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# Chemo-type of essential oil of *Ocimum basilicum* L. from DR Congo and relative *in vitro* antioxidant potential to the polarity of crude extracts

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ABSTRACT

**Objective:** To carry out a phyto-chemical characterization of essential oil from *Ocimum basilicum* L. (*O. basilicum*) harvested in DR Congo and to assess the antioxidant potential of crude extracts with respect to the polarity for comparison reason.

**Methods:** The phyto-chemical characterization of essential oil produced by hydro-distillation was performed by coupled gas chromatography-mass spectrometer analysis and the antioxidant potential evaluation by *in vitro* 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity method.

**Results:** A previously weighed amount of fresh leaves of *O. basilicum* produced 0.65% of essential oil that led to the identification of a set of 84.44% out of 99.98% as major compounds (> 1.5%). The chemo-type of this essential oil was linalool-methyl chavicol. Chemical components of oil were characterized by oxygenated aromatic hydrocarbons (46.00%) and oxygenated monoterpenes (26.75%). With respect to the amount of components, methyl chavicol also known as estragole (35.72%) constituted the very large quantity afterward linalool (21.25%) and then epi-α-cadinol (8.02%), α-bergamotene (6.56%), eugenol (4.60%), 1,8-cineole (4.04%), germacrene D (2.06%), thymol (1.64%), and (E)-citral (1.55%), respectively. Essential oil exhibited antioxidant potential and $IC_{50}$ = (1.180 ± 0.015) mg/mL. Non-polar crude extracts yields were low compared to the one of polar extracts. Only methanol and ethyl acetate had considerably manifested antioxidant potential with $IC_{50}$ values equal to (0.025 ± 0.013) mg/mL and (0.085 ± 0.012) mg/mL, respectively. As concerns to $IC_{50}$ values, essential oil was less active than methanol and ethyl acetate extracts. The methanol crude extract exhibited the highest activity. Non-polar extracts showed insignificant radical scavenging ability that did not allow assessing $IC_{50}$ values. These results highlighted the occurrence of antioxidant potential compounds in polar media.

**Conclusions:** Essential oil and crude extracts of *O. basilicum* growing in DR Congo can be advocated as natural sources of antioxidant potential compounds not only in food but also in pharmaceutical industries. The high antioxidant potential of polar crude extracts highlights antioxidant character of its composition particularly butyl stearate and rosmarinic acid we isolated and identified, respectively in the methanol crude extract.

## 1. Introduction

Last decades researches revealed that oxidation processes in the living systems are mainly due to highly reactive and potentially tissues damaging transient chemical. The famous species produced *in vivo* are superoxide anion, hydroxyl radical and hydrogen peroxide [1–3]. The reported oxidation mechanisms showed that tissues damage is due to an imbalance between oxidant species

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and scavenging systems. Deficiency of such balance is involved in many disorders, including degenerative disorders such as Alzheimer's disease, cancer, atherosclerosis, diabetes mellitus, hypertension, AIDS and aging [2,3]. Consumption of antioxidants has been suggested as one of the ways to shift the balance towards an adequate redox status [4]. Many years ago, synthetic antioxidants were widely used in foods and pharmaceutical industries; however synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (BHT) have been restricted because they were suspected to possess carcinogenic effects [4,5]. Therefore, the search for new natural antioxidant sources grows and is considered to be more suitable to inhibit oxidation in living systems. Plant materials especially attracted researchers' attention in finding natural antioxidants [5–7]. Nowadays antioxidant phytochemicals, notably flavonoids and other polyphenol compounds from plants have been reported to inhibit the propagation of oxidant compounds like free radical and to protect the living system from disease caused by the oxidation process [8–12]. As a result extraction, characterization and the use of natural antioxidants from plants have been known to be a great progress [10–12]. Various aromatic plants' parts and volatile oil of the Lamiaceae family have been reported to be rich in polyphenolic compounds with other secondary metabolites and many of them are known for antioxidant and other medicinal properties [13–15]. Lately, our research team carried out researches of medicinal plants for the management of sickle cell disease and more than 115 plants have been tested [16–22]. Indeed previously we have shown that *Ocimum basilicum* L. (*O. basilicum*), one of member of the Lamiaceae family used as food seasoning and Congolese herbal medicine [16,23], exhibited *in vitro* antisickling activity. This biological activity was mainly displayed by polar fractions. Anthocyanins, organic acids and derivatives were reported to be among the active chemical groups [24–27]. Moreover, polar fractions (methanol and ethyl acetate) of *Ocimum canum* Sims. (*O. canum*) exhibited high antioxidant activity and antisickling activity [28,29]. Relatively, polar fractions of *O. basilicum* and *Ocimum gratissimum* L. displayed the same characteristics [23,29]. Preliminary chemical screening on the crude extract revealed the presence of polyphenols (flavonoids, anthocyanidins, leucoanthocyanins, tannins, quinones), alkaloids, saponins, triterpenoids and steroids, and fractionation of the acidified methanol crude extract of *O. basilicum* led to the identification and characterization of antisickling compounds as rosmarinic acid and butyl acetate active, respectively [23]. Besides, other researches also showed that *O. basilicum* contains high concentrations of phenolic compounds associated to the herb's potent antioxidant capacity [30,31]. However, content of essential oil of *O. basilicum* from DR Congo and the effect of extraction solvents polarity on the antioxidant potential of crude extracts have not been reported. This work analyzes chemical composition and evaluates the antioxidant activity of essential oil of *O. basilicum* growing in DR Congo and that of crude with respect to the polarity.

