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جامعة زيان عاشور- الجلفة
Faculty of Natural and Life Sciences
كلية علوم الطبيعة والحياة
Department of Agronomic and Veterinary Sciences
قسم العلوم الفلاحية والبيطرية

End of Studies Project

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Field: Food Sciences

Specialty: Agricultural Food and Quality Control

Title

Antioxidant efficacy of *Elettaria cardamomum* (cardamom) essential oil: A comparative study

Presented by:

- **BENAISSA Aicha Intissar**
- **RETIMI Aouali Naila**

Defended on: 27/06/2024

In front, the jury composed of:

President	**:**	Dr. REBHI Abdelghani El Mahdaoui	University of Ziane Achour - Djelfa
Supervisor	**:**	Dr. LAHRECHE Talal	University of Ziane Achour - Djelfa
Examiner	**:**	Dr. Mme HEFIAD F	University of Ziane Achour - Djelfa

University year: 2023/2024

Dedication

Thank you, Allah, for the life you have blessed me with. Thank you for the opportunities that I have been given and for the strength to make the most of them. Thank you for guiding me through difficult times and for giving me the courage to face whatever comes my way.

Then I dedicate this humble work to me first, which began with ambition and ended with success;

To my beloved dad, may Allah bless him with a long life for all he has done for me, pushing me towards my goals and instilling in me the value of hard work and education through his sacrifices; Person who showered me with love and care, who stood by me through thick and thin, supporting me and praying for my success, guiding me every step of the way. And the one who always brought a smile to my face, my source of comfort. My mother, may Allah bless you;

To my grandmother, may Allah prolong her life and provide her with good health and well-being, which has always been my support;

To my brother and sister who shared the burden of life with me, who supported me with love in my times and removed all troubles from my path, paving the way for me, my support and the shoulder on which I always lean;

To my lifelong friends and my dear family who showered me with love and guidance, always provided me with strength, and were the place to lean on in all my stumbles.

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I also do not forget my colleague in this journey Naila, I wish her all the best;

To all the faculty members and administrators at the department of agronomic and veterinary sciences;

I hope to Allah Almighty to bring us together in other beautiful days. Congratulations to my beautiful heart for this joy that makes me proud of me. Congratulations on my graduation.

Benaissa Aicha Intissar

Dedication

I dedicate this humble work to:

My dear mother, the woman who suffered without letting me suffers and always supported me during my years of study;

My precious father whom I owe my life and my success;

My late beloved uncle, who passed away a few months ago, who was always encouraging;

Me and loving me;

To my adorable sisters and my brother may Allah protect them and give them luck and happiness;

I also do not forget my colleague in this journey Aicha Intissar, I wish her all the best;

And to everyone who gave me a helping hand from near or far and helped me, even with a word or a prayer;

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Retimi Aouali Naila

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%	Percentage
°C	Degree Celsius
AA	Ascorbic Acid
ABTS	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)
Abs	Absorbance
AFNOR	Association Française de Normalisation
ANOVA	Analysis of Variance
BHT	Butylated hydroxytoluene
COPD	Chronic Obstructive Pulmonary Disease
D	Distilled oil
DPPH	2,2-diphenyl-1-picrylhydrazyl radical
EC_{50}	Half Effective concentration
EO	Essential oil
E. coli	*Escherichia coli*
FRAP	Ferric Reducing Antioxidant Power Assay
g	gram
GC/MS	Gas chromatography/mass spectrometry
GP	Graduation project
h	Hours
IC_{50}	Half maximal inhibitory concentration
ISO	International Organization for Standardization
m	Mass of dry and crushed plant material in g
MAHD	Microwave-assisted hydro-distillation
m_{EO}	Mass of essential oil in g
mg	Milligram

µg	Microgram
min	Minutes
ml	Milliliter
µl	Microliter
mm	Millimeter
mM	Millimolar
nm	Nanometer
ORAC	oxygen radical absorbance capacity
P	Purchased oil
pH	potential of Hydrogen **(**Measure of acidity and alkalinity of a solution)
p-value	Probability value
RP	Reducing Power
rpm	Revolutions Per Minute
RSA	Radical Scavenging Activity
s	Seconds
SD	Standard deviation
SPSS	Statistical Package for Social Science
TAC	Total Antioxidant Capacity
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
UV-Vis spectrophotometer	Ultraviolet–Visible spectrophotometer
W	Watt
Y	Yield

Introduction

Introduction

Cardamom is recognized worldwide as the “queen of spices” due to its pleasant aroma and taste and the third most expensive spice after saffron and vanilla. It is a versatile spice used in sweet and salty foods. For centuries, cardamom capsules have been used for culinary and traditional medicine applications including controlling asthma, teeth and gum infections, digestive and kidney disorders (Hamzaa et al., 2012; Ashokkumar et al., 2014), cataracts, nausea, diarrhea, and cardiac disorders (Gilani et al., 2008; Khan et al., 2011).

