

## Chemical characterization and antioxidant effect of *Ocimum basilicum* L. essential oil grown in Comoros

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**Abstract:** Nowadays, many researches are interested in finding natural and antiradical substances because, in addition to their nutritional values, they could also help to balance the anti-protection systems of antioxidant and to protect against oxidative stress-related diseases, particular cardiovascular and neurodegenerative diseases, diabetes and cancer. Thus, the use of medicinal and aromatic plants for their therapeutic effects, prompted us to perform investigations on the antiradical activity of the essential oil of *Ocimum basilicum*, species used, in particularly, folk medicine in Comoros. The antioxidant activity was demonstrated by the spectrophotometric method of DPPH (2,2-diphenyl-1-picrylhydrazyl). The result showed that the essential oil of *Ocimum basilicum* has an anti-radical effect compared to BHT (Butylhydroxytoluene) as antioxidant reference. Concentration value of IC<sub>50</sub> is 7.75 µg/mL. The major component of the essential oil of *Ocimum basilicum* was methyl chavicol (estragole) with a rate of 80.04%.

### 1. Introduction

The Reactive Oxygen Species (ROS) are molecules produced by the body during normal metabolic processes of the cells. According to the study of some authors [1-4], environmental exposure factors (smoking, pollution, radiation, ion, alcohol, synthetic pesticides ...) increases its production. This overproduction beyond the antioxidant capacity of biological systems leads to oxidative stress that involves the development of several diseases, inflammatory [5, 6], auto-immune diseases [7], neurodegenerative [3], cardiovascular [8] or cancer [9] and diabetes [10]. To avoid these serious dangers of oxidative stress, antioxidant use is necessary. Oxidative stress refers to a disturbance in the cellular metabolic balance in which the generation of oxidants overwhelms led the antioxidant defense system. Antioxidants are widely used as food additives to maintain food quality and protect the phenomena of oxidation. Spices could be used to enhance the flavor of foods and they are known for their antioxidant power [11]. In this context, medicinal and aromatic plants with their essential oils have particularly been the subject of several studies. The use of natural substances such as essential oils with antioxidant activity is very beneficial and effective. The *Ocimum basilicum* is a plant which widely and

heavily used today in traditional medicine around the world. In this study, we are interested to evaluate the ability of essential oil to inhibit free radicals compared to butyl hydroxytoluene (BHT) as antioxidant reference. The antioxidant activity of essential oils of *Ocimum basilicum* from Comoros was assessed using the test of DPPH (2,2-diphenyl-1-picrylhydrazyl). From the values of the percentage of absorbance (optical density) obtained inhibition depends on the concentration of essential oil ([HE]), we determined graphically the concentration corresponding to 50% inhibition ( $EC_{50}$ ) for this species. The value of the antioxidant activity for this essential oil studied has determined with the concentration value corresponding to 50% inhibiting ( $EC_{50}$ ) of DPPH with an antioxidant standard, BHT ( $AA\% = f([EH])$ ).

## 2. Materials and Methods

### 2.1. Plant material

The aerial parts (stems and leaves) of *Ocimum basilicum* L. were collected in March (2008) in Moroni and Dzahani-Tsidé Great Comoro. *Ocimum basilicum* L. identified by Professor Jean-Noel Labat from National Museum of Natural History (Paris, France) and specimens were deposited in the herbarium of Botanic Department at the Faculty of Sciences and Technology, University of Comoros. The taxonomic identification of plant materials was confirmed by A. Aafi, botanist at the Forest Research Centre in Rabat, Morocco.

### 2.2. Distillation of essential oils

The isolation of essential oils from plant materials was achieved by hydrodistillation that was carried out using a Clevenger-type distillation system [12]. In every test, 100g of dries leaves and stems have been treated. The oils obtained were separated from water by decantation, dry over anhydrous sodium sulphate (20% of the total mass essential oil), filtered and stored at 4°C in the dark until testing and analysed by gaz chromatography and gaz chromatography coupled with mass spectrometry.

