blue coloration was read at 595 nm using an MP96 SAFAS spectrophotometer after 10 minutes of incubation at room temperature (Benzie and Strain, 1996). The antioxidant content of each extract was assessed by plotting the absorbance read against the standard curve established with Trolox (Fig. 4).

DPPH radical scavenging activity

A volume of 200 µL of DPPH reagent was added to 50 µL of extract at different concentrations ranging from 0.01 to 1 mg/mL (cascade dilution on the order of

1/2) and the absorbance of the purple coloration was read 10 minutes later at 510 nm against a blank using a SAFAS MP96 spectrophotometer (Dudonné *et al.*, 2009). The antioxidant content of each extract was assessed by relating the absorbance read to the standard curve established with Trolox (Fig. 5).

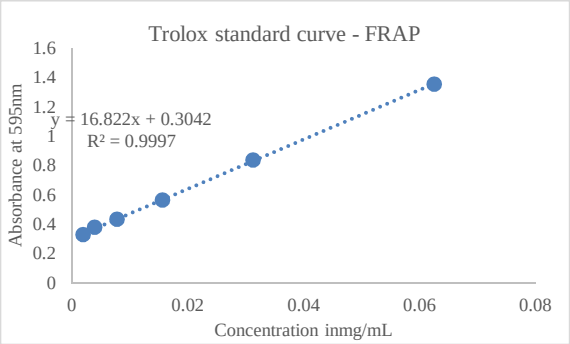

Fig. 4. Trolox standard curve-FRAP.

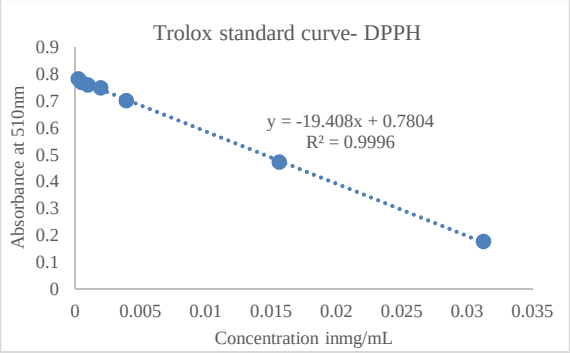

Fig. 5. Trolox standard curve-DPPH.

Antiradical activity of the extracts

The free radical scavenging activity of the extracts was determined by the 50% Inhibitory Concentration (IC50), which is the ratio of half the absorbance of the DPPH solution alone to the regression curve of the extracts and standards. It defines the concentration of antioxidants required to decrease the initial DPPH concentration by 50%. Antioxidant activity was expressed as the Antioxidant Activity Index (AAI), calculated as follows:

$$AAI = \frac{\text{final concentration of DPPH (µg/mL)}}{IC_{50}\ (\mu g/mL)}$$

Thus, the IAA was used to classify the antioxidant activity of the extracts.

- For an IAA < 0.5: the extract presents a low antioxidant activity;
- For an IAA between 0.5 and 1.0: the extract has a moderate antioxidant activity;
- For an IAA between 1.0 and 2.0: the extract has a high antioxidant activity;
- For an IAA > 2.0: the extract has a very high antioxidant activity (Scherer and Godoy, 2009).

Statistical analysis

Data were entered into Microsoft Office Excel 2016 and means and standard deviations were calculated. Results are presented as mean ± standard deviation.

Analyses of variance (ANOVA) were performed using SPSS version 20 software. Means were compared using Fisher's least significant difference (LSD) method with a 95% confidence level.

Results and discussion

Extraction yields

A total of fifteen extracts were obtained by successive exhaustion using solvents of increasing polarity (hexane, dichloromethane and methanol) by the maceration method. The extraction yields' values are presented in Table 1. Analysis of the results in Table 1 shows that the extraction yield varies between 1.50% and 10.21% depending on the plant and the solvent used. The methanol extract of *E. camaldulensis* recorded the highest yield with a value of 10.21% followed by the methanol extract of *C. nardus* with a yield of 8.25%. The DCM extract of *L. camara* recorded the lowest yield with a value of 1.50%.

Table I. Extraction yield of the different plants of the study.

Plants	Solvent	Yield (%)
E. camaldulensis	DCM	3.48
	MeOH	10.21
C. nardus	DCM	2.5
	MeOH	8.25
H. suaveolens	DCM	1.97
	MeOH	5.43
L. camara	DCM	1.50
	MeOH	5.10
L. multiflora	DCM	3.50
	MeOH	1.53

The quality and quantity of the extracts depend on the natural sources, structures of the target compounds, nature of the solvent, and the type of process used (Karacabey *et al.*, 2013).