It is very difficult to give a single definition to essential oils (EOs) because several definitions have (According to the French Standardization Association (AFNOR), and the International Organisation for Standardization (ISO)...etc) (Diass et al., 2021). But generally, they can be defined as it is very intricate compositions that might be made up of hundreds of different components EOs are composed of various types of compounds, including terpenes, sesquiterpenes, alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, ethers, and esters. These compounds impart desirable sensory characteristics, such as flavor and aroma, to food products; hence, EOs are widely used as food ingredients. In addition, some EOs are believed to have antibacterial and antioxidant properties, spurring recent research on their potential health benefits (Sakkaravarthy et al., 2023).

The EO and other bioactive metabolites accumulated in cardamom capsules contribute to their characteristic aroma and utility as a functional food, pharmaceutical, and nutraceutical (Hamzaa et al., 2012). Cardamom EO is produced by distilling powdered cardamom seeds. The kind of distillation, the speed at which it is done, and the amount of time it takes all contribute to the final product's quality (Mani et al., 2017). Distilling high-quality cardamom EO would be too costly since the spice sells for more as a complete commodity (Sakkaravarthy et al., 2023).

The development of new antioxidants with high antioxidant activities is essential to combat oxidation phenomena. For this purpose, the investigation of the cardamom plant represents invaluable potential for the discovery of new substances of an antioxidant nature, if we consider that this plant can contain many secondary metabolites.

Therefore, the present study aims to extract the EO from cardamom seeds using the Clevenger apparatus and comperd with purchased EO to evaluate its antioxidant activities efficacy with different antioxidant assays which are: Free radical scavenging activity (DPPH), Ferric ion Reducing Antioxidant Power (FRAP), and Total Antioxidant Capacity (TAC).

The present study is organized as follows:

CHAPTER 1: Provides a comprehensive overview of cardamom, highlighting their biological activities.

CHAPTER 2: Outlines the materials and methods involved in extracting the EO from cardamom, and the assessment used to evaluate the antioxidant activities.

CHAPTER 3: Will present the results and discussion of the study.

Finally, our dissertation concludes with a conclusion. Also presents potential future perspectives.

CHAPTER 1: Overview of Cardamom

1.1 Cardamom definition

Cardamon also known as *Elettaria cardamomum*, belongs to the Zingiberaceae family. It is native to the Indonesia and Indian subcontinent, Pakistan, Burma, Bangladesh, and tropical and subtropical Asia. It is perennial, herbaceous monocots with 4-5 m in height. Cardamom flowers have a whitish lip at the corolla tube's tip (Ravindran and Madhusoodanan, 2002).

Cardamom seed is known as the “queen of spices” because of its delightful aroma, and adaptable flavoring for various foods and beverages (Delgadillo-Puga et al., 2023). After saffron and vanilla, cardamom is one of the most expensive spices because of its high cost of production and delicate preservation (Neri, 2021).

1.2 Botanical description

During one of their travels to India, the Portuguese discovered cardamom, particularly Vasco da Gama. This spice is widely used in food and traditional medicine and is available in this country. Its past is rooted in antiquity. Many legends were attached to cardamom during the Middle Ages. It was said to ward against scorpion stings and snake bites, to be used in love filters, and to have a strange power that allowed one to see a loved one's face in a dream (Neri, 2021).

The Figure 1.1 represent a botanical illustration of a cardamom plant:

Figure 1.1 : Botanical illustration of a cardamom plant (Schneider, 2024).

Tall perennial herbaceous plants have two distinct aerial growths—flower and leaf shoots—and a creeping rootstock. The leaves are dark green and either glabrous or pubescent. They are arranged in two rows with long sheaths, usually without stalks, and an undivided lanceolate leaf blade.