### 2.3. Chromatographic analysis

The different samples of essential oils isolated from *Ocimum basilicum* were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (FID), equipped with a HP-5 capillary column (30m x 0.25mm x 0.25µm). The temperature was programmed from 50°C after 5 min initial hold to 250°C at 4°C/min. Gas chromatography conditions were as follows: N<sub>2</sub> as carrier gas (1.8 mL/min); split mode was used (Flow: 72.1 mL/min, ratio: 1/50); temperature of injector and detector was 275°C. The machine was led by a computer system type "HP ChemStation", managing the functioning of the machine and allowing to follow the evolution of chromatographic analyses. Diluted samples (1/50 in hexane) of 1.2 µL were

injected manually. GC/MS analyses were performed on a Hewlett-Packard equipped with a HP-5MS (Crosslinked 5% PHME Siloxane) capillary column (30m x 0.25mm i.d, 0.25µm film thickness) and coupled with a mass spectrometer (HP 5973). The temperature was hold from 50 to 250°C at 2°C/min. The carrier gas was He (1.5 mL/min) and used split mode (Flow: 112 mL/min, ratio: 1/74.7). The different compounds were confirmed by reference to their MS identities (Library of NIST98 Spectra). MS operating parameters were: ionization voltages 70eV, ion source temperature 230°C, scan mass range 35-450 nm. Oils constituents were identified by their retention indices relatives to n-alkanes (C<sub>8</sub>-C<sub>24</sub>) and by comparison of their mass spectral fragmentation patterns with those reported in literature [13].

#### 2.4. Antioxidant activity

To evaluate the antioxidant activity, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) spectrophotometric assay was used [14, 15]. The experiment was carried out in a UV/visible spectrophotometer (Littrow 1200 lignes/mm per stage) of type M550 Camspec, controlled by a computing system with a bidirectional numerical interface (RS232C) and a screen of posting LCD backlit (1/4 VGA 320 x240 pixels) to the wavelength 514 nm. We prepared the solution of DPPH by the dissolution of 4 mg of the powder in 100 mL of ethanol (EtOH). The samples of essential oils were prepared in EtOH at a rate of 40µg/2mL. These solutions were diluted to obtain the following concentrations: 1.25, 2.5, 5, 10, 20 and 40 µg/mL and the test was carried out by mixing 4 mL of DPPH solution with 1 ml of essential oils to be tested with various concentrations. The reference antioxidant or positive control (BHT) was also prepared according to the same method. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 514 nm. Inhibition free radical DPPH in percent (I %) was calculated as follows [15, 16]:

$$AA \% = 100 \times (A_{\text{blank}} - A_{\text{test}}) / A_{\text{blank}}$$

A<sub>blank</sub>: Absorbance of blank (containing all the reagents without the product to be tested)

