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# **CHEMICAL PROFILE AND ANTIOXIDANT PROPERTIES OF THE ESSENTIAL OIL OF**  *XYLOPIA AETHIOPICA* **(DUNAL) A. RICH. FROM CÔTE D'IVOIRE**

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### **ABSTRACT**

Essential oils generally possess significant antioxidant activities and can successfully replace synthetic antioxidants, which often present harmful side effects, in the fight against oxidative stress. In this study, we performed the chemical analysis and evaluated the antioxidant activity of the essential oils from the seeds of *Xylopia aethiopica*. The extraction of the oils was carried out by steam distillation using a Clevenger-type apparatus and analyzed by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy (proton and carbon 13). The antioxidant activity of the oils was evaluated by two different assays (the DPPH radical and ABTS<sup>\*+</sup> tests) and compared in each test to Trolox (a reference antioxidant). The analysis results show that the essential oils of dried fruits of *Xylopia aethiopica* are mainly composed of β-pinene (21.3%), α-pinene (9.3%), 1,8-cineole (13.1%), and trans-pinocarveol (7.7%). The oils exhibited negligible antioxidant activity in the DPPH radical test (IC<sub>50</sub> = 182.36 mg/mL) and moderate activity in the ABTS<sup>\*+</sup> test (8.89 mg/mL).

**KEYWORDS**: *Xylopia aethiopica*, Essential oil, Antioxidant, Antioxidant activity, IC<sub>50</sub>.

### **INTRODUCTION**

The human body is the site of numerous chemical reactions that use oxygen (Abdou, 2009). During these reactions, reactive oxygen species (ROS) are produced, such as hydrogen peroxide  $(H_2O_2)$ , singlet oxygen  $(^1O_2)$ , and hydroxyl radical (<sup>\*</sup>OH). Under normal conditions, these species are in equilibrium with the body's antioxidants, protecting it against bacteria and viruses (Benhammou, 2011). Any imbalance in this system leads to severe consequences for vital molecules in the body (Touré, 2015; Choho, 2018). Unfortunately, the level of these radicals easily exceeds that of antioxidants due to their diverse production sources: ingestion of processed foods, exposure to radiation, cigarette smoke, pollution, etc. (Abdou, 2009). To protect the body from the dangers associated with this imbalance, an external supply of antioxidants is necessary. For this purpose, humans must consume antioxidant-rich foods, such as spices, as well as synthetic antioxidants like 2,6-di-ter-butylhydroxytoluene (BHT) and 2-ter-butyl-p-methoxyanisole (BHA) (Oussou *et al.,* 2020). Among these two groups of antioxidants, studies have shown that, unlike natural antioxidants, synthetic antioxidants often pose risks to the body. In fact, they are thought to trigger the overproduction of toxic or carcinogenic microsomal enzymes in hepatocytes (Oussou *et al.,* 2009; Oussou *et al.,* 2020). For this reason, research has intensified in the field of essential oil (EO) antioxidants. It is in this context that our study is situated, with the general objective of evaluating the antioxidant power of the essential oils from *Xylopia aethiopica* seeds. Specifically, the goal is to extract the EO from *Xylopia aethiopica* seeds, characterize them, and assess their antioxidant potential.

### **MATERIALS AND METHODS**

## **1. Materials**

## **1.1 Plant Material**

The plant material used in this study consisted of 2.85 kilograms of dried *Xylopia aethiopica* seeds. These seeds were purchased from the Adjamé market (Abidjan, Côte

d'Ivoire) and were dried on a workbench for three (3) days prior to the extraction process.

#### **1.2 Technical Equipment**

The essential oil extraction and analysis were carried out using several pieces of equipment: a Clevenger hydrodistiller, a hot plate, the Pioner PA202C balance with a capacity of 2100 g and a precision of 0.01 g, a Delsi DI 200 gas chromatograph equipped with a flame ionization detector, gas chromatography coupled with mass spectrometry (GC-MS), a Nuclear Magnetic Resonance (NMR) device, and the HACH DR 2400 spectrophotometer, along with standard laboratory glassware.