An inflorescence is a spike, resembling a panicle of heads, bearing one or more flowers. Every flower has a bract positioned laterally and is enclosed in the aril of a subtending leaf. The flower bears fruit longitudinally, with an outer calyx that is long and tubed and an inner corella that ends in lobes that are brilliantly colored, one of which resembles a helmet.

The staminodium, also known as the "lip" or "labellum," is the flower's more visually appealing method of drawing pollinating insects. It has a pale color. The fruit node is trilocular and inferior. Double-rowed, centrally angled, inverted ovules are present in every cell. The fruit node's thread-like stylus has a funnel-shaped bottom filled with nectar glands. The fruit is a dehiscent, three-compartmentalized berry that has a leathery, fleshy, or juicy texture. The seeds are wrinkled and angled or ellipsoidal.

The flowers of the genus *Elettaria* are composed of spirals with multiple flowers and are borne atop long panicles. Flowers are white or pale green, measuring between 30 and 35 mm in length, with a blue or violet smear on the center lip. Fruits are indehiscent, round, or nearly spherical, fleshy when green, and leathery when dry. The characteristic that sets the genus *Amomum* apart from *Elettaria* is its densely packed, conically-shaped spike of short-stalked or sensile flowers, lacking or slender lateral staminodia. The thick-fleshed, loosely or densely clustering fruits develop a leathery fruit coat that dries out. Fruits are dehiscent, have three chambers, and contain a large number of seeds (Govindarajan et al., 1982; Nair, 2020)

The dry capsule can be green, yellow, greenish-yellow, or light brown. A tiny percentage of the produce is sold as creamy white, bleached produce. The dry capsule has three sides, is ovoid or somewhat long, and has tips that resemble beaks and easily identifiable longitudinal ridge lines. Depending on the variety, growth region, harvest maturity, and drying conditions, the length can range from 7 to 15 mm. Each of the capsules' three paper-thin arils covers clumps of six to eight reddish-brown to dark-brown seeds. The chambers are divided by thin membranes. The irregular, conical seeds have a rounded base. Their surface is slightly wrinkled, and they measure about 3 mm in length.

The husk, which is roughly one millimeter thick, is made up of two or three layers of obliterated thin-walled cells that make up the inner wall and the outer epidermis, which are sandwiched between a thick layer of thin parenchymal cells with an uneven elliptical. There are one

or more calcium oxalate crystals in every parenchymal cell. The parenchyma is dotted with fibrovascular and fibrous bundles as well as rounded oil-resin cells." The tough, fibrous husk has a faint scent (Jadav and Mehta, 2018; White, 1811).

1.3 Cardamom classification

According to the Cronquist system, cardamom is classified as follows (Cronquist, 1981):

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Liliopsida
- Order: Zingiberales
- Family: Zingiberaceae
- Genus: *Elettaria*
- Species: *Elettaria cardamomum*.

1.4 Common names of cardamom

The international names of the word “cardamom” reveal a shared origin (Poirel, 2017):

- In German: Kardamom;
- In English: Cardamom;
- In Spanish: Cardamomo;
- In Italian: Cardamomo;
- In Portuguese: Cardamomo;
- In Greek: Kárdamo;
- In Latin: Amomum;
- In India: Ilaayachee, elaichi, elettari, chhoti elaichi;
- In Sanskrit: Elâ, bâhula, truti, vayastha, chandraravâlâ;
- In Arab: Hil, hal, hab’han;
- In French: Ellataria, cardamome verte.

1.5 Chemical composition of Cardamom seeds

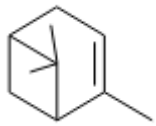
Seeds of *Elettaria cardamom* are rich in volatile oil that mainly includes phenolic and flavonoid components. Starch, protein, waxes, and Sterols are other components of the oil.

The volatile oil is the major component of all the varieties of *Elettaria cardamom*. Sharma et al. (2011) reported that volatile oils for the major varieties (Mysore, Malabar, Vazhukka, and Guatemala) have 7.90, 8.79, 7.90, and 8.60% respectively. Table 1.1 shows the major constituents of the volatile oil of cardamom seed.

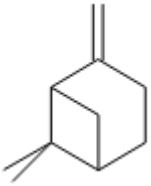
Table 1.1: Main components of volatile oil present in different varieties of cardamom (Sharma et al., 2011).

