



## Extraction and determination of antioxidant properties of essential oils of *Ocimum gratissimum* and *Thymus vulgaris* (Lamiaceae) or (Labiataeae)

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### ABSTRACT

Leaves collected from two species of *Thymus vulgaris* and *Ocimum gratissimum* (Lamiaceae) from Cameroon gave by hydrodistillation method, essential oils yield between 1.3 and 1.6%. Identification of chemical composition by chromatography and chromatography coupled with mass spectrometry was done. It appeared that the majority of compounds of *T. vulgaris* detected were Thymol (39.2%), p-Cymene (25.36%),  $\lambda$ -Terpinene (16.2%), Linalool (3.32%),  $\alpha$ -Thujene (1, 7%) and Camphene (1.3%). For *O. gratissimum*, p-cymene (29.6%),  $\lambda$ -terpinene (21.9%),  $\alpha$ -Thujene (6.2%), Thymol (5.1%), Myrcene (3.9%) and 1,8-Cineol (2.9%). p-Cymene,  $\lambda$ -terpinene, thymol and  $\alpha$ -thujene were found in both species at significant levels. The antioxidant activities of essential oils of the leaves of these two species were highlighted with IC<sub>50</sub> for the DPPH method which gave values of 146.33  $\mu$ g/ml; 121.61  $\mu$ g/ml and 69.99  $\mu$ g/ml and for the FRAP method 342.33  $\mu$ g/ml, 264.93  $\mu$ g/ml, 202.85  $\mu$ g/ml respectively for *O. gratissimum*, *T. vulgaris* and Butyl hydroxy toluene (BHT). These results showed that the essential oils of the leaves of these two plants can be used in the preservation of food against oxidation. For concentration necessary to have a 50% trapping ability, a significant difference ( $p < 0.05$ ) in the increase in average trapping percentages (%IC) of the DPPH radical as a function of BHT concentrations was observed. It can be concluded that the essential oils of the leaves of *Ocimum gratissimum* and *Thymus vulgaris* could be used as antioxidants in food products and protect humans against certain chronic diseases.

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**Keywords:** *O. gratissimum*, *T. vulgaris*, Essential Oil, Chemical Composition, Anti-radical.

### INTRODUCTION

The Lamiaceae or Labiataeae is a family of flowering herbaceous plants. It's a large family of aromatic plants, rich in highly sought-after essential oils thanks to their economic and medicinal interest. Their biological properties are most often due to the essential oil fraction contained in plants

(Abdelli, 2017). They have numerous properties such as anti-radical, anti-inflammatory, and anti-flu activities (Lourens et al., 2004; Jazet et al., 2008). The biological activity of an essential oil depends on its chemical composition, the functional groups of its compounds (alcohols, phenols, terpenes and

ketone compounds) and the possible synergistic effects between these compounds. The nature of the chemical structures which constitute it, and the proportions of these also play a determining role (Lawrence, 2000).

Currently, several questions are being raised regarding the safety of synthetic chemicals used in medicine or the food industry. Indeed, the peroxidation of lipids produced during food manufacturing and storage processes under the action of free oxygen radicals leads to the loss of food quality and safety. Synthetic antioxidants generally used in the food industry to delay lipid oxidation have been found to cause undesirable effects. In addition, the excessive use of chemical antibacterial agents in human medication as well as in animal breeding leads to the appearance of resistant bacterial strains (Mau et al., 2004). The high cost of health services and medicines as well as socio-economic factors push a large part of the population to use medicinal plants for treatment (Agban et al., 2013). For all these reasons, numerous researches for natural compounds are being realized. Hence the present study was aimed at evaluating some biological properties of essential oils from two plant species from Cameroon, namely, *O. gratissimum* and *T. vulgaris* used in Cameroon as a food additive. These species have a very high growth and survival capacity in Cameroon and are generally found in great abundance. They are widely used both in the food industry and in the pharmacopoeia.

## MATERIALS AND METHODS

### Collection of Leaves

*Ocimum gratissimum* (Figure 1) is native from America, Africa, India and Southeast Asia (Kpokame, 2019). It is an herbaceous, perennial and aromatic shrub. With a general growth of 0.4 to 1.5 m high, it can however reach up to 2 m high depending on environmental conditions. The plant has an erect, rounded-quadrangular stem, very

branched and woody. It has an exfoliating epidermis in a strip with a more or less hairless base. It becomes pubescent at the nodes and on the axes of inflorescences. The axes of inflorescences are tomentose with white hairs and often also with sessile glands. Its leaves are spread to deflected, elliptical or ovate to broadly ovate and petiolate. Its size varies in length and in width, tight, often acute acuminate or obtuse at the top, and glabrous. Its lower surface is sometimes tomentose, often blacker than the upper surface, with a petiole 5 to 30 mm long. Its inflorescence appears in a whorled form or in a cluster of 10 to 15 cm, sometimes branched at the base, dense, sessile bract, ovate, lanceolate, colored and quickly deciduous. Its flowering period is between November and May, the plant bears small white, pink-white or carmine red flowers arranged in a long cluster (Manampisoa, 2021).

Fresh leaves of *Ocimum gratissimum* were harvested in December 2023, at a site near the Bonaberi Douala slaughterhouse, having a tropical climate. The average temperature of the coldest month (August) is 25.6°C and that of the hottest month (February) is 28.9°C.

*Thymus vulgaris* L. (Figure 2) is originally from southern Europe. It can be found from the eastern half of the Iberian Peninsula to the south-east of Italy, via the French Mediterranean coast (Özcan and Chalchat, 2004). However, it is now cultivated throughout the world as tea, spice and medicinal plant (Kitajima et al., 2004).