#### **1.3 Reagents and Solvents**

The solvents and reagents used included: pentane, distilled water, methanol, DPPH (2,2-diphenyl-1 picrylhydrazyl), and ABTS (2,2'-azino-bis(3 ethylbenzothiazoline-6-sulfonic acid)).

#### **2. METHODS**

#### **2.1 Extraction of Essential Oil from** *Xylopia aethiopica*

The essential oils (EOs) from *Xylopia aethiopica* seeds were extracted by steam distillation using a Clevengertype apparatus. Steam distillation is one of the three methods for distilling plant essences. The process involves placing the plant material (2.85 kg of *Xylopia aethiopica* seeds) on a perforated grid or plate positioned at an appropriate distance above the bottom of a still containing water, which is heated. The steam produced passes through the plant material, carrying its volatile compounds into the vertical tube and then into the cooling coil where condensation occurs. The condensed mixture in the coil is collected in an Erlenmeyer flask. Since the essential oil of *Xylopia aethiopica* has a lower density than water, it floats and is collected through simple filtration. Distillation was performed twice using 3 liters and 1 liter of water, respectively, for a duration of 3 hours for each run. The EO collected at the end of the extraction process was stored at 3°C in a freezer to prevent possible degradation.

### **2.2. Essential Oil Extraction Yield Calculation Method**

The essential oil yield is the ratio of the mass of essential oil obtained  $(m<sub>oe</sub>)$  to the mass of plant material subjected to extraction  $(m_0)$ . It is expressed as a percentage and calculated using the formula in Equation 1.

$$
Rd = \frac{M_{0\ell}}{M_0} X 100
$$
 (Equation 1)

#### **2.3. Characterization Methods**

The extracted essential oil was characterized in Corsica (France) using the following techniques: proton and carbon-13 nuclear magnetic resonance  $(^{1}H$  and  $^{13}C$ NMR), gas chromatography (GC), and gas chromatography-mass spectrometry (GC-MS). NMR spectra were recorded using a Bruker instrument (Bruker

BioSpin AG), equipped with a 5 to 10 mm probe, operating at 400.132 MHz for proton and 100.623 MHz for carbon-13. Chemical shifts  $(\delta$  in ppm) were compared to tetramethylsilane (TMS) used as an internal reference. The carbon-13 spectra were recorded under the following conditions: 5 mm probe, 45° pulse angle, 2.73 s acquisition time corresponding to a 64 K acquisition, with a spectral width (SW) of 25,000 Hz (250 ppm), and a digital resolution of 0.183 Hz/pt. The number of accumulations ranged from 2000 to 5000 for each recording. Decoupling was performed using the "Composite Phase Decoupling" pulsed field.

### **2.3. Antioxidant Activity Evaluation Methods of the Extracted Essential Oil**

To assess the antioxidant activity of the essential oil, two chemical tests were performed.

- The scavenging effect of an antioxidant on the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>\*</sup>),
- The free radical "2,2'-azino-bis(3ethylbenzothiazoline-6-sulfonic acid)"  $(ABTS^{\bullet+})$ test.

### **2.3.1 DPPH● Free Radical Test**

This method is based on evaluating the ability of antioxidants to neutralize the DPPH● radical. The impact of each extract on this radical is measured according to the procedure described by Seung-cheol et al. (2004). First, a 0.4 mM methanolic solution of DPPH is prepared, with an optical density (OD) measured at 1.683. Using a 200 µL micropipette, a series of five 2 fold dilutions is made from 200 µL of essential oil (EO). Then, 3 mL of DPPH $^{\bullet}$  reagent is mixed with 100 µL of solution from each tube containing the dilutions. These tubes are incubated at 30°C in the dark for 30 minutes. For another calibration range, Trolox, a reference antioxidant (6-hydroxy-2,5,7,8-tetramethylchroman-2 carboxylic acid), is used instead of EO. Methanol serves as a negative control for the preparation of dilutions. The absorbance or optical density of the tested essential oil extracts and Trolox is measured by spectrophotometry. To evaluate antioxidant efficiency, the inhibition percentages are calculated using Equation (2):

$$
I(\%) = \frac{D_{control} - D_{sampling}}{D_{control}} \times 10
$$
 (Equation 2)  
Where:

DO<sub>control</sub>: represents the optical density of the methanolic DPPH<sup>•</sup> solution;

DOsample : the optical density of the tested sample.

Furthermore, the 50% inhibitory concentration (IC50) is determined. This value represents the concentration of the sample required to reduce the DPPH<sup>•</sup> radical by 50%. IC50 values are established by linear regressions of graphs plotting inhibition percentages against the concentrations of the tested extracts. It should be noted that a lower IC50 indicates better antioxidant activity.

## **2.3.2 ABTS●+ Test**

The inhibition percentage of the  $ABTS^{\bullet+}$  radical is evaluated using the method described by Ramful et al. (2010). To produce the ABTS $^{\bullet+}$  radical, a 7 mM ABTS solution is mixed with a 2.6 mM potassium persulfate solution. This mixture is then kept in the dark at room temperature for four hours. After this period, 1 mL of the resulting solution is diluted with 60 mL of methanol to obtain a solution with an optical density (OD) measured at 1.587. For the test, a series of five dilutions is prepared from 200 µL of the essential oil (EO) in pure methanol. Then, 3 mL of  $ABTS^{\bullet+}$  reagent is added to 100 µL of solution from each tube. The tubes are incubated in the dark for 30 minutes to allow the reaction. Trolox is used as a positive control, while methanol serves as a negative control for the preparation of the different EO dilutions. As with the DPPH<sup>•</sup> test, the absorbance or optical density of the tested essential oil extracts and Trolox is measured by spectrophotometry. The results from this

test are also expressed as inhibition percentages, and the 50% inhibitory concentration (IC50) values are calculated to assess the antioxidant activity of the tested samples (Equation 2).

### **RESULTS AND DISCUSSION**

## **1. Results**

### **1.1. Yield and Physicochemical Characteristics of the Essential Oil**

### **1.1.1. Crude Extract Yield**

The volume of essential oil obtained after extraction was 42 mL, corresponding to a mass  $m_{oe} = 42g$ . The yield, calculated using Equation 1, was 1.5%.

#### **1.1.2. Crude Extract Yield**

The chemical composition analysis of the essential oil from *Xylopia aethiopica* fruits identified thirty-five (35) compounds. The identified constituents are listed in Table 1.