Component (%)	**Mysore**	**Malabar**	**Vazhukka**	**Guatemala**
α-Pinene	0.36	0.07	0.14	0.11
Sabinene	1.32	0.31	0.41	0.55
β -Myrcene	0.85	0.37	0.25	0.53
Limonene	2.23	1.82	1.57	1.92
1,8 cineole	11.76	7.55	7.23	9.29
γ-Terpinene	0.23	0.03	_	0.11
Terpinolene	0.31	0.09	_	0.18
Linalool	0.94	4.53	0.86	5.96
Linalyl acetate	0.33	1.92	0.42	1.63
Terpinen-4-ol	1.51	1.18	1.34	1.53
α- Terpineol	5.51	3.69	2.91	4.92
β-Terpineol	0.64	0.2	0.21	0.22
α-Terpinyl acetate	64.45	63.32	68.19	61.65
Octyl Acetate	0.06	0.07	0.11	0.11
Nerly Acetate	2.11	1.61	1.75	1.58
Nerolidol	0.88	3.75	1.71	2.17
Geraniol	1.66	2.5	1.52	2.47
Geranial	0.49	0.51	0.54	0.65
B-caryophyllene	0.12	0.23	0.4	0.24
Cis, trans Farnesol	0.16	0.84	0.14	0.38
Cis, Cis Farnesol	0.17	0.36	0.19	0.21

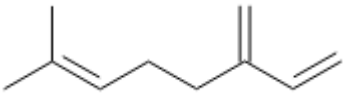
The Figure 1.2 shows the chemical structures of major constituents of cardamom (Sharma et al., 2011).



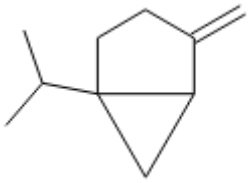
Alpha-pinene



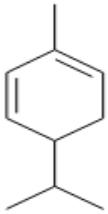
beta-pinene



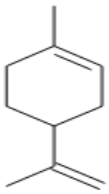
myrcene



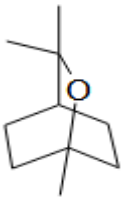
sabinene



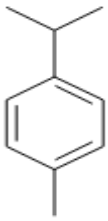
alpha-phelandrene



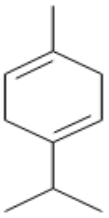
limonene



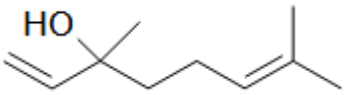
1,8- Cineole



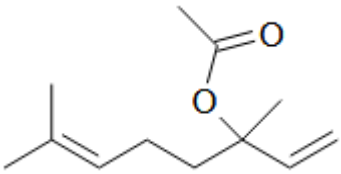
p-cymene



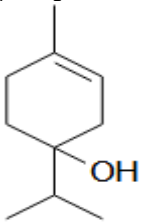
γ-terpinene



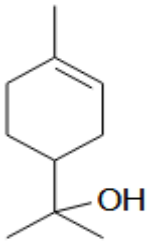
Linalool



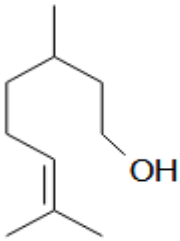
linalyl acetate



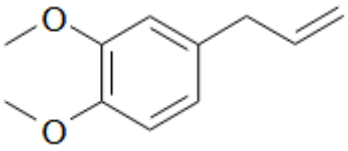
terpinen-4-ol



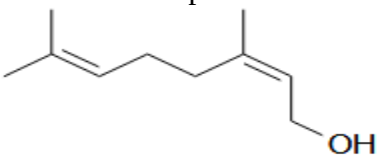
α –terpineol



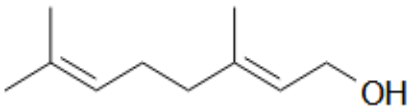
citronellol



methyl eugenol



Nerol



geraniol

Figure 1.2: Chemical structures of major constituents of cardamom (Sharma et al., 2011).

1.6 Cardamom types

There are two main types of cardamom:

- Small green cardamom (*Eletteria cardamomum*);
- Large red/black cardamom (*Amomum subulatum Roxb*).