## 2. Materials and methods

### 2.1. Plant material

Aerial parts, especially leaves of *O. basilicum* were harvested in the vicinity quarters of the University of Kinshasa, Kinshasa, DR Congo in June 2011 for crude extracts and in May 2013 for essential oil. Leaves were authenticated and voucher specimens (425/Devred) were deposited at the herbarium of the Faculty of Science, University of Kinshasa. Leaves of plants for extraction were dried at the room temperature and finely grounded in a high speed mill (Retsch ZM 100 Model) to 0.02 inches size [23]. The powders were stored in the dark at the room temperature and used for solvent extraction. Other parts of fresh leaves were used for essential oil distillation.

### 2.2. Essential oil distillation

Essential oil has been produced by hydro-distillation as earlier reported [29]. A weighed amount of leaves was immersed in a 500 mL round bottom flask of water and hydro-distilled. Water and essence were recovered in a decant bowl, and anhydrous magnesium sulfate was used for drying trace of water. Oil was stored in a dark glass bottle at 4 °C before gas chromatography (GC) and gas chromatography-mass spectrometer (GC–MS) analyses described below. The process was repeated for many times in order to increase the yield.

### 2.3. GC analysis

GC analysis of the essential oil was performed in a Hewlett-Packard (series HP 5880A) chromatograph equipped of a flame ionization detector and a non-polar capillary column OV-1 (30 m × 0.25 mm × 0.25 μm). Helium with a flow rate of 1 mL/min was used as vector gas. The split mode ratio of 1/50 was the injection mode and the injected volume was 1 μL. The column was initially kept at a temperature of 40 °C during the first 5 min, programmed at a range of 40–200 °C owing to 4 °C/min, and at 200–300 °C owing to 10 °C/min. Injector and detector temperatures were kept at 250 and 280 °C, respectively. An internal gauging solution composed of a mixture of *n*-alkanes (C9–C20) was injected under the same conditions for calculating of retention indices of different peaks of the chromatograms.

### 2.4. Coupled GC–MS analysis

Coupled GC–MS analysis was carried out on a Varian CP 3800 chromatograph equipped with a non-polar capillary in melted silica OV-1ms (30 m × 0.25 mm × 0.25 μm) coupled to Kratos MS 80 RFA mass spectrometer (MS) equipped with a DS90 data system. The spectra were recorded between 50 and 240 uma. Fragmentation was carried out by electron impact under a field of 70 eV. The temperature of the transfer line was 295 °C. Chromatographic analysis conditions were the same as described. The identification of most of the components of the essential oil was done by comparison of their mass spectra with spectra stored in Wiley Registry 10th libraries data base [32] and by comparison of retention indices to those of literature [33].

### 2.5. Preparation of plant extracts

Plant extracts were produced by increasing gradually the polarity of extraction solvents as previously reported [29]. Sequence of liquid–liquid extractions using *n*-hexane, dichloromethane, and ethyl acetate and methanol were performed. About 100 g of plant powder was macerated sequentially in *n*-hexane, dichloromethane, ethyl acetate and methanol separately for 48 h.

The final solutions were filtered and concentrated under reduced pressure using a rotary evaporator. The different extracts were submitted to antioxidant test.