**Table 1: Chemical Composition of the Essential Oil of** *Xylopia aethiopica* **Fruits.**

$\overline{\mathbf{N}^{\circ}}$	Composés identifiés	<b>IKA</b>	<b>IKP</b>	<b>Teneurs</b>
1	$\alpha$ -Thujene	921	1021	1.1
$\overline{2}$	$\overline{a}$ -pinene	930	1019	9.3
$\overline{3}$	Camphene	942	1062	0.3
$\overline{4}$	Verbenene	945	1121	0.8
$\overline{5}$	Sabinene	964	1117	$\overline{2}$
$\overline{6}$	$\overline{\beta}$ -pinene	971	1108	$\overline{21.8}$
$\overline{7}$	Myrcene	979	1159	0.6
$\overline{8}$	$c$ arene-3	1005	1147	0.5
9	$\alpha$ -Terpinene	1008	1174	0.6
10	p-cymene	1011	1265	1.1
11	Limonene	1020	1195	0.9
12	<b>Cineole</b>	1020	1207	13.1
13	$\gamma$ -terpinene	1047	1239	$\mathbf{1}$
14	trans-hydrate de Sabinene	1051	1455	0.7
15	Terpinolene	1078	1280	0.3
16	Linalol	1081	1538	0.4
17	cis-hydrate de sabinene	1081	1540	0.8
18	$\beta$ -thujone	1090	1428	0.1
19	$\alpha$ -compholenal	1104	1174	0.5
20	Nopinone	1106	1574	2.7
21	trans-pinocarveol	1124	1647	7.7
22	trans-verbenol	1127	1669	$\overline{2}$
23	Pinocarvone	1138	1563	3.4
24	Pinocompohone	1150	1544	0.4
$\overline{25}$	Terpineol-4	1161	1594	2.7
26	iso pinocampheol	1161	1718	0.5
27	Myrtenal	1170	1623	4.8
28	$\alpha$ -terpineol	1171	1687	1.3
29	Myrtenol	1179	1783	4.8
30	neoiso Menthol	1179	1628	0.7
$\overline{31}$	$\gamma$ -terpineol	1180	1702	1.3
$\overline{32}$	$\beta$ -campholenol	1186	1782	0.5
$\overline{33}$	trans-carveol	1196	1826	0.6
34	Nérol	1207	1790	$\overline{0.3}$
$\overline{35}$	bicyclogermacrene	1494	1727	0.55
	Total content			



With **IKA**: Kovats Index on Apolar Column and **IKP**: Kovats Index on Polar Column

The above Table 1, presenting the various compounds of the extracted essential oil, indicates that.

- Only compounds with a content greater than or equal to 0.1% were identified:
- There are two classes of compounds: monoterpenes and sesquiterpenes.

The compounds highlighted in bold represent the major constituents identified, with concentrations close to or exceeding ten percent. The contents of the major oxygenated compounds are lower compared to those of hydrocarbon compounds. The proportions of the main identified constituent classes are presented in Figure 1 below.



**Figure 1: Proportion of Identified Compounds in the Essential Oil of** *Xylopia aethiopica.*

The above figure indicates that the essential oil extracted from the dried fruits of *Xylopia aethiopica* is more concentrated in monoterpenes (73.4%) compared to sesquiterpenes (16.75%).

### **1.2. In vitro Antioxidant Activity of the Extracts 1.2.1. DPPH● Radical Test**

The absorbance values and inhibition percentages obtained are recorded in Table 2.





The different DO and inhibition percentage values (I%) in Table 2 show a decrease in the concentration of the DPPH<sup>•</sup> radical depending on the concentrations of the extracts. This decrease is more significant with the Trolox extracts compared to the essential oil.

The calibration curves for DPPH<sup>•</sup> radical as a function of the different concentrations of essential oil fractions and Trolox tested are presented in Figures 2 and 3.



**Figure 2: Calibration Curve of DPPH● Radical with Essential Oil of** *X. aethiopica.*





**Figure 3: Calibration Curve of DPPH● Radical by Trolox Extracts.**

This figure demonstrates the linear relationship between the concentration of Trolox and its ability to inhibit the DPPH<sup>•</sup> radical. The curve shows a stronger antioxidant activity in comparison to *Xylopia aethiopica*, as reflected in the lower  $IC_{50}$  value of Trolox (0.20 mg/mL).





The IC<sup>50</sup> values in Table 3 clearly indicate that *Xylopia aethiopica* essential oil has a lower antioxidant activity compared to Trolox.

## **1.2.2. ABTS●+ Radical Test Table 4: Absorbance Measurements of ABTS●+ in Essential Oil Extracts and Trolox.**



Similar to the DPPH<sup>•</sup> test, the percentage of inhibition of the ABTS<sup>\*+</sup> radical increases with the concentration of essential oil extracts and Trolox. The inhibition percentages in this test are higher compared to those in the DPPH● test, both for the essential oil and Trolox at equivalent concentrations.