Small green cardamom is the most common variety, while large cardamom is mostly grown in India with some also found in Nepal and Bhutan as shown in Figure 1.3. They are both members of the plant family Zingiberaceae (Rajathi et al., 2017).

(a) **(b)**

Figure 1.3 : Small (a) and large (b) Cardamom (Rajathi et al., 2017).

1.7 Cardamom uses

Cardamom has many uses such as:

1.7.1 Medicinal and pharmacological uses

Cardamom possesses many medicinal properties such as antiseptic (pulmonary), antispasmodic (neuromuscular), aphrodisiac, expectorant, anthelminthic, antibacterial (variable), cephalic, cardiotonic, diuretic, emmenagogue, sialogogue and stomachic. Moreover, it also can be used as (Rajathi et al., 2017):

- **Anti-inflammatory:** Cardamom can be used broadly to treat infections in teeth and gums, to prevent and treat throat troubles, congestion of the lungs and pulmonary tuberculosis, inflammation of eyelids, and also digestive disorders;
- **Antidote to snake venom:** Reportedly, the cardamom is also used as an antidote for both snake and scorpion venom;
- **Hepatoprotective:** The components in the volatile oil, e.g., 1,8-cineole, terpinene, terpineol, sabinene, α-pinene, and limonene, act as a tonic for the heart and liver, an appetizer, promote the elimination of bile and help reduce congestion of the liver.

1.7.2 Other uses

- **Alimentary uses:** the cardamom is a spice rarely used in cooking. On the other hand, it is often used throughout the world in various recipes and is sometimes found in drinks such as coffee and tea (Poirel, 2017);
- **Perfumery and cosmetics:** Cardamom oil is also used in cosmetics because of its cooling properties and it is a pale to colorless liquid that can be easily incorporated into different solutions. The taste is warm and spicy and can be used as a flavor for chewing gum. The essential oil can be blended with vegetable oils such as coconut oil to develop essential lotions and creams for healthy skin cell functioning. Due to its powerful cleansing, purifying, and insect-repellent activities, cardamom essential oil can be mixed with lemon essential oil to develop natural wood preservatives and polish (Jadav & Mehta, 2018).

1.8 Biological activities of cardamom

The majority of the biological activity research on cardamom has focused on identifying the plant's antibacterial, anti-inflammatory, anticancer, and antioxidant properties (Sarac, 2021).

1.8.1 Antibacterial activities

Antibiotics are used to treat microbial infections (both bacterial and fungal). *Elettaria cardamom* contains a wide range of secondary metabolites, including tannins, alkaloids, and flavonoids that have antimicrobial properties. Cardamom extracts in petroleum ether demonstrated antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* bacteria (Kumar et al., 2010).

Similar studies show that the seeds EO was discovered to have a significant inhibitory effect on various keratinophilic and dermophytic fungi (Jain & Agarwal, 1976). Similarly, acetone, methanolic, and ethanol extracts (Hussain et al., 2011). Cardamom inhibits two bacteria that cause dental caries, *Streptococcus mutans* and *S. aureus*, as well as two fungi, *Candida albicans* and *Saccharomyces cerevisiae* (Aneja and Joshi, 2009).

1.8.2 Anti-inflammatory activities

Various studies have revealed the cardamom plant's anti-inflammatory activity. In a study on the anti-inflammatory activity of cardamom essential oil on rats, it was discovered that cardamom essential oil extract demonstrated anti-inflammatory activity in a model of acute right paw inflammation induced by lambda-carrageenan in rats. In a similar study, it was discovered that 1,8-

cineole found in cardamom essential oil has anti-inflammatory properties against inflammatory airway diseases such as asthma and Chronic Obstructive Pulmonary Disease (COPD). Other studies have suggested that this component is primarily responsible for the cardamom plant's anti-inflammatory activity. Cardamom EO contains α-pinene, which has been linked to gastric protection in studies. This suggests that the cardamom plant has gastro-protective activity (Sarac, 2021).

1.8.3 Anticancer activities

A study on the cardamom plant revealed that it inhibits platelet aggregation. Also, the cardamom extracts prevent colon and skin cancers (Sengupta et al., 2005; Qiblawi et al., 2015). The chemo-preventive function of cardamom has also been demonstrated to regulate colorectal cancer (Bhattacharjee et al., 2007).

