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Physicochemical characterization and evaluation of the antioxidant activities of essential oil extracted from *Eucalyptus globulus*

Hamza Belkhodja [1]*, Djilali Bouhadi [1], Bouchra Medjadel [2], Amel Brakna [2]

[1] Laboratory of Bioconversion, Microbiology Engineering and Health Safety, Department of Biology, University of Mustapha Stambouli, Mascara, 29000, Algeria

[2] Department of Biology, University of Mustapha Stambouli, Mascara, 29000, Algeria

* Corresponding author: hamzabelkhodja@yahoo.fr; hamza.belkhoja@univ-mascara.dz

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ABSTRACT: This work was conducted as part of evaluation of the antioxidant activities of the essential oil extracted from a plant that belongs to the family of Myrtaceae: *Eucalyptus globulus*. The extraction of the essential oil was carried out by hydrodistillation and followed by extraction yield determination and physicochemical analysis. Then, the evaluation of the antioxidant activity was performed according to the method of DPPH free radical scavenging and the determination of total antioxidant capacity. The extraction of the essential oil gave a content of 0.41 ± 0.01%. The analytical study of the physicochemical properties of the essential oil of *E. globulus* showed that this plant presented an essential oil of acceptable quality and in conformity with the standard. The results of the evaluation of the antioxidant activity showed that this essential oil has interesting antiradical properties. It was manifested by a low value of IC_{50} (0.017 mg/ml) compared to the standard antioxidant (ascorbic acid). It was noticed that the essential oil of *E. globulus* has an antioxidant capacity of the order of 19 ± 0.01 mg AAE/g. This result showed that the essential oil of *E. globulus* has a powerful antioxidant power by reducing phosphomolybdate. Thus, the essential oil of *E. globulus* appeared effective in reducing oxidative reactions.

Keywords: Extraction; *Eucalyptus globulus*; Antioxidant; Essential oil; Oxidative stress.

1. INTRODUCTION

Humans have always been interested in plants to solve their health problems. They form the basis of traditional medicine because they are rich in secondary metabolites used as active compounds [1]. According to the World Health Organization, nearly 80% of the population rely on traditional medicine to provide primary health care [2]. Due to its different climatic stages and biological geographic location, Algeria has a large number of natural species which represent a very important phytogenetic heritage [3]. In fact, plants have therapeutic potential, which will enable them to play a beneficial role in preventive actions that are very important to human health [4]. This therapeutic ability is related to the activity of many biologically active molecules such as flavonoids, tannins and essential oils [5, 6]. Currently, essential oils from plants have a

considerable advantage and played popularity by the progressive discovery of their medicinal properties: antimicrobial, anti-inflammatory, antioxidants, anti-tumor, insecticides, as well as their uses in other fields of the interest such as cosmetics, the perfumery, the aromatherapy and food [7].

The oxidative stress is involved in many diseases as a trigger or related to complications. Most of the diseases induced by the oxidative stress appear with age because aging decreases antioxidant defenses and increases the multiplication of free radicals [8]. Additionally, there is a relationship between the excessive production of those free radicals within the body and therefore the installation of the inflammatory mechanism [9]. But given the side effects of the anti-inflammatory drugs, it causes the look for natural substances with biological and / or pharmacological activities which are of interest in bio-pharmacology.

Eucalyptus globulus, known as blue gum, occupies a very important place in pharmacology. The main component is volatile oil. So far, a variety of biologically active compounds have been identified in essential oils. The main components of the essential oil are eucalyptol (1,8-cineole), α-pinene, δ-limonene, α-terpineol, globulol, α-terpineol acetate and alloaromadendrene. *E. globulus* is commonly used in many cold remedies. The bactericidal and antiseptic effect is especially related to the presence of 1,8 cineole. It acts on *Escherichia, Proteus* and *Staphylococcus aureus*. Applying *E. globulus* essential oil to the painful area can help relieve rheumatism (acute pain, neuralgia, bacterial skin infections) [10].

In this context, this present work is aimed to the evaluation of the antioxidant activity by the method of DPPH free radical scavenging and the determination of total antioxidant capacity.

2. MATERIALS AND METHODS

2.1. Plant

It consisted of the aerial part (leaves and flowers) of the species: *Eucalyptus globulus,* which was harvested during the month of February 2019. The plant was identified by botanists of the Department of Biology of the University of Mustapha Stambouli, Mascara.