This spontaneous plant grows abundantly in arid, stony and sunny places from the seaside to the mountains (Iserin, 2001). Found almost everywhere in the world in domestic and forest form, it adapts to various climatic conditions (hot and cold) and soil (dry and humid). The yield and chemical composition of essential oils vary from one species to another. This variability can be linked to intrinsic and extrinsic factors. The organ of the plant used and extrinsic conditions such as environmental factors, in particular

temperature, light, rainfall and soil conditions, affect the chemical composition of aromatic and medicinal plants (Aprotosoie et al., 2010). Cultivation conditions such as harvesting techniques, sowing date, use of fertilizers, phytosanitary treatments also influence the composition and yield of essential oils (Aprotosoie et al., 2010).

The leaves of *Thymus vulgaris* were purchased in Yaoundé at the Sandaga-Douala market provided from Dschang West region 344.6 km. The climate Dschang is equatorial with an average annual rainfall of 1790.5 mm. There are two types of seasons, a rainy which lasts from March to November and a dry season lasting from October to February.

### Extraction of Essential Oils

The essential oils were obtained by hydrodistillation in a Clevenger type device. Before any extraction, the plant material was pretreated and introduced into the flask (reactor) with a quantity of water approximately 3 times the weight of the plant material to be extracted. The mixture was then brought and allowed to boil using a heating cap for six hours. During heating, the water vapor loaded with essential oil rose in the column, condensed at the level of the refrigerant where cold water circulates continuously, and resulted in the formation of a distillate made up of two phases in the decanting column, the lower aqueous phase which was constantly recycled and the higher organic phase containing essential oils.

Traces of water was eliminated in this essential oil by filtration on an anhydrous sodium sulfate column. The essential oil was then stored in dark bottles at laboratory temperature. The extraction yield was calculated relative to the mass of the dry plant material before extraction according to the following formula:

$$\text{Yield} = \frac{\text{mass of essential oil}}{\text{mass of plant}} \times 100$$

### Analysis of the chemical composition of essential oils

The analysis of the chemical composition was carried out by Gaseous Phase Chromatography (GPC) then by Gaseous Phase Chromatography coupled with Mass Spectrometry (GPC/MS). This operation was carried out at the National Polytechnic Institute Houphouët Bobigny (INPHB), Chemical and Process Engineering Laboratory Ivory Coast.

The principle of the gaseous phase chromatography (GPC) is based on the separation by heating of volatile compounds or compounds likely to be volatilized without decomposition according to their affinities between two phases: one stationary and the other mobile. The gas mixture was introduced into the column using an injector, where it was separated according to its retention time and its affinity with the column support in a high temperature oven. The sample components were then detected at the column exit using an appropriate detector.

The chromatogram used in the present study the Varian-HP5890, was a capillary column (length of 30 m and 0.25 mm internal diameter) characterized by an apolar stationary phase of the methylsilicone type (DB-1, film thickness 0.25  $\mu\text{m}$ ). 40  $\mu\text{l}$  of essential oils were dissolved in 500  $\mu\text{l}$  of hexane to constitute a "stock solution". 0.3  $\mu\text{l}$  of this solution was introduced using a syringe at the head of the column where it was vaporized by the Split type injector. The volatile solutes were pushed by the carrier gas (nitrogen) into the column where they travelled at different speeds depending on their differences in affinity between the stationary phase and the mobile phase. Molecules were detected at the output by a flame ionization detector (FID), and revealed by the integrator-recorder which was a Star Chromatography Workstation (STARWS) type software which signaled the presence of peaks each characterized by a retention time and an area.

The operating conditions were as follows:

- Temperature of the injector and that of the detector 200°C;
- Oven temperature: 60 to 246°C with a gradient of 3°C/min;

Carrier gas was nitrogen with a flow rate of 1ml/min.

Identification was made by comparing the retention indices with those in the laboratory's own database (Adams, 2007).

Mass Spectrometry (MS) is based on the fragmentation and ionization of EO compounds under the influence of an electric or magnetic field, followed by separation of the ions formed according to the individual mass to charge ratio (m/z).

Gas chromatography coupled with mass spectrometry is an analytical method that combines the performance of gaseous phase chromatography and mass spectrometry in order to precisely identify and/or quantify many substances.

The gaseous phase chromatography/mass spectrometry coupling was carried out using Hewlett-Packard (HP) 5890 A equipment, equipped with an apolar capillary column 30 m long, 0.25 mm in diameter, and covered with a film 0.25 micrometer (µm) thick. The injections were made in split mode. The quadrupole type detector (thermo) equipped with an ionization energy source of 70 electron-volts allowed the mass spectra of each of the constituents to be obtained.

The handling conditions were as follows:

- Injection temperature 220°C;
- Detector temperature 180°C;
- Temperature programming 70-200°C with a gradient of 10°C/min;
- The carrier gas was helium with a flow rate of 0.6 ml/min;
- The injection volume was 0.1 µl of an essential oil solution prepared at 10% in pentane.

As the oven temperature rises, the integrator-recorder records the different eluted

compounds in the form of peaks each characterized by a retention time and an area. These compounds are then identified by calculating linear retention indices (Figure 3). This requires the injection of a solution of linear alkanes spread over the entire chromatogram and under strictly the same conditions. The retention indices of the different constituents were calculated in relation to the retention times of a series of n-alkanes and their relative percentages calculated by electronic integration without taking into account possible differences in their response coefficients. Identification was made by comparing the retention indices with those in the laboratory's own database (Adams, 2007).