1.8.4 Antioxidant activity

Antioxidants are systems that eliminate the harmful effects of free radicals (Faydaoğlu and Sürücüoğlu, 2011; Bal et al., 2017; Çelik and Ayran, 2020). A plant's antioxidant potential is determined by the presence of components such as ascorbic acid, carotene, anthocyanin, and tocopherol, as well as phenolic compounds such as flavonoids and phenolic acids (Khalaf et al., 2008; Sarac et al., 2021).

Cardamom fruits and seeds contain a high concentration of antioxidants, which neutralize free radicals and prevent oxidation at the earliest stage. A study found that cardamom essential oil extract and methanol, ethanol, chloroform, and diethyl ether oleoresins exhibit substantial antioxidant activity. Another investigation on the antioxidant activity of methanol extracts from key plants, including *E. cardamomum*, found that the cardamom plant has modest antioxidant activity (Khalaf et al., 2008).

In a study investigating both antioxidant and antidiabetic properties of methanol and water extracts of cardamom fruits, it was determined that water and methanol extract of the plant exhibited in vitro antidiabetic and antioxidant activities. In addition, it was detected that the water extract exhibited better activity among the extracts (Ahmed et al., 2017). In a similar study with water extract of cardamom fruits, it was determined that the plant exhibits antioxidant, antiobesity, and antidiabetic activities (Al-Yousef et al., 2021).

Additionally, research on diabetic rats has shown that several cardamom extracts can decrease blood sugar and cholesterol and enhance hippocampus functions including learning and memory that are compromised (Winarsi et al., 2014; Gomaa et al., 2019). One of the goals of using antioxidant-based medications is to prevent or cure serious conditions like diabetes, stroke, and Alzheimer's, which can arise from oxidative or cellular damage brought on by free radicals (Khalaf et al., 2008; Ahmed et al., 2017).

CHAPTER 2: Materials and Methods

2.1 Objectif

The objective of this study is to evaluate the antioxidant activity of the EO and purchased EO of cardamom (*Elettaria cardamomum*) by three methods namely DPPH, FRAP, and TAC assays.

2.2 Workplace

This research was done to accomplish the Graduation project (GP) in the laboratory of agronomic and veterinary sciences department, faculty of natural and life sciences, Ziane ACHOUR University of Djelfa.

2.3 Reactants and materials

2.3.1 Equipment's

- Clevenger apparatus.
- UV-Vis spectrophotometer (Beckman, DU520).
- Incubator.
- Precision balance.
- Mill laboratory.
- Refrigerator.
- Centrifuge.

2.3.2 Physical material

- Glass test tubes.
- Test tube racks.
- Adjustable pipettes.
- Mortar and Pestle.
- Beakers.
- Graduated measuring cylinder.
- Parafilm tape.
- Spatula.
- Round Bottom Flask, Single Neck.
- Pipette tips.

2.3.3 Chemical's products

All chemical substances, reagents, solvents, reference standards, and materials used in this work are of the highest quality available for good results. These substances were procured from Sigma-Aldrich and include:

- Methanol (CH_3OH).
- 2,2-diphenyl-1-picrylhydrazyl radical (DPPH).
- Butylated hydroxytoluene (BHT) ($C_{15}H_{24}O$).
- Ascorbic Acid (AA) ($C_6H_8O_6$).
- Dipotassium hydrogen phosphate (K_2HPO_4).
- Potassium dihydrogen phosphate (KH_2PO_4).
- Potassium ferricyanide ($K_3Fe(CN)_6$).
- Trichloroacetic acid (TCA) ($C_2HCl_3O_2$).
- Iron (III) chloride ($FeCl_3$).
- Sodium phosphate (Na_3PO_4).
- Sulfuric acid (H_2SO_4).
- Ammonium molybdate ($(NH_4)_6Mo7O_{24}$).
- Distilled water.

2.4 Plant material and purchased EO

The cardamom seed was purchased from a local market in Djelfa in March 2024. Upon arrival at the laboratory, peels were removed and the obtained cardamom pods were grounded to get a good yield;

The purchased EO was purchased from French website "Oshadhi cardamom Essential Oil ".