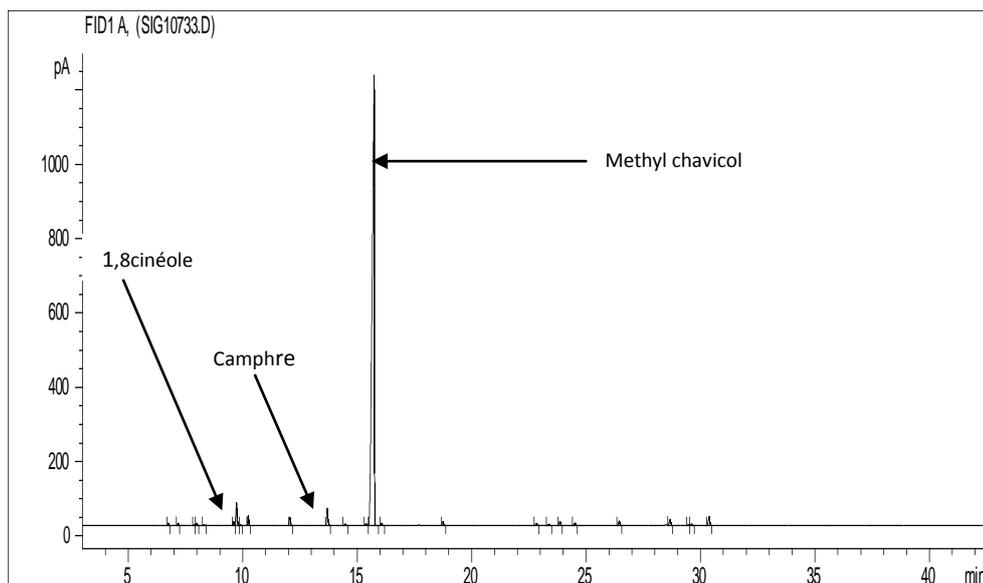
A<sub>test</sub>: Absorbance of test

The graph of the percentages of inhibition according to the concentration of essential oil makes it possible to determine the IC<sub>50</sub> and the value obtained is compared with that found for the compound of reference (BHT).

### 3. Results and discussion

#### 3.1. Chemical composition

The essential oil of *Ocimum basilicum* was analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID) and GC-Mass Spectrometry (GC-MS) (Fig.1).



**Figure 1:** Chromatographic profile of essential oil of *Ocimum basilicum*.

Analytic conditions:

Apparus: Hewlett-Packard (Series HP 6890)

Column: HP-5 (5% phenyl methyle siloxane: 30m x 0,25mm x 0,25 $\mu$ m); Detector: FID 250°C; Injector: 250°C; Gaz vector: N<sub>2</sub>; T: 50 to 200°C grounds 4°/min by 5min.

The chromatographic profile of the *Ocimum basilicum* essential oil gives twenty five constituents representing approximately 99.97%.

**Table 1:** Essential oil of *Ocimum basilicum* from Moroni and Dzahani-Ntsidjé Comoros.

N°	IR	Constituents	OB (%)
1	939	$\alpha$ -pinene	0,23
2	944	Camphene	0,21
3	969	1-octen-1-ol	0,08
4	973	$\beta$ -pinene	0,17
5	984	2-octanone	0,14
6	1028	Limonene	0,39
7	1033	1,8-cineole	2,30
8	1030	Z- $\beta$ -ocymene	0,13
9	1050	E- $\beta$ -ocymene	1,00
10	1095	Linalol	0,89

11	1141	Camphor	2,00
12	1163	Trans- $\beta$ -terpinol	0,26
13	1189	$\alpha$ -terpinol	0,25
14	1199	estragole (methyl chavicol)	87,04
15	1208	p-cymen-7-ol	0,27
16	1382	$\alpha$ -terpin-7-al	0,51
17	1396	$\beta$ -longipinene	0,27
18	1414	cis- $\alpha$ -bergamotene	0,20
19	1430	2,5-diméthoxy-para-cymene	0,52
20	1452	$\alpha$ -humulene	0,35
21	1513	$\Gamma$ -cadinene	0,55
22	1582	oxyde de caryophyllene	0,76
23	1606	epoxyde II d'humulene	0,15
24	1611	E-acetate d'isoeugenol	0,19
25	1636	epi- $\alpha$ -cadinol	1,11
	Total		99,97

N°: Number of constituent, IR: Retention Indices.

For the essential oil of Comorian *Ocimum basilicum*, the main compound is methyl chavicol (87.04%). Other components such as low levels of 1,8-cineole (2.30%), camphor (2.00%),  $\alpha$ -epi-cadinal (1.10%) and E- $\beta$ -ocymene (1.00 %) exist in this species. Other compounds have concentrations below 1% (Table 1). Many investigations have been carried out on the species basilicum in various countries found and the French basil oil is composed by methyl chavicol (87.3%), linalool (5.4%),  $\beta$ -caryophyllene (2.4%), methyl eugenol (1.5 %),  $\alpha$ -pinene (1.0%), the  $\beta$ -pinene (0.8%), limonene (0.5%) and camphene (0.2%) [17-21].