For identification, the retention indices (Kovats Indices) were calculated by comparing the retention times (TR) with those of a series of alkanes allowing calibration of the chromatogram, according to the following formula:

$$IR = 100 \left[ n + \frac{TR(X) - TR(C_n)}{TR(C_{n+1}) - TR(C_n)} \right]$$

Where:

TR (x): retention time of compound x;

TR (C<sub>n</sub>): retention time of hydrocarbon at n carbon atom;

TR (C<sub>n+1</sub>): retention time at n+1 carbon atom.

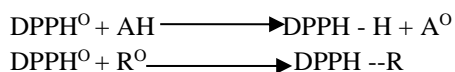
The quantitative and qualitative analysis of the chemical composition of essential oils is a step in the valorization of an essential oil. It remains a delicate operation requiring the implementation of various techniques.

### **Antioxidant activities**

#### ***2,2diphenyl-1-picryl hydrazine (DPPH) method***

The evaluation of the anti-radical activity was carried out by the DPPH (2,2diphenyl-1-picryl hydrazine) method. This method consists of reacting the compound to be tested (AH) with the free

radical of DPPH. This trapping is visualized by the disappearance of the purple color of DPPH which turns yellow when it is passed into its stable hydrogen form. The reaction can be followed spectrophotometrically at 517 nm (UV-mc2 spectrophotometer, SAFAS Monaco; quartz cells (1 cm x 1 cm x 4.5 cm)). The reference used butylated hydroxytoluene, is a synthetic monophenol type anti-free radical. (Brand-Williams et al., 1995).



#### Determination of anti-radical activity

The tubes were left in water bath thermostatically controlled at 300°C and the absorbance of the solutions was measured every 10 minutes for 2 hours. In each measurement series, the behavior of the solutions with the test substance was compared to that of the DPPH solution without the essential oil extracts. All measurements were repeated three times.

The calculation of the inhibition percentage was done using the following formula:

$$\text{Trapping percentage: IC (\%)} = \frac{A_{\text{ref}} - A_{\text{sam}}}{A_{\text{ref}}} \times 100$$

Where;

Aref: Absorbance at t=20 min of the blank (DPPH solution without the test substance),

Ames: Absorbance at t=120 min of the DPPH solution containing the test substance (essential oils in the case of the present study).

Results were expressed as IC50, the lower the IC50 values the higher the antioxidant power the extract had. The synthetic antioxidant butylated hydroxytoluene was used as references.

#### Reducing power “FRAP”

The FRAP method is based on the reduction of the ferric ion (Fe<sup>3+</sup>) to ferrous ion (Fe<sup>2+</sup>). This method evaluates the reducing

power of compounds (Bassène, 2012). The presence of reducing agents (HA) in plant extracts causes the reduction of Fe<sup>3+</sup>/ferricyanide complex to the ferrous form. Therefore, Fe<sup>2+</sup> can be assessed by measuring and monitoring the increase in density of cane blue color in the reaction medium at 700 nm. Indeed, the FeCl<sub>3</sub>/K<sub>3</sub>Fe(CN)<sub>6</sub> system gives the method sensitivity for the “semi-quantitative” determination of the concentrations of antioxidants, which participate in the redox reaction (Amarowicz et al., 2004).

The protocol used was based on the method described by Oyaizu, 1986. Test tubes having the FRAP solution and the essential oil at different concentrations (31.25, 61.5, 125, 250, 500 ppm) were prepared. The whole was heated to 50°C in a water bath for 20 minutes. A volume of 500 µl of trichloroacetic acid (10%) was then added and the mixture was centrifuged at 3000 rpm for 10 minutes. An aliquot of 500 µL of the supernatant was pipetted and transferred to another tube to which 500 µL of distilled water and 100 µL of freshly prepared 1% FeCl<sub>3</sub> in distilled water were added. A blank without the sample was prepared under the same conditions by replacing the extract with methanol.

The absorbance of the reaction medium was read at 700 nm against a similarly prepared blank, replacing the extract with methanol which allowed the device to be calibrated (UV-VIS spectrophotometer). The positive control was a solution BHT whose absorbance was measured under the same conditions as the samples. An increase in absorbance corresponded to an increase in the reducing power of the extracts tested. Results were expressed as mM equivalent BHT.

#### Data analysis

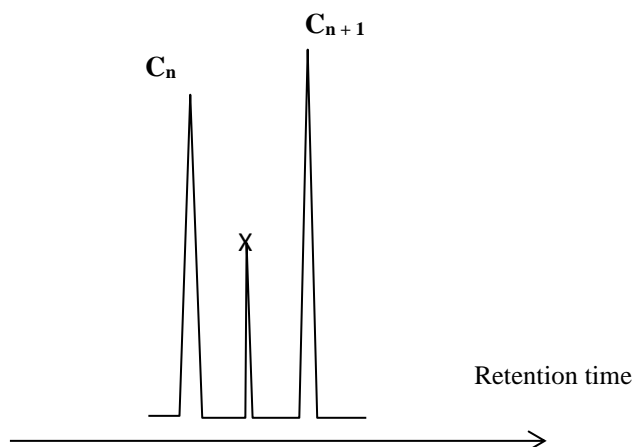
One-way analysis of variance (ANOVA) and Duncan's post-hoc test were used to make comparisons for the same column; numbers with different letters were statistically significant at the 5% level.