The calibration curves of the  $ABTS^{\bullet+}$  radical for the essential oil extracts and Trolox are presented in Figures 4 and 5 below.



**Figure 4: Calibration Curve of ABTS●+ Radical by Essential Oil Extract of** *Xylopia aethiopica.*



**Figure 5: Calibration Curve of ABTS●+ Radical by Trolox Extract.**

This figure illustrates the calibration curve showing the inhibition percentage of the ABTS●+ radical by different concentrations of Trolox. As the concentration of Trolox increases, the inhibition percentage rises, demonstrating its potent antioxidant activity across various concentrations.

**Table 5: IC<sup>50</sup> Values of Essential Oil Extracts and Trolox in the ABTS●+ Test.**

<b>Extract</b>	$CI_{50}$ (mg/mL)
Xylopia aethiopica	8 89
Trolox	O 17

The  $IC_{50}$  values presented in Table 5 indicate that Trolox exhibits significantly greater antioxidant activity than the essential oil extracted from the dried fruits of *Xylopia aethiopica*. These values suggest that both the essential oil extracts and Trolox demonstrate enhanced antioxidant activity against the ABTS●+ radical.

#### **2. DISCUSSION**

The essential oil yield from the dried fruits of *Xylopia aethiopica* in this study is 1.5%. This yield is higher than that reported by Oussou (2009), which is 1.2%, but lower than the 3.5% obtained by Agbodan *et al.* (2014). These differences could be attributed to factors such as temperature, extraction methods, and the quality of the fruits subjected to extraction (Oussou, 2009). In this study, thirty-five (35) compounds, representing 90.15% of the constituents, were identified. These compounds are categorized into two main classes: monoterpenes, which include 40.3% hydrocarbons and 33.1% oxygenated compounds, and sesquiterpenes, consisting of 16.2% oxygenated and 0.55% hydrocarbon compounds. The predominance of these two main classes of compounds (monoterpenes and sesquiterpenes) supports the high quality of our essential oils. Indeed, monoterpenes and sesquiterpenes constitute the primary class of essential oil constituents (Franchomme & Pénoël, 1990). The predominance of our essential oil in monoterpenes (73.4%) aligns with the results of Noudjou *et al.* (1999) from Cameroon, where their samples of essential oil from *Xylopia aethiopica* fruits were primarily composed of monoterpenes (60%). However, the percentage of oxygenated monoterpenes they found

(12.9%) is significantly lower than our 33.1%. A comparison of the major compounds in our essential oil with those in the literature reveals that β-pinene (21.8%), the major constituent in our sample, is also prevalent in many prior studies. Kéïta *et al.* (2003) in Mali reported β-pinene (19.1%), γ-pinene (14.7%), trans-pinocarveol (8.6%), and p-cymene (7.3%). Yéhouénou et al. (2010) identified β-pinene (38.9%), valerianol (7.7%), myrtenal (7.4%), and elemol (5.1%) as the major compounds in their sample. In Cameroon, Noudjou *et al.* (2007) identified sixty-three (63) compounds in the essential oil of the species harvested from four different areas, comprising 47 to 84% hydrocarbon monoterpenes, predominantly β-pinene (44.11%) and β-phellandrene (13.89%). They reported for the first time a diterpene (ent-13-epimanoyl oxide) in the essential oil from the fruits of *X. aethiopica*. A unique feature of our essential oil is the presence of myrtenol and myrtenal, while βphellandrene and germacrene D are absent. Large-scale exploitation of the essential oil from this plant should consider the collection site, as the chemical composition of the oil can vary significantly by region. Indeed, Noudjou *et al.* (2007) highlighted this in Cameroon in their study on the chemical composition of *X. aethiopica* fruits from various localities (Baffoussam, Douala). They showed that the predominant β-pinene in samples from Douala (39.39%), Ngaoundéré (38.17%), and Yaoundé (44.11%) constitutes only 8.22% in the Baffoussam sample, which is rich in β-phellandrene and 1,8-cineole (31.42%).