2.2 Extraction of the essential oil

Extraction of the essential oils (EO) was carried out by hydro-distillation in a Clevenger apparatus. 100 g of *E. globulus* leaves and flowers was boiled. When the temperature stabilizes, the distillate was collected. The sodium chloride (NaCl) was added to the distillate. Then, the mixture was placed in a separating funnel and three successive washes (10, 10, 20 ml) of Cyclohexane were achieved. After agitation, the organic phase was taken to undergo rotary evaporation to remove the Cyclohexane and obtain the essential oil. The essential oil was stored at +4°C after the calculation of yield [11].

2.3 Sensory properties and physicochemical indices

The sensory and physicochemical properties of EO (aspect, color, odor, solubility, density, refractive index, optical rotation, acid index, saponification and ester indices) were carried out in *E. globulus* essential oil to determine the quality. All the parameters were determined according to the method of European Pharmacopeia [12].

The determination of Relative density was performed using a pycnometer with a volume of 1 ml and temperature of 20°C. While, Rotator power was obtained using a polarimeter type VISTA C25. Light source (sodium-vapor lamp), for obtaining a light wavelength of 589.3 nm ± 0.3 and a viewing tube 100 ± 0.5 mm in length. For the Refractive index, it was carried out using a refractometer ABBE with a precision of ± 0.0002, direct reading of refractive index between 1.3000 and 1.7000 situated. The Miscibility test was determined by the volume

(V) of ethanol 96% necessary to form a homogeneous mixture with 0.5 ml of EO. The acid Index (AI) expressed the number of milligrams of potassium hydroxide (KOH) to neutralize the free acids contained in one gram of essential oil. 2 g of essential oil was added to 5 ml of ethanol 95% and 0,5 ml of phenolphthalein 0.2%. The solution was neutralized by the solution of potassium hydroxide (0.1 mol/L).

The calculation of AI was given by the formula: AI = 5.61 x V/M

5.61: Corresponds to 0.1 mol/L of KOH

M: mass of the essential oil

V: Volume in milliliters of potassium hydroxide solution (0.1 mol/L) used for titration

The ester index (EI) was the number of milligrams of potassium hydroxide to neutralize the free acids by hydrolysis of esters contained in one gram of essential oil. 2 g of essential oil was added to 25 ml of potassium hydroxide solution (0.5 mol/L). After heating the mixture for one hour, the solution was added to 20 ml of distilled water and 0.5 ml of phenolphthalein 0.2%. The excess of KOH solution was titrated with hydrochloric acid 0.5 mol/L a blank test was carried out under the same conditions and with the same reagents.

The calculation of EI was given by the formula: EI = (28.05 x (V0-V1) / M)-IA

28.05 g/L: corresponding to 0.5 mol/L KOH

M: mass of the essential oil

V0: Volume in ml of the HCl solution (0.5 mol/L) used for the blank

V1: volume in ml of the HCl solution (0.5 mol/L) used to determine the EI of the EO.

To determine the carbonyl index (CI), 1 g of EO was added to 20 ml of hydroxyl ammonium chloride solution and 10 ml of KOH solution 0.5 M. After 24 hours 0.5 ml of bromophenol blue was added (Blue color). Then, the excess of KOH was titrated with HCl solution 0.5 M (greenish yellow). At the same time a blank test was carried out under the same conditions.

The carbonyl index was given by the formula: CI = 56.1 x C x (V0-V1) / m

C: Concentration of the HCl solution

m: Mass in g of the essential oil

V0: Volume in ml of the HCl solution used for the blank test

V1: Volume in ml of the HCl solution used for the determination of CI

2.4 DPPH Free radical scavenging activity

The antiradical activity of *E. globulus* EO has been determined according to the method of Sanchez-Moreno [13] which uses DPPH as a relatively free radical which absorbs in the visible at the wavelength λ of 517 nm. The DPPH solution was prepared in advance by solubilizing 2.4 mg of DPPH in 100 ml of absolute methanol. 25 µl of the essential oil at different concentrations are added to 975 µl of DPPH. Standard antioxidant solution (ascorbic acid) was also prepared under the same conditions to serve as a positive control. The negative control consisted of DPPH and methanol. The mixture was left in the dark for 30 minutes. The assay was performed spectrophotometrically at a wavelength of 517 nm. The percentage of antiradical activity was estimated according to the equation:

Anti-radical activity [%] = [(A1-A2) / A1] x 100

A1: Absorbance of the negative control

A2: Absorbance in the presence of the extract

2.5 Calculation of the IC_{50}

Inhibitory Concentration 50 (IC_{50}) was the concentration of the test sample required to reduce 50% of the DPPH radical. The IC_{50} values were calculated graphically by inhibition percentage based on different concentrations of the extracts [14]. For the entire experiment, each test was performed in triplicate.