It appears that although the content of the lead compound, methyl chavicol (Figure 1) the Comorian essential oil is approximately the same as that of French origin, but it is different for the other minority components (1,8-cineole, Camphor, epi-cadinal and E- $\beta$ -ocymene). Concentrations of linalool,  $\alpha$ -pinene, limonene and camphene are significantly lower for the sample studied compared to basil from France. Qualitatively, the essential oil of *Ocimum basilicum* study is similar to that from Lome, Togo studied by Kofi et al (2009) [22] and that the contents of two main constituents are methyl chavicol or estragole (85.50%) and 1,8 cineole (2.25%). The high content of methyl chavicol the essence of *Ocimum basilicum* has been cited by several authors [23]. However, it differs from that of Comorian origin studied by Céline

(2007) [24], methyl chavicol and methyl eugenol is present equally. In this case a methyl eugenol/methyl chavicol chemotype [25-27]. Oil *Ocimum basilicum* from Comoros is considered one methyl chavicol chemotype. For oil from *Ocimum basilicum* Turkey, only 88.1% of oil could be identified as major compounds with methyl eugenol (78.02%) in addition to the  $\alpha$ -cubebene (6.17 %). Moreover, Keita and colleagues (2000) [20] reported that oil *O. basilicum* native of Guinea as major compounds: linalool (69%), eugenol (10%), (E)- $\alpha$ - $\beta$  bergamotene (3%) and thymol (2%). Thus, investigations of Akgül (1989) [17] on the essence of this species from Turkey showed that linalool (45.7%), eugenol (13.4%), methyl eugenol (9.57 %) and alcohol fenchyl (3.64%) were the main constituents. According to several authors [24, 29], the work carried out on samples of *Ocimum basilicum* oil from Nigeria and Benin have shown that these species are linalool chemotype-estragole. This type of basil essential oil contains as main components, estragole (22.17%) and (E)- $\alpha$ -bergamotene (7.56%), linalool (4, 21%). Of these three compounds, estragole only exists in the essential oil from Comoros with a very high concentration (87.04%). Several chemotypes of essential oils such as types exist:

- Methyl eugenol chemotypes (78.02%) (sample of Turkey) with  $\alpha$ -cubebene (6.17%).
- Methyl eugenol chemotype / t-anethole (sample from Adeticopé Togo) which major compounds are: methyl eugenol (41.10%) and t-anethole (32.56%).
- t-anethole chemotype (sample from Bassar Togo), with other major components t-anethole (74.64%), linalool (17.30%) and carvacrol (2.69%).
- Chemotype estragole or methyl chavicol from Lome in Togo: estragole (85.5%) and 1,8 cineole (2.25%). This sample basil Lomé region is similar to the same species from Comoros.

Marotti et al. (1996) [29] reported the presence of linalool, methyl eugenol and chavicol as main components for certain essential oils *O. basilicum* provenance in Italy. In another study, the main compounds were linalool and methyl chavicol [30]. The chemical composition of basil oil has been the subject of several studies [23, 28, 31]. This research indicates that the chemotypes estragole and linalool/estragole are the most widely encountered. There is a wide variety of components in the essential oils of basil and several chemotypes have been established by various investigations of phytochemicals Methyl chavicol, linalool, methyl cinnamate, methyl eugenol, eugenol and geraniol are reported as major components of oils from different chemotypes of *Ocimum basilicum*. These chemical races are generally known by names based on geographical origins such as the Egyptian type, European type and sweet basil are considered as the aroma of high quality, containing linalool and methyl chavicol as dominant components [32]. Egyptian basil is very similar to that of Europe, but it contains a high percent of methyl chavicol as the type of chemical Reunion, Comoros, Madagascar, Thailand and Vietnam [29]. However, the basil high in methyl cinnamate was produced in Bulgaria [32], India, Guatemala, and Pakistan [29]. As for basilisks Java [31], Russia and North Africa, they are dominated by eugenol [29]. As a result, the basil of Comoros is methyl chavicol or estragole chemotype which major compound (exotic type). These results are corroborated by those published in the literature [29, 33].