Phytochemistry profile of extracts

Phytochemical screening of the plants 'extracts revealed the presence of a wide range of secondary metabolites, including tannins, flavonoids, saponosides, terpenes and sterols. The results are recorded in Table 2.

Except for the absence of saponosides in the methanol extract of *C. nardus*, chemical compounds such as tannins, flavonoids and saponosides were present in most of the methanol extracts. In addition, flavonoids were also detected in the DCM extracts. Moreover, compounds such as terpenes and sterols were only detected in the DCM extracts. Tube reactions and observation of Thin Layer Chromatography plates after using Dragendorff's reagent did not reveal detectable amounts of alkaloids in the extracts.

Table 2. Phytochemical screening of some chemical groups of the extracts.

Plants	Solvent	Chemical group tested				
		Tannins	Flavonoids	Alkaloids	Terpenes and sterols	Saponosides
E. camaldulensis	Hexane	-	-	-	+	-
	DCM	-	+	-	+	-
	MeOH	+	+	-	-	+
C. nardus	DCM	-	+	-	+	-
	MeOH	+	+	-	-	-
H. suaveolens	DCM	-	+	-	+	-
	MeOH	+	+	-	-	+
L. camara	DCM	-	+	-	+	-
	MeOH	+	+	-	-	-
L. multiflora	DCM	-	+	-	+	-
	MeOH	+	+	-	-	+

+: presence of the chemical group - : absence of the chemical group

Authors have reported that methanolic extracts of the aerial part of *C. nardus* revealed in addition to tannins and flavonoids, other chemical groups such as irridoids, proanthocyanidins and phenolic acids (Gebashe *et al.*, 2020). A previous study found that Petroleum ether, methanol and ethanol extracts of *E. camaldulensis* leaves contain in addition to tannins and flavonoids, chemical groups such as alkaloids, saponins, steroids, carbohydrates and cardiac glycosides (Chuku *et al.*, 2016).

Phytochemical screening of methanolic extracts of *H. suaveolens* leaves revealed chemical groups such as coumarins, mucilages, sterols, triterpenes, cardiotonic heterosides, oses, and holosides in addition to tannins, flavonoids, and saponosides (Kone, 2009). Chemical groups such as tannins, saponins and sterols/ triterpenes were detected in methanolic extracts of *L. camara* twigs (Bangou, 2012). Phytochemical analysis of the DCM extract of *L. multiflora* revealed the presence of chemical groups such as alkaloids, carbohydrates and tannins (Dabire *et al.*, 2015).

The observed differences in phytochemical composition with the reported results could be justified by the fact that the phytochemical composition of the same plant collected from different locations is influenced by environmental factors (Borokini and Ayodele, 2012).

Total phenolic compounds content of extracts

The results of colorimetric analysis of total phenolic compounds (TPCs) of DCM and MeOH extracts are presented in Fig. 6.

Fig. 6 shows that the contents of total phenolic compounds varied in the extracts in the range of 9.97 to 68.62 mg GAE/g extract. For the five species studied, the MeOH extracts recorded the highest contents compared to the DCM extracts. Thus, the MeOH extracts of *E. camaldulensis* and *H. suaveolens* showed the highest contents with a value of 68.62 ± 0.99 mg GAE/g extract and 52.88 ± 0.53 mg GAE/g extract respectively followed by *C. nardus* with a content of 36.20 ± 1.16 mg GAE/g extract.

The lowest content was recorded by the MeOH extract of *L. multiflora* with a value of 13.73 ± 0.45 mg GAE/g extract. As for the DCM extracts, of *E. camaldulensis* and *H. suaveolens* recorded the highest phenolic compound contents with a value of 40.51 ± 1.12 mg GAE/g extract and 22.36 ± 0.60 mg GAE /g extract respectively, followed by *C. nardus* with a content of 14.59 ± 0.12 mg GAE/g extract. The lowest content was recorded by the DCM extract of *L. camara* in the order of 13.73 ± 0.45 mg GAE/g extract.

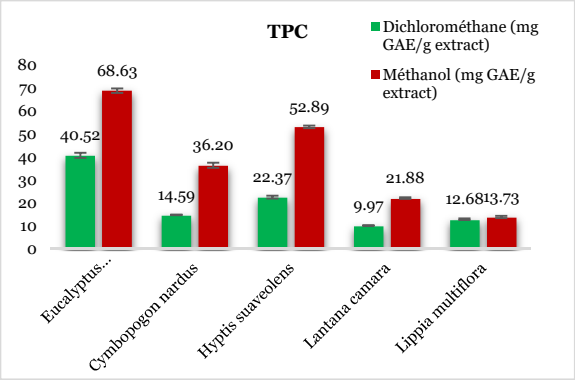

Fig. 6. Quantitative compounds of total polyphenols contents.