2.6 Total Antioxidant Capacity

The Total Antioxidant Capacity (TAC) (Phosphomolybdate test) was a variant of the DPPH test. It was evaluated by the method of Prieto et al. [15]. The method was based on the reduction of molybdenum Mo (VI) present in the form of molybdate MoO_4^{2-} to molybdenum Mo (V) MoO_2^{+} ions in the presence of EO to form a green to yellowish complex of phosphate/Mo (V) at acid pH. A volume of 0.3 ml of EO was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then the tubes were incubated at 95°C for 90 min. After cooling, the absorbance of the solutions was measured at 695 nm against the blank which contained 3 ml of the reagent solution and 0.3 ml of the methanol and incubated as the sample. Total antioxidant capacity was expressed in milligram equivalent of ascorbic acid per gram (mg AAE/g). The test was performed in triplicate.

2.7 Statistical analysis

The values were expressed as mean ± standard deviation (Mean ± SD). The results were analyzed by ANOVA single factor for multiple comparisons. The P values less than 0.05 ($p < 0.05$) were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Extraction yield

The essential oil of *E. globulus* presented a yield of 0.41 ± 0.01%. It became nearly identical to that of Taleb-Toudert [16] who recorded a value of 0.48%. However, the results obtained were much lower than those reported by Pereira et al. [17] and Mulyaningsiha et al. [18], which were 1.57% and 0.71%, respectively. It was also lower than that of Aouidet [19] where they estimated a yield of 1.4%.

An essential oil was very fluctuating in its composition, on which intervene a large number of parameters, of intrinsic origin (genetic, vegetative stage), of extrinsic origin (soil, climate, latitude) or of technological order [20]. The essential oil contents were generally very low, it sometimes takes several tons of plants to obtain a liter of essential oil [21]. Indeed, Emara and Shalaby [22] observed changes in the conformation of the secretory channels of *E. globulus* through the seasons. In addition, the extraction efficiency, as well as the quality of an EO were influenced by several factors such as temperature, relative humidity and duration of insolation exerting a direct influence [23] as well as the operating pressure, the method and the duration of distillation [24, 25].

3.2 Sensory properties and physicochemical indices

Physicochemical properties were useful in determining the quality of essential Oils [36]. The essential oil of the *E. globulus* plant was fractionated in such a way that its sensory and physicochemical properties can be determined. For the EO of *E. globulus*, the sensory properties showed that the essential oil was of a liquid appearance, pale yellow in color with a very strong, peculiar, camphoric, fresh, striking and persistent odor. According to AFNOR and article 31 (EC) n° 1907/2006, the essential oil of *E. globulus* should have a liquid,

colorless to pale yellow appearance with a camphoric and fresh odor. So, after comparison with these standards, the results suggested an essential oil of very good quality. Table 1 groups the specific results to the measurement of the physicochemical parameters.

For the EO of *E. globulus*, the pH estimation gave a value of 4.5 ± 0.01. This value showed up in the reference standard which required a pH value of 4 to 6 for essential oils. It was even slightly higher than that obtained by Rabiai [26] where they recorded a value of 3.4. This pH was probably linked to the chemical composition of the essential oil by the presence of substances and molecules with acid nature. EO of *E. globulus* gave a density value of 0.632 ± 0.01. This value was lower than those obtained by Mendes Silva et al. [27] which were 0.908 for the EO of the fruit and 0.909 for the EO of the leaves of *Eucalyptus*. Thus, the analytical study carried out by Ben Hassine [28] recorded a relative density value at 20°C equal to 0.92.

For the rotating power, this index recorded a value of +4.1°± 0.02. According to the AFNOR standard for essential oil of *E. globulus* which required that the value of the optical rotation must be in the range of +0° to +10°, it was found that this essential oil complied with this standard. The refractive index was considered a criterion of purity, also used to identify essential oils. Each substance has its refractive index. Indeed, the AFNOR standard provided for essential oil the refractive index of between 1.460-1.476. In our case, this index reached a value of 1.4428 ± 0.001 (Table 1). This value was slightly lower than those obtained by Mendes silva et al. [29] which were 1.463 for the EO of the fruit and 1.458 for the EO of the Eucalyptus leaves. The refractive indices changed essentially with the content of monoterpenes and oxygenated derivatives. A high content of monoterpenes will give a higher index. According to Hussain et al. [29], refractive index and density were not affected by the variation of the seasons. For some researches, a low refractive index of EO indicated its low refraction of light, which could promote its use in cosmetic products [36].