**Figure 1:** *Ocimum gratissimum* plant.



**Figure 2:** Fresh and dry *Thymus vulgaris* leaves.



**Figure 3:** Retention index (Kovats indice).

## RESULTS

Extraction by the hydrodistillation method made it possible to calculate percentage yields per plant species and the results are represented in Table 1 from which it can be seen that the extraction yields of essential oils vary from 1.3 to 1.6 for the leaves of two plants. The essential oils of *Ocimum gratissimum* had a higher yield than that of *Thymus vulgaris* (1.6%) and (1.3%) respectively. These results are quite similar with those of other similar studies carried out using the two plant species in Cameroon.

In regards to the Chemical composition identified in the essential oils extracted as shown in Table 2, it can be seen that there exists qualitative and quantitative difference between chemical compounds found in both plant species. Hence, it can be observed that *Ocimum gratissimum* essential oil extract was richer in hydrocarbon monoterpene compounds (77.00%) than *Thymus vulgaris* (46.94%) while *Thymus vulgaris* was richer in oxygenated monoterpenes (46.73%) than *Ocimum gratissimum* (10.2%). The majority chemical compounds found respectively in *Thymus vulgaris* and *Ocimum gratissimum* were: p- Cymene (25.36%), (29.6%),  $\gamma$  - Terpinene (16.2%), (21.9%),  $\alpha$ -Thujene (1.7%), (6.2%),  $\alpha$ -Terpinene (1.02%), (6.8%), Thymol (39.2%), (5.1%), E-B-Caryophyllan (3.82%). Also, (1.1%). Carvacrol found in *Thymus vulgaris* (3.2%) was absent in

*Ocimum gratissimum* and on the other hand *Ocimum gratissimum* contained 1.8 Cineole (2.9%) which was absent in *Thymus vulgaris*.

Statistical analysis allowed to observe a positive correlation between the percentage of free radical scavenging and the concentration of essential oils in all extracts. The analysis of the means by ANOVA and Duncan's, showed a statistically significant influence of the concentrations and extracts of essential oils on the percentage of trapping of the DPPH free radical ( $<0.05$ ). The percentages of free radical trapping at each concentration of the four EO extracts calculated from their respective absorbance are recorded in Tables 3, 4, 5 and 6. It can be seen that an increase in the percentage of trapping power is a function of the essential oil concentration, the greater the concentration of the extract the bigger the trapping percentage.

The anti-radical activities by the DPPH and FRAP method (as it can be seen in Figures 4 and 5 respectively) of all the extracts studied were significantly lower in *O. gratissimum* (146.33  $\mu\text{g/ml}$ ) and *T. vulgaris* (121.61  $\mu\text{g/ml}$ ), ( $P<0.05$ ) than that of BHT (IC50: 69.99  $\mu\text{g/ml}$ ) which is considered here as a reference anti-radical agent. The most effective extract of the species studied presented reducing and anti-radical power close to that of BHT. This allows them to be used as anti-radicals.

**Table 1:** Extraction yield of essential oils.

Vegetal species	Place of harvest	organs	Yield (%)
<i>Ocimum gratissimum</i>	Bonaberie, Cameroon	Leaves	1,6
<i>Thymus vulgaris</i>	Dschang, Cameroon	Leaves	1,3

Chemical Composition of Essential Oils.

**Table 2:** Chemical composition (%) of essential oils of *Ocimum gratissimum* and *Thymus vulgaris* leaves.

N°	Identified compounds	TR	T.V	O.G
<b>Hydrocarbonated monoterpens</b>			<b>46,94%</b>	<b>77,00%</b>
1	$\alpha$ -Thujene	922	1.7	6.2
2	$\alpha$ -Pinene	930	-	2.3
3	Camphene	945	1.01	0.7
4	Sabinene	971	0.21	0.4
5	$\beta$ -Pinène	974	0.12	0.5
6	Myrcene	985	1.03	3.9
7	$\alpha$ – Phellandrene	1002	0.09	0.8
8	$\delta$ – 3 – Carene	1008	-	0.3
9	$\alpha$ -Terpinene	1014	1.02	6.8
10	<i>p</i> – Cymene	1021	25.36	29.6
11	Limonene	1025	0.2	3.6
12	(Z)- $\beta$ -ocimene	1031	-	-
13	$\gamma$ – Terpinene	1056	16.2	21.9
<b>Oxygenated Monoterpens</b>			<b>46,73%</b>	<b>10,2%</b>
14	1,8 Cineole	1028	-	2.9
15	Linalool	1095	3,32	0,4
16	(Z)-Epoxy-ocimene	1122	-	-
17	E-pinocarveol	1137	-	-
18	Camphor	1143	1.01	-
19	Nerol oxide	1151	-	-
20	Menthone	1154	-	-
21	Isomenthol	1159	-	-
22	$\alpha$ – Terpineol	1163	-	-
23	Myrtenol	1187	-	1.8
24	Cis-Carveol	1195	-	-
25	Geraniol	1226	-	-



26	Piperitone	1249	-	-
27	E – Cinnamaldehyde	1253	-	-
28	Menthyl acetate	1270	-	-
29	Thymol	1291	39.2	5.1
30	Carvacrol	1293	3,2	-
31	Eugenol	1299	-	-
32	2-hexyl-(Z)- Cinnamalehyde	1355	-	-