### 2.6. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

DPPH free radical scavenging assay was carried out as reported [29]. About 3.5 mL of 0.3 mmol/L solution of DPPH radical in methanol were added to either 0.5 mL solution of essential oil or to 0.5 mL of crude extracts solutions. Bioactive essential oil and extracts solutions were used at the same values of concentration in methanol for comparison. Each mixture was submitted to spectrophotometry (UV–vis 320/Safas Monaco Spectrophotometer) analysis. Mixture of essential oil or crude extracts solution with DPPH radical solution in methanol were shaken vigorously and absorbances were recorded at 517 nm during 35 min as equilibrium time. DPPH radical scavenging has been determined by percentage of reduction that provides $IC_{50}$ (concentration of essential oil or extract or ascorbic acid that reduces 50% of DPPH radical concentration) by extrapolation as antioxidant effectiveness [29]. Ascorbic acid was tested as standard for comparisons. Percentages of reduction were calculated according to the following equation:

$$I\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where $A_{blank}$ is absorbance of blank and $A_{sample}$ is absorbance of the tested sample.

## 3. Results

### 3.1. Essential oil yield

Hydro-distillation of 100 g of fresh leaves produced a total of 0.648 g (0.65%) of oil, relatively to the initial amount of fresh herbs used.

### 3.2. Chemical profile of essential oil

Chemical profile of the hydro-distillated oil and analyzed by GC and GC–MS are shown in Table 1. Compounds are listed according to the elution order from the column.

### 3.3. Crude extracts yields

Yields in percentage (%) of polar and non-polar extracts from dried and powdered herbs of *O. basilicum* extracts after evaporation were 11.500%, 11.500%, 1.797% and 2.171% for methanol, ethyl acetate, dichloromethane and *n*-hexane, respectively.

### 3.4. DPPH radical reduction by essential oil and crude extracts

Percentages of reduction of the DPPH radical *vs*. concentration by essential oil are shown in Figure 1. The ascorbic acid has been plotted as standard. In order to compare the potential antioxidant of oil and that of extracts, the percentages of reduction of the DPPH radical *vs*. concentration by polar and non-polar extracts were also plotted together with the percentages of reduction of both oil and ascorbic acid. Figure 2 shows the plot of percentages of reduction of the DPPH free radical *vs*. concentration values of essential oil, various extracts (methanol and ethyl acetate) and ascorbic acid.

**Table 1**
Structural assignment of the essential oil components.

| Compounds | $RI^a$ | $RI^b$ | % | Identification |
|---|---|---|---|---|
| α-Thujene | 921 | 928 | 0.02 | MS, RI |
| α-Pinene | 931 | 932 | 0.13 | MS, RI |
| Camphene | 942 | 946 | 0.04 | MS, RI |
| Oct-1-en-3-ol | 965 | 964 | 0.04 | MS, RI |
| Sabinene | 966 | 968 | 0.03 | MS, RI |
| β-Pinene | 970 | 972 | 0.20 | MS, RI |
| Myrcene | 982 | 984 | 0.03 | MS, RI |
| *p*-Cymene | 1013 | 1015 | 0.02 | MS, RI |
| 1,8-Cineole | 1020 | 1023 | 4.04 | MS, RI |
| *trans*-Ocimene | 1035 | 1038 | 0.16 | MS, RI |
| γ-Terpinene | 1048 | 1050 | 0.02 | MS, RI |
| Hydrate de cis-sabinene | 1083 | 1054 | 0.02 | MS, RI |
| Unidentified ($C_{10}H_{12}O$) | – | – | 0.01 | MS: *m/z*: 44, 59, 65, 91 (100%), 118, 120, 131 |
| Linalool | 1084 | 1085 | 21.25 | MS, RI |
| Camphre | 1126 | 1125 | 0.23 | MS, RI |
| δ-Terpineol | 1143 | 1148 | 0.02 | MS, RI |
| Terpineol-4 | 1161 | 1164 | 0.02 | MS, RI |
| Estragole | 1173 | 1178 | 35.72 | MS, RI |
| Nerol | 1213 | 1216 | 1.25 | MS, RI |
| (Z)-Citral | 1217 | 1220 | 1.40 | MS, RI |
| Geraniol | 1235 | 1237 | 1.01 | MS, RI |
| (E)-Citral | 1242 | 1247 | 1.55 | MS, RI |
| Thymol | 1275 | 1272 | 1.64 | MS, RI |
| Eugenol | 1335 | 1340 | 4.60 | MS, RI |
| Unidentified ($C_{12}H_{18}O$) | – | – | 0.23 | MS: *m/z*: 41, 48, 56, 66, 79, 121, 137, 146, 164, 178 (100%), 187, 195 |
| α-Copaene | 1372 | 1374 | 0.53 | MS, RI |
| *cis*-α-Bergamotene | 1408 | 1410 | 6.56 | MS, RI |
| *cis*-Caryophyllene | 1415 | 1419 | 0.61 | MS, RI |
| α-Humulene | 1451 | 1450 | 0.85 | MS, RI |
| Unidentified: ($C_{15}H_{24}O$) | – | – | 0.15 | MS: *m/z*: 53, 80, 81, 91, 105, 117, 119, 131, 137, 147, 161 (100%) |
| Alloaromadendrene | 1452 | 1459 | 1.30 | MS, RI |
| Unidentified ($C_{15}H_{24}O$) | – | – | 0.65 | MS: *m/z*: 41, 53, 66, 69, 79, 81, 91 (100%), 93, 107, 119, 121, 133, 147, 161, 175, 204 |
| Unidentified ($C_{15}H_{24}O$) | – | – | 0.84 | MS: *m/z*: 41, 55, 67, 79, 81, 91 (100%), 93, 105, 107, 108, 119, 121, 135, 139, 147, 148, 161, 167, 189, 204 |
| Germacrene D | 1471 | 1476 | 2.06 | MS, RI |
| δ-Cadinene | 1515 | 1514 | 1.23 | MS, RI |
| (E)-α-Bisabolene | 1532 | 1534 | 1.27 | MS, RI |
| 1,10-di-epi-Cubenol | 1612 | 1606 | 0.95 | MS, RI |
| epi-α-Cadinol | 1622 | 1626 | 8.02 | MS, RI |
| β-Eudesmol | 1630 | 1631 | 0.72 | MS, RI |
| α-Eudesmol | 1637 | 1641 | 0.52 | MS, RI |