Table 1. The physicochemical parameters of the EO of *E. globulus.*

	Properties	**EO of *E. globulus***	**Standard values**	**References**
Physical properties	Relative density	0.632 ± 0.01	0.905-0.921	NF ISO 279
	Rotary power	+4.1°± 0.02	+0° à +10°	NF ISO 592
	Refractive index	1.4428 ± 0.001	1.460-1.476	NF ISO 280
	Mixibility	V_{EO} / $3V_{Ethanol}$	/	NF ISO 875
Chemical properties	pH	4.5 ± 0.01	4-6	AFNOR
	Acid index	03.18 ± 0.05	0.84-3.74	AFNOR
	Ester index	196.35 ± 0.5	/	/
	Saponification index	199.53 ± 0.41	/	/
	Carbonyl index	392.77 ± 0.9	/	/

The acid index represented the amount of free fatty acids resulting from the hydrolytic reactions of triglycerides. It was considered as a quality criterion making it possible to account for the conservation of an essential oil; an essential oil of a good quality should have low acidity [30]. In fact, the AFNOR standard provided for essential oil the acid index of between 0.84-3.74. According to the results, this index reached a value of 03.18 ± 0.05 (Table 1). It was close to that obtained by Ben hassine [28] and Taleb-Toudert [16] who recorded an acid value of 3.58 and 2.3 (mg KOH / g EO) respectively. A low acid index indicated that essential oils were stable and do not cause oxidation because the oil, by oxidizing, degraded quickly and caused an increase in the acid index [31]. From the both indices of saponification and acid, can deduce the ester number. It was noted that the higher the ester value, the better the quality of the oil [32]. The saponification index of a fatty

substance was higher as the carbon chain of fatty acids was short. In our case, this index hit a value of 199.53 ± 0.41 and the carbonyl index has a value of 392.77 ± 0.9 for the essential oil of the *E. globulus* (Table 1). Usually, a large value for both indices showed that the essential oils were of good quality.

3.3 DPPH Free radical scavenging activity

From these results, it can be seen that the inhibition percentage of the free radical has a proportional relationship with the concentration of essential oil of *E. globulus* and Ascorbic acid. The essential oil showed significant antioxidant activity ($p < 0.05$) compared to the ascorbic acid. It reached a value of 93.57% for the essential oil of *E. globulus* and 97.38% for Ascorbic Acid. Therefore, it can be deduced that the both samples (EO and ascorbic acid) exhibited remarkable antioxidant activity, nevertheless the DPPH inhibition rates recorded in the presence of the low concentrations of essential oil of *E. globulus* appeared to be superior to ascorbic acid. While Ascorbic Acid remained more active than essential oil in high concentrations (Figure 1).

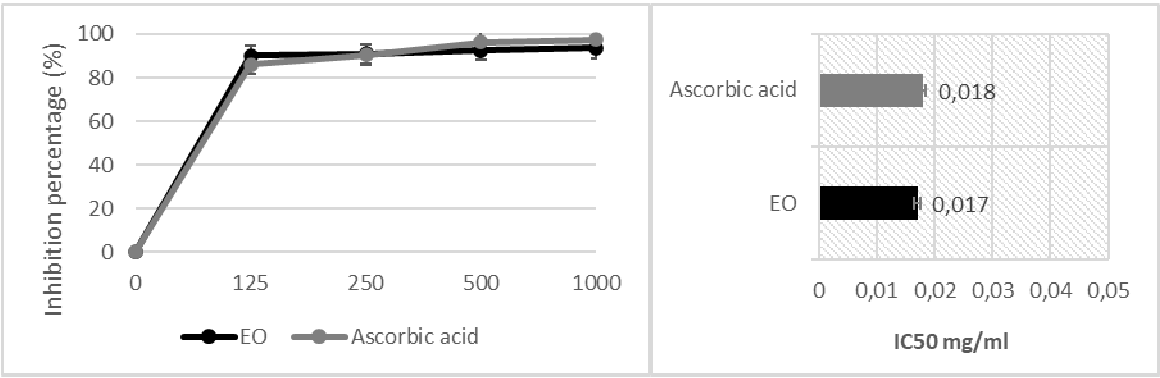

Figure 1. Inhibition percentage and IC_{50} of EO of *E. globulus* and ascorbic acid.