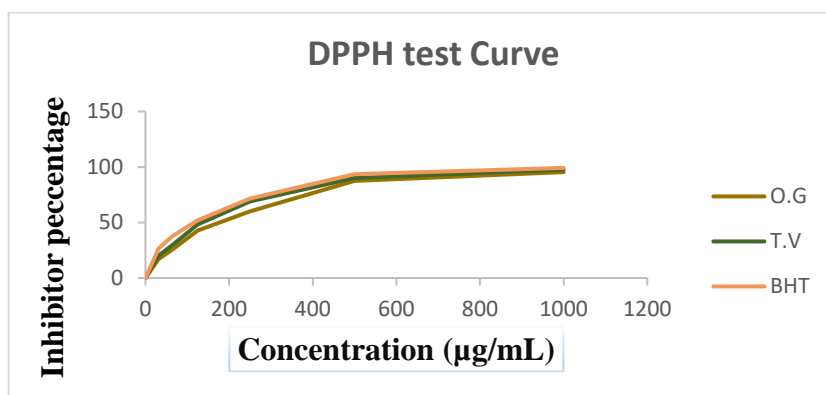
<b>Hydrocarbonated sesquiterpens</b>		<b>5,35%</b>	<b>1,96%</b>	
33	$\alpha$ -Cubebene	1346	0,16	-
34	$\alpha$ – Copaene	1371	0,36	0.7
35	$\beta$ – Cubebene	1378	0.11	-
36	$\beta$ – Bourbonene	1385	-	-
37	Z-Caryophyllene	1408	-	-
38	$\beta$ -Cedrene	1420	-	-
39	E-B-Caryophyllene	1423	3.82	-
40	(E)- 9-epi-Caryophyllene	1464	-	1.1
41	D – Germacrene	1477	-	-
42	$\alpha$ – Murolene	1483	0.5	-
43	$\alpha$ – selinene	1498	-	-
44	$\gamma$ – Cadinene	1515	0.21	0.5
45	$\delta$ – Cadinene	1524	0.19	1.5
<b>Oxygenated Monoterpens</b>		<b>0,8 %</b>	<b>5,98%</b>	
46	Caryophellene oxide	1587	0.8	-
47	Guaiol	1613	-	1,93
48	Iso caryophyllene	1621	-	2,9
49	14-oxy- $\alpha$ -Murolene	1763	-	1,15
<b>Composés aromatiques</b>		<b>0,18%</b>	<b>3,6%</b>	
50	Chloroacétophénone	1872	-	0,8
51	Fluoro-o-xylène	2025	0.18	2,8

**O.G:** essential oils from the leaves of *Ocimum gratissimum*, **T.V** essential oils from the leaves of *Thymus vulgaris*, **TR:** Retention Time, **-:** absent.

**Table 3:** DPPH activities.

Substances	Concentrations					
	31,25 ppm	62,5 ppm	125 ppm	250 ppm	500 ppm	1000 ppm
BHT	6,5± 0,02 <sup>c</sup>	37,2± 0,002 <sup>c</sup>	52,1± 0,08 <sup>c</sup>	71,6± 0,17 <sup>c</sup>	93,4± 0,03 <sup>d</sup>	99,2± 0,03 <sup>b</sup>
<i>Ocimum gratissimum</i>	17,1± 0,01 <sup>d</sup>	25,06± 0,01 <sup>d</sup>	42,7± 0,01 <sup>cd</sup>	60,2± 0,17 <sup>c</sup>	87,4± 0,003 <sup>b</sup>	95,4± 0,01 <sup>c</sup>
<i>Thymus vulgaris</i>	0,3± 0,003 <sup>c</sup>	29,2± 0,02 <sup>C</sup>	48,3± 0,03 <sup>a</sup>	69,1± 0,08 <sup>b</sup>	90,05± 0,02 <sup>c</sup>	97,6± 0,002 <sup>a</sup>

Data were presented as mean ± standard deviation (SD)



**Figure 4:** Percentage of free radical trapping by BHT, *O. gratissimum* and *T.vulgaris* as a function of concentration.

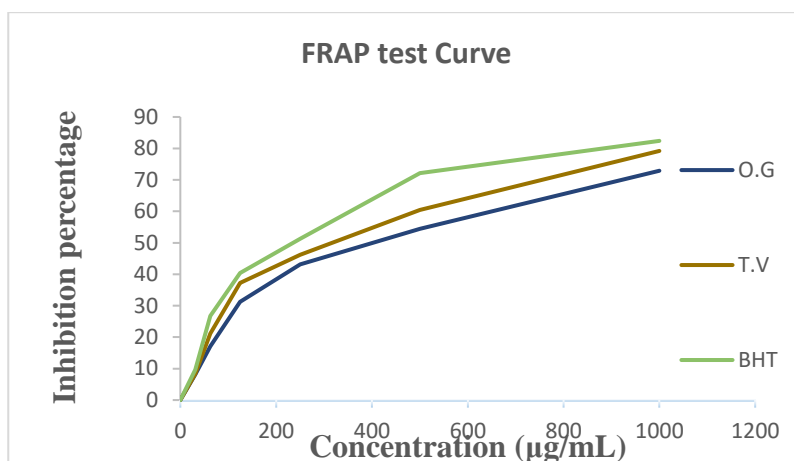
**Table 4:** SC50, IC50 and anti-radicalary power of essential oils from leaves of *O. gratissimum* and *T. vulgaris*.

Samples	IC50 (µg/ml)	CE50 (µg HE/µg DPPH)	PA
O.G	146,3308	3,6582	0,2733
T.V	121,6127	3,0403	0,3289
BHT	69,9999	1,7499	0,5714

**Table 5:** FRAP test table.

Substances	Concentrations					
	31,25 ppm	62,5 ppm	125 ppm	250 ppm	500 ppm	1000 ppm
BHT	9,6± 0,01 <sup>b</sup>	26,8± 0,04 <sup>c</sup>	40,42± 0,01 <sup>b</sup>	51,3± 0,01 <sup>b</sup>	72,1± 0,03 <sup>c</sup>	82,4± 0,08 <sup>b</sup>
<i>Ocimum gratissimum</i>	8,12± 0,01 <sup>d</sup>	17,05± 0,01 <sup>d</sup>	3,2± 0,01 <sup>cd</sup>	43,1± 0,17 <sup>c</sup>	54,4± 0,003 <sup>b</sup>	72,9± 0,01 <sup>c</sup>
<i>Thymus vulgaris</i>	8,31± 0,003 <sup>c</sup>	21,2± 0,02 <sup>C</sup>	37,2± 0,03 <sup>a</sup>	46,2± 0,08 <sup>b</sup>	60,45± 0,02 <sup>c</sup>	79,2± 0,002 <sup>a</sup>

Data were presented as mean ± standard deviation (SD)



**Figure 5:** Percentage of free radical trapping by BHT, *O. gratissimum* and *T.vulgaris* as a function of concentration.