$RI^a$: Retention indices measured on non-polar column (OV-1); $RI^b$: Retention indices from literature [34,35]. –: There is no $RI^b$ to be compared to $RI^a$.

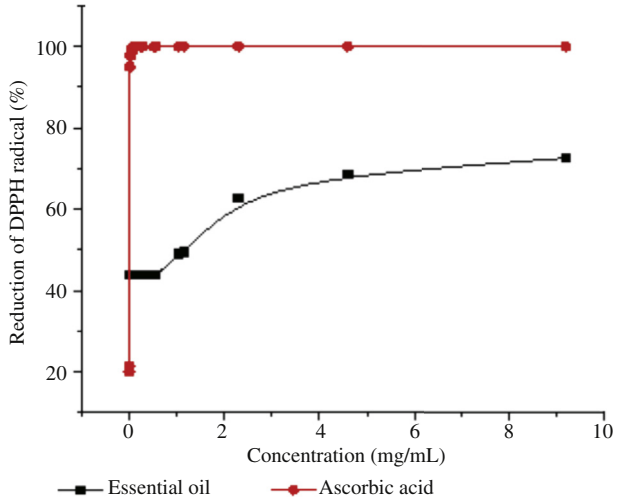


**Figure 1.** Reduction of DPPH radical by essential oil and ascorbic acid.

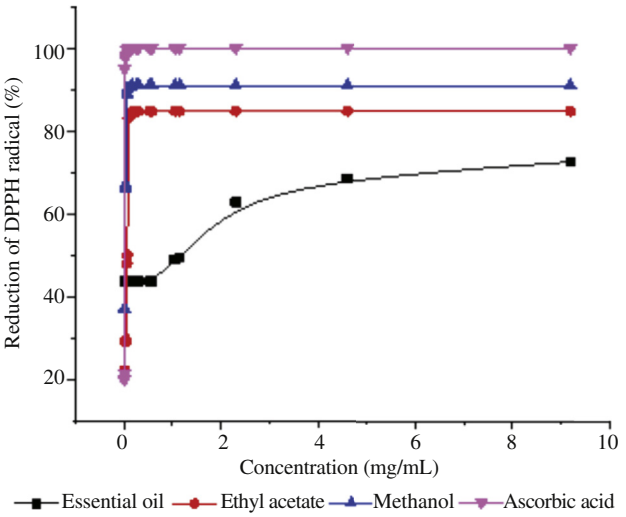


**Figure 2.** Reduction of DPPH free radical by essential oil, polar extracts and ascorbic acid.

### 3.5. Antioxidant activity

$IC_{50}$, the concentration of oil, extracts and ascorbic acid that reduces 50% of DPPH radical concentration and $1/IC_{50}$ were evaluated and are shown in Table 2. Low $IC_{50}$ value indicates high antioxidant activity.