This was in perfect agreement with the results of Mishra et al. [33] where they showed that the trapping effects with high essential oil concentrations of *E. globulus* were found to be relatively less important than those of ascorbic acid. This reversed action may be due to the presence of 1,8-cineole in high concentration in the *E. globulus*. Aidi Wannes et al. [34] and Bagheri et al. [25] showed that the presence of monoterpene derivatives such as 1,8-cineole in high percentage in the essential oil of *E. globulus* may be attributed to decreased anti-free radical activity. In this method of trapping the DPPH radical, the decoloration of the DPPH in the presence of the antioxidant was carried out by accepting an electron or a hydrogen atom donated by an antioxidant compound [35]. Thus, the strong reducing capacity of this radical was due to the hydrogen / electron donor capacity of the compounds present in this essential oil, which endows them with a good antioxidant acting as an inhibitor of free radicals. The essential oil of *E. globulus* exhibited an interesting anti-free radical property manifested by a low IC50 value (0.017 mg/ml). Comparison with ascorbic acid showed that the essential oil of *E. globulus* presented greater antioxidant power than ascorbic acid which exhibited a significantly high IC_{50} value (0.018 mg/ml) (Figure 1).

In contrast, the work carried out by Mishra et al. [33] on the essential oil of the leaves of *E. globulus* showed a recorded IC_{50} value of 0.033 mg/ml. Which significantly exceeded our results. In addition, the IC_{50} value that was recorded remained lower than the value mentioned in the work of Bey-Ould Si Said et al. [36], where they recorded an IC_{50} value of 0.027 mg/ml. This antioxidant power due to the richness in phenolic compounds where the hydroxyl groups in the phenolic compounds can serve as an electron donor. According

to Pietta [37], the flavonoids, procyanidins and propelargonidins were responsible for the strong anti-free radical and antioxidant activity. The essential oil of *E. globulus* was made up of several compounds, each of which contributed to their biological activities. According to the study of Hasegawa et al. [38], among the main bioactive compounds of *E. globulus*, they identified two compounds called globulusin A and eucaglobulin. They demonstrated a suppressive effect on the development of free radicals of DPPH. In fact, these molecules scavenged the free radical of DPPH in a concentration-dependent manner and showed more potential inhibitory activity than ascorbic acid.

According to Vazquez et al. [39], the anti-free radical activity was attributed to the action of some phenolic compounds (thymol, carvacrol). These results were confirmed by the results of Akolade et al. [40] where they showed the relationship between the powerful antioxidant effect of the essential oil of *E. globulus* and the presence of phenolic compounds and some monoterpene derivatives (1,8-cineole) in low concentrations. Therefore, the presence of these phenolic compounds during phytochemical screening increased the antioxidant activity. A moderate antioxidant activity of terpinene and its derivatives has been cited in the literature. Terpenes such as α-epinene, β-epinene, limonene, β-myrcene, sabinene and terpinolene were known to have good antioxidant properties, but depending on the antioxidant mechanism [41] (Martins et al., 2014). In addition, a strong antioxidant activity of EOs was attributed to their phenolic groups such as thymol, carvacrol and probably to 1,8-cineole [40, 42]. A study on the anti-free radical power of the essential oil of *E. globulus* has shown that essential oils from the fruits of *E. globulus* were more effective than those of its leaves with an IC_{50} value of 0.033 for fruits and 0.067 mg/ml for leaves [43].

3.4 Total Antioxidant Capacity

It was noted that the essential oil of *E. globulus* has an antioxidant capacity of around 19 ± 0.01 mg AAE/g. This result showed that the essential oil of *E. globulus* has a powerful antioxidant power by reducing phosphomolybdate. The strong antioxidant activity of the essential oil of *E. globulus* was attributed to the presence of phenolic compounds. The study of Leela et al. [44] on a few species of Myrtaceae showed that the antioxidant capacity was linked to the presence of some compounds derived from phenylpropene eugenol. Recent studies have shown that many related polyphenols significantly contribute to phosphomolybdate scavenging activity [45, 46]. Flavonoids were also responsible for efficient free radical scavenging activities [47].

4. CONCLUSIONS

The results of sensory and physicochemical analyzes has proven that this plant has an essential oil of acceptable quality and complies with standards. thus, the antioxidant activity revealed that this oil has interesting anti-free radical properties. It was manifested by a low IC_{50} value compared to the standard antioxidant (Ascorbic acid).

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