Previous studies conducted on the methanolic extracts of *E. camaldulensis* presented phenolic compound contents of 75.21 mg GAE/g extract (Meksem, 2018). Methanolic extracts of *L. camara* exhibited phenolic compound contents of 10.03 mg GAE/100 mg extract (Bangou, 2012). DCM extracts of *L. multiflora* exhibited phenolic compound contents of 6.71 µg GAE /mg extract (Dabire *et al.*, 2015). The phenolic content of a plant depends on a number of intrinsic and extrinsic factors (Falleh *et al.*, 2008; Osama, 2018). The high solubility of phenols in polar solvents could justify the high concentration of these compounds in extracts obtained using polar solvents for extraction (Andersen and Markham, 2005). This could explain the high content of the MeOH extracts compared to the DCM extracts of the studied plants.

Total flavonoid (FT) content of extracts

The results of the total flavonoid (TF) content of the DCM and MeOH extracts are reported in Fig. 7. The flavonoid contents of the extracts ranged from 114.19 to 265.02 mg QE/g extract. Regardless of the plant, DCM extracts recorded the highest flavonoid contents compared to MeOH extracts. With respect to fig. 2, the DCM extracts of *H. suaveolens* and *E. camaldulensis* contained the highest flavonoid contents with a value of 265.02 ± 7.67 mg QE/g extract and 250.70 ± 8.50 mg QE/g extract respectively, followed by *C. nardus* with a content of 234.79 ± 3.61 mg QE/g extract. The DCM extract of *L. multiflora* had the lowest flavonoid content with a value of 163.01 ± 8.54 mg QE/g extract. As for the MeOH extracts, *H. suaveolens* and *L. camara* showed the highest flavonoid contents with a value of 157.20 ± 1.60 mg QE/g extract and 139.33 ± 1.51 mg QE/g extract, respectively, followed by *E. camaldulensis* with a content of 122.59 ± 1.79 mg QE/g extract. The MeOH extract of *C. nardus* had the lowest flavonoid content with a value of 102.04 ± 1.44 mg EQ/g extract. The concentration of flavonoids in the extracts depends on the polarity of the solvents used in the extract preparation (Gao and Liu, 2005). The type of standard used (quercetin, rutin) can also change the results (Djeridane *et al.*, 2010).

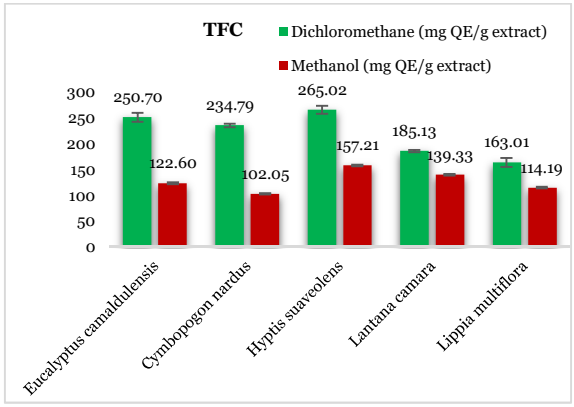

Fig. 7. Quantitative composition of total flavonoids compounds.

Furthermore, a negative correlation (R = -0.26) between the total polyphenol content of 7 species of *Stachys taxa* and that of flavonoids has been reported (Bilušić Vundać *et al.*, 2007). Indeed, there is a diversity of classes of phenolic compounds: quinones, coumarins, anthocyanins, tannins, flavonoids, acid-phenols, etc. (Abedinia, 2013). Phytochemical screening revealed the presence of flavonoids in the DCM extracts and the absence of tannins in these extracts.

This would suggest that flavonoids are the predominant class of phenolic compounds present in the DCM extracts, and therefore justifies their high content of these compounds.

Antioxidant content of extracts by ABTS method

The antioxidant content (OAC) of the extracts by ABTS method is recorded in Fig. 8. An analysis of the results in fig. 8 shows that the antioxidant contents varied in the extracts in the range of 7.25 to 28.99 mg TE/g extract. The MeOH extracts recorded the highest antioxidant contents compared to the DCM extracts. Thus, the MeOH extracts of *E. camaldulensis*; *H. suaveolens* and *C. nardus* showed the highest contents with a value of 28.99 ± 0.17 mg TE/g extract; 25.83 ± 0.26 mg TE/g extract and 23.98 ± 0.18 mg TE/g extract respectively. The lowest content was recorded by the MeOH extract of *L. multiflora* with a value of 12.93 ± 0.11 mg TE/g extract. As for the DCM extracts, *E. camaldulensis*; *H. suaveolens* and *C. nardus* showed the highest contents in the order of 11.93 ± 0.23 mg TE/g extract; 10.55 ± 0.17 mg TE/g extract and 7.83 ± 0.15 mg TE/g extract respectively. The lowest content was recorded by the DCM extract of *L. multiflora* with a value of 7.24 ± 0.04 mg TE/g extract.