**Table 2**
Antioxidant activities of essential oil, ascorbic acid and polar crude extracts.

| Groups | | $IC_{50}$ (mg/mL) | Antioxidant activity ($1/IC_{50}$) |
|---|---|---|---|
| Control | Ascorbic acid | 0.022 ± 0.011 | 45.455 |
| Extracts | Methanol | 0.025 ± 0.013 | 40.000 |
| | Ethyl acetate | 0.085 ± 0.012 | 11.765 |
| | Dichloromethane | – | – |
| | *n*-Hexane | – | – |
| Essential oil | | 1.180 ± 0.015 | 0.847 |

–: Insignificant activities.

## 4. Discussion

### 4.1. Essential oil yield

The yield of this oil is low regarding to earlier works on extraction of essential oil of *O. basilicum* [36,37], however it is high in the study of Zheljazkov *et al.* [38]. This can be explained by difference of the natural environment for each of the studied *O. basilicum* to grow and the management of the sample.

### 4.2. Chemical profile of essential oil

A total of 40 compounds (99.98%) were identified by GC, and only 35 compounds (98.10%) were characterized by coupled GC–MS (MS and RI), and five compounds (1.88%) were unidentified. For those unidentified compounds only fragmentations (MS) are reported. Chemical composition of this essential oil is mainly dominated by terpenes and hydrocarbon compounds. The overall composition (99.98%) can be described as follow: non-oxygenated monoterpenes (0.67%), oxygenated monoterpenes (26.75%), non-oxygenated sesquiterpenes (14.41%), oxygenated sesquiterpenes (10.21%), oxygenated hydrocarbons (0.04%), aromatic hydrocarbons (0.02%), and oxygenated aromatic hydrocarbons (46.00%). The remaining compounds (1.88%) were unidentified among spectra stored in Wiley Registry 10th libraries. Major compounds (> 1.5%) of this chemical composition are methyl chavicol also known as estragole (35.72%), linalool (21.25%), epi-α-cadinol (8.02%), α-bergamotene (6.56%), eugenol (4.60%), 1,8-cineole (4.04%), germacrene D (2.06%), thymol (1.64%), and (E)-citral (1.55%). They represent 84.44% of the overall composition of oil; while minor compounds (0.5%–1.5%) are (Z)-citral (1.40%), alloaromadendrene (1.30%), (E)-α-bisabolene (1.27%), δ-cadinene (1.23%), nerol (1.25%), geraniol (1.01%), 1,10-di-epi-cubenol (0.95%), α-humulene (0.85%), β-eudesmol (0.72%), *cis*-caryophyllene (0.61%), α-copaene (0.53%), α-eudesmol (0.52%), camphre (0.23%), β-pinene (0.20%), *trans*-ocimene (0.16%), α-pinene (0.13%), camphene (0.04%), oct-1-en-3-ol (0.04%), sabinene (0.03%), myrcene (0.03%), α-thujene (0.02%), *p*-cymene (0.02%), γ-terpinene (0.02%), *cis*-sabinene hydrated (0.02%), δ-terpineol (0.02%), terpineol-4 (0.02%).

This chemical composition reveals that essential oil of *O. basilicum* growing in DR Congo is rich in oxygenated aromatic hydrocarbons (46.00%) and oxygenated monoterpenes (26.75%). The occurrence of methyl chavicol (35.72%) and linalool (21.25%) as most abundant compounds indicates that the chemo-type of this essential oil is linalool-methyl chavicol. The oxygenated and non-oxygenated sesquiterpenes represent 14.41% and 10.21%, respectively. Only 0.67% of non-oxygenated monoterpene was identified. The unidentified compounds and aromatic hydrocarbons represent 1.88% and 0.04%, respectively. Similar studies on the analysis of the chemical composition of essential oil from *O. basilicum* growing in others countries have reported the occurrence of either some compounds we found in *O. basilicum* from DR Congo or with notably differences [39,40]. Many reasons can explain chemical plants contents differences as well as yields of extraction [36,37]. Indeed weather conditions of vegetative stage, harvesting methods, drying and storage conditions of plant material, the part of the plant, the process of distillation, storage conditions of the samples before experiences, *etc.* can influence the chemical contents and yield of plant material

[41,42]. It is also known that composition of essential oil is not reproduced similarly in all plants of the same species. Climate, histological and ecologic parameters are also involved in the plant life. Therefore the same vegetal species can show two or more chemical races called chemo-types [41]. The chavicol-linalool chemo-type that we have found in this oil from *O. basilicum* species was reported for the first time on the chemical composition of the essential oils of *O. basilicum* from Brazil [38,39].