### 3.2. Antioxidant activity

The EC<sub>50</sub> value of the essential oil of *Ocimum basilicum* in Comoros is 7.75 µg /mL (Fig. 2).

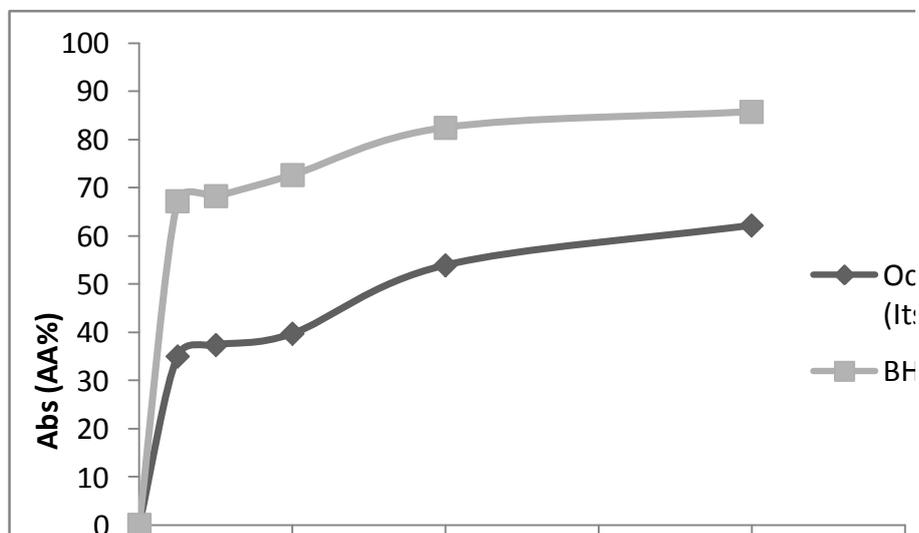


Figure 2: Antioxidant effect of *Ocimum basilicum* essential oil.

The essential oil of *Ocimum basilicum* was reported cytotoxic to human cancer cells [34]. It also has anti-tumor activity in mice [35]. With the IC<sub>50</sub> value of 7.75 mg/mL, the antioxidant effect of this species of *Ocimum basilicum* can be explained by its high methyl chavicol (87.04%). Compounds such as eugenol and methylchavicol or derivatives are provided antioxidant power [36]. Indeed, antioxidant and antitumor of basil oil has been attributed to phenolic compounds [33, 36, 37]. In addition, the inhibitory effect of this oil could be explained by the antiradical compounds such as terpene alcohols (terpinol, linalol etc.), but also to monoterpenes (limonene, pinene and cymene) and sesquiterpenes: oxyde de caryophyllene, [38-42]. These compounds may play an important role in inhibiting and neutralizing free radicals and singlet oxygen triplets or decomposing peroxides [43]. However, according to several studies, the minor compounds may also have an inhibitory radical effect and therefore the factor of synergy between the different compounds can exist [41, 44]. We note that studies by Taie Aly et al. (2010) [45] showed that with IC<sub>50</sub> = 0.0616 ppm and 0.0642 ppm IC<sub>50</sub> = effective inhibitors had a dead human cells respectively 81.05% and 77.89% for samples extracted essential oils of the same species from Egypt. Similarly, Manosroi and colleagues (2006) [33] found that the oil of basil leaves had an inhibitory effect on cancer cells P388 from a concentration of 0.0362 ppm. Our results are confirmed with these investigations on the antioxidant properties of the essential oil of *Ocimum basilicum* growing in Comoros.

## Conclusion

The essential oil of *Ocimum basilicum* from Comorian species used in folk medicine is very rich in methyl chavicol or estragol (87.04%). In comparison with an antioxidant of reference (BHT), essential oil of *Ocimum basilicum* studied shows an antioxidant power for free radicals. This oil can be used as resource of natural antioxidant. This characteristic deserves further investigations for the use of this oil for therapeutics, cosmetics and alimentary industries. Therefore, the valorization of aromatic and medicinal plants could provide an added economic value for Comoros development.

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