**Table 6:** IC50, CE 50 and antiraducalary power of essential oils from leaves of *O. gratissimum* and *T. vulgaris*.

Samples	IC50 (µg/ml)	CE50(µgHE/µg Fecl3)	PR
O.G	342,3384	0,05705	17 ,5284
T.V	264,9391	0,044156	22,64697
BHT	202,8567	0,033809	9,5779

## DISCUSSION

The present study was carried out to identify the various chemicals found in *Ocimum gratissimum* and *Thymus vulgaris* essential oils from their respective leaves and evaluate their ferrous trapping as well and their anti-oxidant potentials. It appeared that the leaves of *Ocimum gratissimum* had a higher yield than those of *Thymus vulgaris*, (1.6%) and (1.3%) respectively. Previous studies have been done on *Ocimum gratissimum* and *Thymus vulgaris*. The present results are similar to other studies involving various *Thymus* species that reported 1.6% from France by Satyal et al. (2016), and 2.7% from Ain Defla in Algeria by Sidali et al. (2014). However, these results are higher than those obtained in Algeria (0.64%) by Ahmia (2020), from Morocco (0.50%) by El Ouali et al. (2013), in Brazil (0.25%) by Alexandre et al. (2008), in Serbia (1%) by Satyal et al. (2016), and in Iran (0.81%) by Pirbalouti et al. (2013). The variability of the present results with these

other studies carried out can be justified by the harvest period and by local environment and climate and soil conditions. According to several authors, the harvest origin of the species, the harvest period, the organ of the plant, the drying time and the extraction method are factors among others which can also have a direct impact on essential oil yields (Vekiari et al., 2002).

Gaseous phase chromatography in association in with mass spectrometry revealed a wide variety of chemicals in varying concentrations. It is also important to note that the present study showed qualitative and quantitative variability of the majority chemical compositions with other studies. Florentine et al. (2016) from Cameroon obtained p-cymene (45.90%), thymol (23.72%) cis-sabinene hydrate (5.9%), and linalool (3.90%) while Sidali et al. (2014) in Algeria showed Carvacrol (55.1%),  $\gamma$ -terpinene (12.6%), p-cymene (9.2%), and linalool (3.8%). Also, the results obtained from the present study are similar to those obtained by

Tchoumbounang et al. (2009) which showed thymol (40.1%),  $\gamma$ -terpinene (15.1%) p-cymene (23.4%) from *Thymus vulgaris* from other parts of Cameroon. Similarly, El Ouali Lalami et al. (2013) reported, thymol (41.39%),  $\gamma$ -terpinene (22.25%) p-cymene (15.59%)  $\alpha$ -terpinene (3.25%) from *Thymus vulgaris* and *Thymus vulgaris* in Morocco. David (2019) showed Thymol (55-36%, p-cymene (28-15%),  $\gamma$ -terpinene (10-5%), linalool (6.5-4%) in a study carried out in France. However, it is important to note that variability in chemical content of essential oils may exist due to numerous ecological factors such as temperature, relative humidity, insolation and the nature of the soil can influence the chemical composition of essential oils (Oliveira et al. 2005).

The ferric reducing and anti-radical activities of the essential oils of both plant species used in the present study were significantly lower than that of BHT which is a reference anti-oxidant substance. It is established in numerous works that the activity of an essential oil is related to the majority compounds and the possible synergistic effects between the constituents (Oussou et al., 2010, Kalemba, Kunicka, 2003). In general, essential oils rich in oxygenated compounds present a more marked anti-radical activity than those with hydrocarbon terpenes (Kunicka, 2003). The manifestation of these activities is very consistent with the above hypothesis. In addition, compounds such as thymol are known for their strong antioxidant property. These compounds, thanks to their redox properties, act as reducing agents, donors of hydrogen and singular oxygen (Tepe et al., 2005). According to studies by Zeghad and Merghem (2013), Benabed et al. (2017), the essential oil of *T. vulgaris* has high antioxidant activities due, in part, to the presence of several compounds, such as thymol, thymol-methyl-ether, linalool and carvacrol in its Chemical compositions. The essential oils from these two Cameroonian plants therefore show strong anti-radical activity thanks to compounds such as thymol. However, this is only observed in certain studies because of qualitative and quantitative variability of those majority compounds

## Conclusion

This work was carried out with the aim of evaluating the yield rate, quantity and quality of chemical compounds of essential oils of plants of the Lamiaceae or Labiaceae family of Cameroon namely *O. gratissimum* and