### 4.3. Crude extracts yields

Extraction yields reveal that non-polar crude extracts are lower than polar ones. Recently we extracted crude extracts from *O. canum* from DR Congo with the same extraction's solvents and reported the same tendency *i.e.* high extraction yields for polar crude extracts [29].

### 4.4. DPPH radical reduction by essential oil and crude extracts

Essential oil exhibited antioxidant potential in presence of DPPH radical, however ascorbic acid showed high activity. Ascorbic acid reduced DPPH radical around 100% in low range of concentrations; meanwhile, essential oil active in the same range concentrations did not reduce more than 80% of DPPH radical. Other tests of DPPH scavenging activity carried out in which BHT has been used as standard essential oil was less active than BHT [43,44].

Qualitatively the ascorbic acid as well as methanol and ethyl acetate extracts showed a high DPPH radical reduction. DPPH radical reduction by ascorbic acid, methanol and ethyl acetate extract occurred mainly in weak ranges of concentrations. Two other extracts namely dichloromethane and *n*-hexane have been tested, however the percentages of reduction of DPPH free radical are under 50% and are not plotted. Regarding to the two polar fractions, these results are similar to our antioxidant activity study of *O. canum* from DR Congo [29]. Indeed, the methanol crude extract was more active than ethyl acetate extract. In order to know quantitatively the effectiveness of reduction of each extract or essential oil and that of standard, $IC_{50}$ values are determined, *i.e.* the concentration of extract or oil that reduces 50% of the initial amount of DPPH radical. Antioxidant potential was evaluated as reverse of $IC_{50}$.

### 4.5. Antioxidant activity

Comparison of the two polar crude extracts indicates a weak value of $IC_{50}$ = (0.025 ± 0.013) mg/mL for methanol crude extract, follow by the $IC_{50}$ = (0.085 ± 0.012) mg/mL for ethyl acetate. However, ascorbic acid has the weakest $IC_{50}$ of (0.022 ± 0.011) mg/mL. The essential oil provided the highest value of $IC_{50}$ = (1.180 ± 0.015) mg/mL. This comparison provides more understanding about the antioxidant potential of our samples. Indeed, all samples exhibited high potential. Methanol extract exhibited the highest antioxidant activity, followed by ethyl acetate and then essential oil. Dichloromethane and *n*-hexane extracts showed insignificant activities. Their $IC_{50}$ could not be reached because of the weakest values of DPPH free radical percentages of reduction under 50%. Our previous evaluation of antioxidant potential of *O. canum* crude extracts from DR Congo reported that only hexane extract reduced DPPH free radical under 50% [29]. Hence, the polar extracts exhibited stronger antioxidant activity than the non-polar. Indeed this can be explained by the evidence that compounds known for their radical scavenging activity like polyphenols are soluble in polar solvents [16]. Recently, from the methanol extract of *O. basilicum*, we isolated butyl stearate and identified rosmarinic acid [23,24]. The essential oil from *O. basilicum* exhibited a good antioxidant activity but less active than extracts.

This work aimed to evaluate the chemical composition, antioxidant activity of essential oil and that crude extracts from fresh and dried leaves of *O. basilicum*, respectively. The GC and GC–MS analyses of the essential oil of *O. basilicum* showed that terpenes and hydrocarbon compounds are the main compounds. The *in vitro* DPPH free radical scavenging activity assessment showed that essential oil, methanol and ethyl acetate extracts exhibited high antioxidant activity. Methanol extract is the highest active and then ethyl acetate extract. The highest antioxidant activity of the methanol extract indicates the occurrence of active antioxidant compounds, for instance butyl stearate and rosmarinic acid we previously isolated and identified, respectively in methanol extract. This pharmacological property of *O. basilicum* from DR Congo represents a rational reason to motivate the use of this species by the traditional healers. This species can be recommended as natural sources of active antioxidant candidate compounds for food and pharmaceutical industries. Isolation of active compounds from polar crude extracts is going on.

## Conflict of interest statement

We declare that we have no conflict of interest.

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