2.5 Preparation of plant EO

Fifty grams (50 g) of grounded cardamom was mixed with 500 ml of distilled water. The hydrodistillation process was performed using the Clevenger apparatus type A following the method recommended by the European Pharmacopoeia (Council of Europe, 1996) for 3 h (Figure 2.1), and the obtained EO was collected in a locked tube and then kept to the refrigerator (4-6°C).

Figure 2.1 : The Clevenger apparatus.

2.6 Determination of extraction yield

The yield of EO expressed as a percentage (%), is the ratio between the mass of EO obtained and the mass of the plant material used (AFNOR, 2000). The percent yield can be calculated using the formula:

$$\boldsymbol{Y}(\%) = \frac{\boldsymbol{m_{EO}}}{\boldsymbol{m}} \times \mathbf{100}$$

$\boldsymbol{m_{EO}}$: Mass of essential oil in g.

$\boldsymbol{m}$: Mass of dry and crushed plant material in g

2.7 Assessment of the antioxidant activities

The antioxidant capacity of the tested EO was evaluated in this work by a series of 3 assays: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), Ferric ion reducing antioxidant power (FRAP), and the total antioxidant capacity (TAC).

2.7.1 Free radical scavenging activity (DPPH) assay

- **Principle**

At room temperature, the DPPH• radical presents, in alcoholic solution, an intense purple color that disappears on contact with a proton donor substance. This discoloration, which turns pale yellow, highlights the antioxidant power of a sample through its capacity to trap the free radical and results in a decrease in absorbance at 517 nm, and the intensity of the color is inversely proportional to the antioxidant power (Moon and Shibamoto, 2009).

- **Experimental procedure**

The free radical scavenging activity of DPPH was determined using the Blois method (1958). 500 µl of different concentrations of EO were mixed with 1000 µl of 0.2 mM DPPH solution. The resulting mixture was vigorously shaken and incubated for 30 minutes at room temperature. Subsequently, the absorbance was recorded at 517 nm against a blank using a UV-Vis spectrophotometer (Beckman, DU520).

The percentage of free radical scavenging capacity was calculated using the following equation:

$$\boldsymbol{RSA}\ (\%) = [(\mathbf{1} - \boldsymbol{Sample\ Abs}) / \boldsymbol{Control\ Abs}] \times \mathbf{100}$$

With:

- Control Abs is the absorbance of the control reaction (methanol+DPPH);
- Sample Abs is the absorbance of the samples

The half-maximal inhibitory concentration (IC_{50}) was determined by calculating the concentration at which the trend curve extracted from the regression graph of percentage RSA against EO concentration intersected the 50% inhibition mark. The reference values for ascorbic acid (AA) and butylated hydroxytoluene (BHT) RSA were also estimated.

2.7.2 Ferric ion Reducing Antioxidant Power (FRAP) assay

- **Principle**

The reducing power of an EO is evaluated by the redox reaction between the EO and transition metal ions notably iron. Potassium ferricyanide $K_3Fe(CN)_6$ provides Ferric ions (Fe^{3+}) which will be reduced to Ferrous (Fe^{2+}) by the antioxidants present in the plant EO. After this reduction, the EO form a complex ($Fe_4[Fe(CN)_6]_3$ colored blue which absorbs at 600 nm. The

decrease in absorbance at this wavelength indicates the reduction in the effect of the studied EO (Miguel, 2010; Alam et al., 2013).

- **Experimental procedure**

The reducing power of iron (RP) was assessed using the method described by Oyaizu (1988). 400 µl of various sample concentrations were mixed with 2000 µl of phosphate buffer (2 mM, pH 6.6) and 1000 µl of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was then incubated for 20 minutes at 50°C. Subsequently, 1000 µl of 10% trichloroacetic acid (TCA) was added to the solution, which was then centrifuged at 3000 rpm for 10 minutes. The supernatant was filtered, and 1000 µl of the supernatant was mixed with 3000 µl of distilled water and 1000 µl of 0.1% $FeCl_3$. The mixture was vigorously shaken and incubated for 30 minutes at room temperature. The absorbance was recorded at 700 nm using a Beckman DU-520 UV-Vis spectrophotometer and compared to the standards (AA and BHT).

The percentage of iron's RP was determined using the following equation:

$$\boldsymbol{RP}(\%) = [(\mathbf{1} - \boldsymbol{sample\ Abs}) / \boldsymbol{control\ Abs}] \times \mathbf{100}$$

With:

- Control Abs is the absorbance of the control reaction (methanol+ FRAP);
- Sample Abs is the absorbance of the samples.