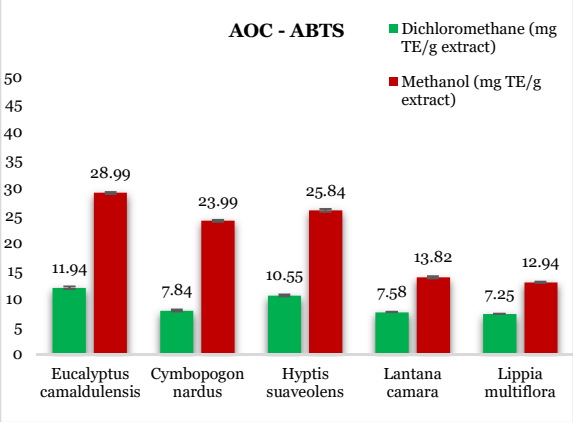

Fig. 8. Antioxidant content of extracts by ABTS method.

Antioxidant content of extracts by FRAP

The antioxidant content (OAC) of extracts by the FRAP method is shown in Fig. 9. From the analysis of the results presented in Fig. 9, it appears that the antioxidant contents vary in the extracts from 9.87 to 54.41 mg TE/g extract.

The MeOH extracts recorded the highest antioxidant contents compared to the DCM extracts. Thus, the MeOH extracts of *H. suaveolens* and *C. nardus* showed the highest contents with a value of 54.41 ± 2.18 mg ET/g extract and 48.91 ± 2.06 mg ET/g extract respectively, followed by the MeOH extract of *L. multiflora* with a value of 28.75 ± 3.01 mg ET/g extract. The lowest content was recorded by the MeOH extract of *E. camaldulensis* with a value of 17.35 ± 0.84 mg TE/g extract. As regards the DCM extracts, *H. suaveolens* and *C. nardus* showed the highest contents with a value of 19.09 ± 1.15 mg TE/g extract and 17.88 ± 0.36 mg TE/g extract, respectively, followed by *E. camaldulensis* with a value of 11.39 ± 0.17 mg TE/g extract. The lowest content was recorded by *L. camara* with a value of 9.87 ± 1.08 mg TE/g extract. These results show that the extracts contain substances that can reduce the ferric ion to ferrous ion. The mechanism of FRAP reactions is totally based on electron transfer rather than a mixture of electron and proton transfer. In combination with other methods, it can be very useful in distinguishing the dominant mechanisms with different antioxidants (Prior *et al.*, 2005). Phenolic compounds are electron-donating substances that play an important role in demonstrating reducing capacity. Therefore, reducing power and total phenolic content may be a reason for the high reducing power activity of extracts (Ebrahimzadeh *et al.*, 2018).

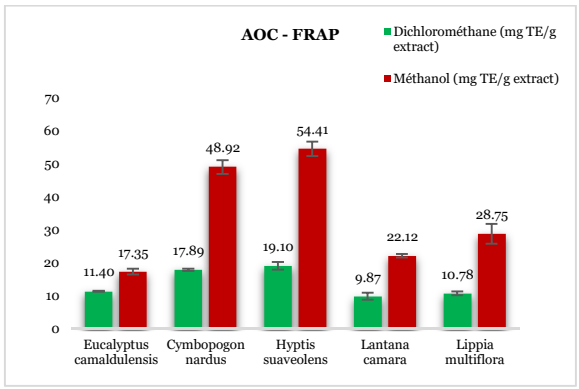

Fig. 9. Antioxidant content of extracts by FRAP.

Antioxidant content of extracts by DPPH method

The antioxidant content (AOC) of extracts by DPPH method is shown in Fig. 10.