*T. vulgaris*. At the end of the study, it can be concluded that the essential oils were obtained with yields of (1.6%) and (1.3%) respectively for the leaves of *O. gratissimum* and *T. vulgaris*. In terms of chemical composition, both leaf oils showed quantitative variability. In fact, Compounds identified from *T. vulgaris* included Thymol (39.2%), p- Cymene (25.36%),  $\lambda$ - Terpinene (16.2%) Lenalool (3.32%),  $\alpha$ - Thujene (1.7%) and Camphene (1.3%) while those identified from *O. gratissimum* were p- Cymene (29.6%),  $\lambda$ - Terpinene (21.9%),  $\alpha$ -Thujene (6.2%), Thymol (5.1%), Myrcene (3.9%) and 1.8- Cineol (2.9%). The anti-radical activities of the leaves of essences were highlighted with IC50 values of 146.33  $\mu$ g/ml; 121.61  $\mu$ g/ml and 69.99  $\mu$ g/ml respectively for *O. gratissimum*, *T. vulgaris*. and BHT. These results give the essential oils an anti-radical power that can be used to preserve food against oxidation. Concerning the concentration necessary to have a 50% trapping effect, a significant difference ( $p < 0.05$ ) in the increase in average trapping percentages (%IC) of the DPPH radical as a function of BHT concentrations was also noted. The extracts reveal activities close to the reducing power and anti-radical power compared to those of the reference molecules studied BHT. Thus, this opens a way for possible valorization due to the side effects and non-adaptation of BHT, with the aim of making a real contribution to the problems of production and conservation by chemical products such as BHT which poses a lot of problems for human and animal health and environmental degradation.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

Design: SMAA, MM, PMJ; Investigation: SMAA; Data analysis: TBMM. Writing of the original version of the article: SMAA and ED.

## REFERENCES

Abdelli M. 2017. Caractérisation Chimique de quelque Activités Biologique des Huiles Essentielles de *Juniperus phoenicea* et de *Thymus vulgaris*. These de doctorat 3ieme cycle LMD Microbiologie

- appliquée. Université Abdelhamid Ibn Badis Mostaganem, p.104.
- Adams RP. 2007. *Identification of Essential Oil Components by Gas Chromatography Mass Spectrometry*. Allured Publishing Corporation: Illinois, p. 495.
- Agban A, Gbogbo KA, Hoekou YP, Atchou K, Tchacondo T, Batawila K, Souza C, Gbeassor M. 2013. Evaluation of Antifungal Activity of *Cassia alata* L. *Piliostigma thonningii* (Schumach.) Milne Redh extracts. (Fabaceae) on *Candida albicans*. *Int. J. Biol. Chem. Sci.*, **7**(3): 1041-104, DOI: <http://dx.doi.org/10.4314/ijbcs.v7i3.12>.
- Ahmia S, Fethallah F. 2020. Inventaire de quelques Lamiacées et Caractérisation de l'Huile Essentielle de Thym (*Thymus vulgaris*) dans différentes régions. Mémoire de Master. Université de Blida1, Algérie, p. 20-22.
- Alexandre P, Ronoel LO. 2008. Chemical composition of *Thymus vulgaris* L. (thyme) Essential Oil from the Rio de Janeiro state. *Journal of the Serbian Chemical Society*, **73**(3): 307-310. URL: <https://doaj.org/article/86dec9fed4664b50ab2070e55e51f0c6&ved>
- Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. 2004. Free Radical Scavenging Capacity and Antioxidant Activity of selected plant species from the Canadian prairies. *Food Chemistry*, **84**: 551–562. URL: <https://www.sciencedirect.com/science/article/pii/S0308814603002784&ved>
- Aprotosoia AC, Şpac AF, Miron A, Floria V, Dorneanu V. 2010. The Chemical Profile of Essential Oils Obtained from Fennel Fruits (*Foeniculum Vulgare* MILL.). *Farmacia*, **58**(1): 46-53. URL: <https://www.scirp.org/reference/referenc espapers%3Freferenceid%3D2354224&ved>
- Bassène E. 2012. *Initiation à la Recherche sur les Substances Naturelles Extraction Analyse Essais Biologiques*. Presse Universitaire de Dakar: Dakar, p. 94-96, 140. URL: <https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url>
- Benabed KH, Gourine N, Ouinten M, Bombarda I, Yousfi M. 2017. Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oils of Three Algerian Lamiaceae Species. *Current Nutrition and Food Science, Bentham Science Publishers*, **13**(2): 97–109. URL: <https://hal.science/hal-01777681/document&ved>
- Brand Williams W, Cuvelier ME, Berset C. 1995. Use of Free Radical method to evaluate Antioxydant Activity. *Lebensm-wiss. U. Technol.*, **28**: 25-30. URL: <https://www.sciencedirect.com/science/article/pii/S0023643895800085&ved>.
- David M. 2019. Thymol sources, properties and applications. Doctoral thesis, Pharmacological faculty, University of Limoges, France, p. 19-26.
- El ouali lalami A, El-AKhal F, Ouedrhir W, Ouazzani Chahdi F, Guemmouh R, Greche H. 2013. Chemical Composition and Antibacterial activity of Essential Oils of two Aromatic Plants from central northern Morocco: *Thymus vulgaris* and *Thymus satureioidis*. *Laboratory Technologies*, **8**:31. URL: <https://revues.imist.ma/index.php/technol ab/article/download/724/598/2595&ved>
- Florentine MC, Ndoye F, Maximilienne A, Sylvain L.S, Riwom, HS, François Xavier Etoa. 2016. Chemical Composition, Antioxidant Effects and Antimicrobial Activities of some spices Essential Oils on food Pathogenic Bacteria. *African Journal of Biotechnology*, **15**(16): 649-656. DOI: <https://doi.org/10.5897/AJB2015.15070>
- Iserin P. 2001. *Encyclopédie des Plantes Médicinales* (2ème Edn). Larousse: Londres ; 143 et 225-226.
- Jazet DPM, Kuate J, Ngoouana V, Damesse F, Tchinda E Amvam zollo PH, Menut C. 2008. Analyse Comparative des Propriétés Antiradicalaires et Anti-inflammatoires des Huiles Essentielles de *Citrus reticulata* var. Madagascar et *Citrus sinensis* var. Casagrande du Cameroun, **63**(4): 201-208. URL: <https://academicjournals.org/journal/AJB/article-references/81971A658082&ved>.
- Kalemba D, Kunicka A. 2003. Antibacterial and Antifungal properties of Essential Oils. *Curr. Med. Chem.*, **10**: 813-829. DOI: <http://dx.doi.org/10.2174/0929867033457719>.