The decrease in optical density of the  $ABTS^{\bullet+}$  and DPPH<sup>•</sup> radicals in the essential oil extracts in this study highlights the antioxidant capacity of the essential oil from *Xylopia aethiopica*. This antioxidant activity increases with the concentration of the tested oil extract, which is observed in all antioxidant substances, whether synthetic or natural (Oussou, 2009; Benhammou, 2011; Touré, 2015). Trolox, the reference antioxidant in this study, exhibited significantly greater antioxidant activity than the essential oil extract in both tests. Its radicalscavenging capacity was more pronounced in the ABTS<sup>\*+</sup> test (IC<sub>50</sub> = 0.17 mg/mL), where 85% of its concentration reduced fifty percent of the DPPH<sup>•</sup> radical. Similarly, the essential oil extract displayed a higher

antioxidant capacity in the ABTS<sup>\*+</sup> test (IC<sub>50</sub> = 8.89 mg/mL), where approximately 5% of its concentration reduced fifty percent of the DPPH $^{\bullet}$  radical. The IC<sub>50</sub> values of the essential oil extract compared to those of Trolox indicate a lower antioxidant activity for our essential oil. In the case of the  $ABTS^{\bullet+}$  test, only the equivalent of 1.9% of the essential oil's  $IC_{50}$  in Trolox reduced 50% of the ABTS<sup>\*+</sup> radical. For the DPPH<sup>\*</sup> test, less than 1% of the essential oil's  $IC_{50}$  in Trolox inhibited 50% of the DPPH<sup>•</sup> radical. The difference observed in  $IC_{50}$  values across the two different tests can be explained by the fact that the antioxidant activity of a substance depends on the method employed to assess its antioxidant capacity (Touré, 2015). The low antioxidant activity of the essential oil highlighted by our study, which has also been reported by Agbodan *et al.* (2014) with an  $IC_{50} = 9752$  ppm, or  $IC_{50} = 9.752$  mg/mL, could be attributed to the low proportions of oxygenated constituents in the oil. In fact, the antioxidant effectiveness of essential oils is thought to depend on their richness in oxygenated compounds, particularly phenolic compounds (Oussou *et al.,* 2009). The important role of oxygenated compounds in the antioxidant activity of essential oils is underscored by Benhammou (2011), who states that to accurately determine the true antioxidant capacity of a substance, one should measure the  $EC_{50}$  parameter, a direct quantitative measure of antioxidant activity. It is defined as the concentration of the crude extract of phenolic compounds required to reduce 50% of the initial concentration of the radical.

### **CONCLUSION**

To achieve the objectives set forth in this study, materials were selected based on the methods employed. The extraction of essential oils from the dried fruits of the studied plant was conducted using steam distillation with a Clevenger apparatus, yielding 1.5%. The chemical composition analysis of the essential oils was carried out using gas chromatography, coupled with GC-MS and NMR  $(^1H$  and  $^{13}C)$ . This analysis revealed thirty-five (35) compounds, representing 90.15% of the essential oils. The major compounds identified are β-pinene (21.8%),  $\alpha$ -pinene (9.3%), cineole (13.1%), and transpinocarveol (7.7%). The antioxidant tests conducted on the DPPH<sup>•</sup> and ABTS<sup>•+</sup> radicals demonstrated a doseresponse effect of our essential oil against these radicals. The inhibitory effect of the essential oils is negligible against the DPPH● radical but moderate against the  $ABTS^{\bullet+}$  radical. Therefore, the results of this study warrant further refinement, and it would be interesting to continue this research with in vivo applications on the toxicity of these essential oils. Additionally, it will be necessary to complement the antioxidant activity with other tests to highlight the molecules responsible for these effects. Lastly, investigating other biological activities such as antimicrobial and anticancer activities of these essential oils would also be of great interest.

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