The analysis of the results in Fig. 10 shows that the antioxidant content in the extracts ranged from 3.43 to 30.74 mg TE/g extract. The MeOH extracts recorded the highest antioxidant contents compared to the DCM extracts. MeOH extracts of *E. camaldulensis; H. suaveolens* and *C. nardus* showed the highest contents with a value of 30.74 ± 2.59 mg TE/g extract; 30.10 ± 0.28 mg TE/g extract and 29.61 ± 0.10 mg TE/g extract respectively. The lowest content was recorded by the MeOH extract of *L. multiflora* with a value of 12.21 ± 0.09 mg TE/g extract. With regard to DCM extracts, *E. camaldulensis*; *H. suaveolens* and *C. nardus* showed the highest contents in the order of 9.82 ± 0.01 mg TE/g extract; 5.84 ± 0.47 mg TE/g extract and 4.74 ± 0.04 mg TE/g extract respectively. The lowest content was recorded by *L. multiflora* with a value of 3.43 ± 0.01 mg TE/g extract.

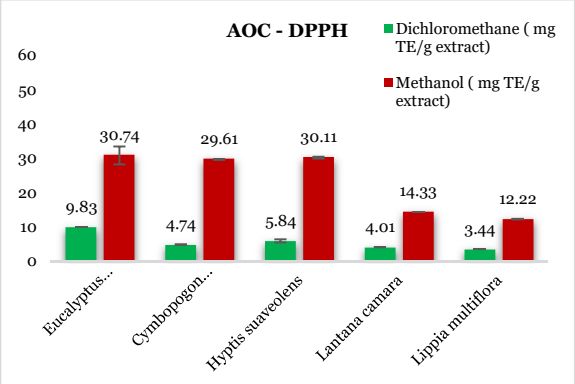

Fig. 10. Antioxidant content of extracts by DPPH method.

In sum, the observed antioxidant activity of the studied plant extracts varied from one extract to another depending on the extraction solvent and the method of determination. These differences in activity could be attributed to the various compounds extracted by the solvents of different polarity (Lafka *et al.*, 2013). Analysis of the antioxidant content of the extracts showed that the MeOH extracts exhibited the highest levels. This activity could be related to their richness in phenolic compounds. In addition, phytochemical screening of the extracts revealed the presence of tannins and flavonoids, which are chemical compounds with antioxidant properties, in the MeOH extracts.

The presence of these two chemical groups, which are generally soluble in polar solvents (Andersen and Markham, 2005), could justify this antioxidant property of these extracts. Furthermore, the proven activity of an extract may reflect a small amount of highly active constituents as well as a large amount of constituents with low activity. The activity of a crude extract depends on the intrinsic activity of the active products and their relative quantity in the extract (Dabire *et al.*, 2015).

Antiradical activity of the extracts

The results of the antiradical activity from the Inhibitory Concentration 50% (IC50) and the Antioxidant Activity Index (AAI) of the extracts of the studied plants are mentioned in Table 3. A comparison of IC50 values of the different extracts and standards used (Table 3) shows that the antiradical activity of the extracts of the plants studied is lower than the DPPH- radical scavenging capacity of the standards. A low value of IC50 of an extract indicates its high antiradical activity (Sahgal *et al.*, 2009). Thus, among the studied plant extracts, the MeOH extract of *E. camaldulensis* recorded the best antiradical activity towards DPPH- radical with an IC50 of 0.87 µg/mL followed by the MeOH extract of *H. suaveolens* with an IC50 of 1.03 µg/mL. Based on the results, the MeOH extract of *E. camaldulensis* exhibited very high antioxidant activity with an IAA of 45.97 followed by the MeOH extract of *H. suaveolens* with an IAA of 38.83.

Table 3. IC50 and IAA values of extracts and standards.

		Solvent	IC_{50} (µg/mL)	AAI
Plants	*E. camaldulensis*	MeOH	0.87	45.97
	C. nardus	MeOH	1.46	27.39
	H. suaveolens	MeOH	1.03	38.83
	L. camara	MeOH	1.78	22.47
	L. multiflora	MeOH	2.05	19.51
Standard	Trolox	MeOH	0.37	108.10
	Quercétine	MeOH	0.36	111.11
	BHT	MeOH	0.40	100
	Acide gallique	MeOH	0.35	114.28

Conclusion

The determination of phenolic compounds revealed that MeOH extracts have the highest content of phenolic compounds; while DCM extracts have the highest content of flavonoids.

The evaluation of the antioxidant content of the MeOH and DCM extracts showed that the studied plants have an antioxidant activity that varies from one species to another depending on the test used. This evaluation revealed that MeOH extracts were the most active compared to DCM, regardless of the test used. In addition, the extracts that exhibited high scavenging capacities towards ABTS and DPPH free radicals, also exhibited Fe3+ reducing capacity. These plants constitute a potential source of natural antioxidant. In perspectives, it would be interesting to isolate and elucidate the structure of the compounds responsible for the antioxidant activity.

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