- Kitajima J, Ishikawa T, Urabe A, Satoh M. 2004. Monoterpenoids and their Glycosides from the leaf of Thyme. *Phytochemistry*, **65**: 3279-3287. DOI: <https://doi.org/10.1016/j.phytochem.2004.09.010>.
- Kpokame M. 2019. Comparative study of the effect of the essential oil of *Ocimum gratissimum* seeds and Amitraz on the *Rhipicephalus microplus* tick. BSc Thesis, Option: Animal Production and Health, Department of Animal Production and Health, Ecole Polytechnique d'Abomey-Calavi, (Abomey-Calavi, Benin), p. 42.
- Lawrence BM. 2000. Progress in Essential Oils- Ravensara oil. *Perfumer and Flavorist*, **25**(5): 68-71. URL: [https://img.perfumerflavorist.com/files/base/allured/all/document/2016/02/pf.PF\\_26\\_06\\_044\\_13.pdf&ved](https://img.perfumerflavorist.com/files/base/allured/all/document/2016/02/pf.PF_26_06_044_13.pdf&ved).
- Lourens ACU, Riddy D, Baser KHC, Viljoen AM, Van Vuuren SF. 2004. *In vitro* Biological Activity and Essential Oil Composition of four indigenous South African *Helichrysum* species. *Journal of Ethnopharmacology*, **95**(2-3): 253-258. DOI: 10.1016/j.jep.2004.07.027
- Manampisoa ND. 2021. *Ocimum gratissimum* essential oils: Extraction and formulation of a balm. Master's Thesis in Inorganic Chemistry - Process Engineering, Faculty of Sciences, University of Antananarivo, (Antananarivo, Madagascar), p. 46.
- Mau JL, Huang PN, Huang SJCC. 2004. Antioxidant Properties of Methanolic Extracts from two kinds of *Antrodia camphorata* mycelia. *Food Chemistry*, **86**: 25-31. DOI: <https://doi.org/10.1016/j.foodchem.2003.08.025>.
- Oliveira MJ, Iani FPC, Oliveira CBA, Santos MR, Souza PS, Santos SC, Seraphin JC, Ferri PH. 2005. Influence of Growth Phase on the Essential Oil composition of *Hyptis suaveolens*. *Biochemical Systematics and Ecology*, **33**: 275-285. DOI:10.1016/j.bse.2004.10.001.
- Oussou KR, Youlou S, Kanko C, Tue BiB, Kanko C, Boti JB, Ahibo C, Casanova J. 2010. Etude Chimique Bio-Guidée de L'huile Essentielle de *Ocimum gratissimum* (Lamiaceae). *European Journal of Scientific Research*, **1**: 50-59. URL: <https://hal.science/hal-00593500>
- Özcan M, Chalcha JC. 2004. Aroma profile of *Thymus vulgaris* L growing wild in Turkey. *Journal of Agricultural & Food Chemistry*, **49**: 4619-4626. URL: <https://www.scirp.org/journal/paperinformation?paperid=94896>
- Pirbalouti AG, Hashemi M, Ghahfarokhi FT. 2013. Essential Oil and Chemical Compositions of Wild and Cultivated *Thymus daenensis* Celak and *Thymus vulgaris* L. *Industrial Crops and Products* **48**: 43-48. DOI: 10.1016/j.indcrop.2013.04.004.
- Tchoumboungang F, Dongmo PMJ, Sameza ML, Mbanjo EGN, Fotso GBT, Zollo PHA, Menut C. 2009. Larvicidal activity on *Anopheles gambiae* Gil and Chemical Composition of Essential Oils extracted from four plants cultivated in Cameroon. *BAZE*, **13**(1): 77-84. URL: <https://www.researchgate.net/deref/http%3A%2F%2Fwww.pressesagro.be%2Fbase%2Ftext%2Fv13n1%2F77.pdf>.
- Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. 2005. Antimicrobial and Antioxidant Activities of the Essential Oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chemistry*, **90**(3): 333-340. DOI: <https://doi.org/10.1016/j.foodchem.2003.09.013>
- Satyel P, Murray BL, McFeeters RL, Setzer WN. 2016. Essential Oil Characterization of *Thymus vulgaris* from Various Geographical Locations. *Foods*, **5**(4): 70. DOI: 10.3390/foods5040070
- Vekiari SA, Protopapadakis EE, Papadopoulou P, Papanicolaou D, Panou C, Vamvakias M. 2002. Composition and Seasonal Variation of the Essential Oil from leaves and peel of a Lemon variety. *Journal of Agricultural and Food Chemistry*, **5**(1): 147-153. DOI: 10.1021/jf001369a
- Zeghad N, Merghem R. 2013. Antioxidant and Antibacterial Activities of *Thymus vulgaris* L. *J. Biol.-Plant*, **58**: 27-36. URL: <http://www.sciencewebpublishing.net/maprj/archive/2013/September/pdf/Nadia%2520and%2520Rachid.pdf&ved>.