The half-maximal effective concentration (EC_{50}) for iron reduction was determined by calculating the concentration using the equation derived from the regression curve of the percentage of RP against the concentration of EO. The iron RP of AA and BHT was also estimated as a reference.

2.7.3 Total Antioxidant Capacity (TAC) assay

- **Principle**

Total Antioxidant Capacity (TAC), is a test that was first presented by Fiske & Subbarrow (1925) who developed it to determine inorganic phosphate by the formation of phosphate-molybdenum complex V with a green color measurable by spectrophotometer. This method was revised and modified by Chen et al. in 1956, and by Prieto et al. in 1999 who adapted it to determine all types of reducing agents.

TAC assay is based on reducing molybdenum VI (Mo^{6+}) to molybdenum V (Mo^{5+}) by a sample extracted from a plant. This reduction induces, at acidic pH, the formation of a green-colored phosphate/Mo^{5+} complex (Alam et al., 2013).

- **Experimental procedure**

The total antioxidant capacity (TAC) was assessed following the method outlined by Prieto et al. (1999). 200 µl of various sample concentrations were combined with 2000 µl of molybdate reagent containing 28 mM sodium phosphate, 4 mM ammonium molybdate, and 6 mM sulfuric acid. After incubating the tubes for 90 minutes at 95°C, the mixture was cooled to room temperature, and the absorbance was measured at 695 nm against a blank using a UV-Vis spectrophotometer (Beckman, DU520). The TAC of AA and BHT was also determined for comparative purposes.

$$\boldsymbol{TAC}\ (\%) = [(\mathbf{1} - \boldsymbol{Sample\ Abs}) / \boldsymbol{Control\ Abs}] \times \mathbf{100}$$

With:

- Control Abs is the absorbance of the control reaction (methanol+TAC);
- Sample Abs is the absorbance of the samples.

The half-maximal inhibitory concentration (IC_{50}) was calculated using the trend line equation extracted from the regression graph of TAC percentage versus EO concentration. The TAC of the AA and the BHT has also been estimated for reference.

2.8 Statistical analyses

All mean and standard deviations were determined from triplicate data. Analysis of variance (ANOVA) was applied followed by Duncan's Multiple Range Tests at a P–value of < 0.05 to conclude the significant differences. Statistical analyses were conducted with the Statistical Package for Social Science (SPSS v.20.0 for Windows, SPSS Inc., Chicago, IL, USA).

CHAPTER 3: Results and Discussion

3.1 Results of extraction yield

The yield of our EO obtained from 52.93g of and ground cardamom at the ratio of 1/10 of distilled water, and distilled using a Clevenger apparatus for 3 hours at 100 °C, was 5.34%. The obtained yield was highest compared to Kapoor et al. (2008) who reported a value of 2.20 % for *Amomum subulatum* distilled using Clevenger apparatus for 6h at 60 °C and a ratio of 1/12. Teresa-Martínez et al. (2022) reported that the extraction yield of *Elettaria cardamom* obtained by hydrodistillation for 30 s at 140 °C and a ratio of 1/12 was 4.43 %. Al-Thbity & Gobouri (2023) reported that the extraction yield of *Elettaria cardamom* was 7.88 % for EO obtained by hydrodistillation for 150 min at 100 °C and a ratio of 1/10 and was 7.22 % for EO obtained by Microwave-assisted Hydro-distillation (MAHD) for 40 min at 500 W and the ratio of 1/10.

3.2 Results of assessment of the antioxidant activities

The antioxidant activity of cardamom EOs that obtained by the Clevenger apparatus was estimated by three different assays:

- Free radical scavenging Activity (DPPH) assay;
- Ferric ion Reducing Antioxidant Power (FRAP) assay;
- Total Antioxidant Capacity (TAC) assay.

3.2.1 Free radical scavenging Activity (DPPH) assay

The antioxidant scavenging activity of purchased cardamom EOs as well as that obtained by the Clevenger apparatus was estimated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay using ascorbic acid (AA) and 2,6- di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT) as standards.

Figure 3.1 shows Free radical scavenging percentages of cardamom extract compared to purchased oil, ascorbic acid, and BHT at different concentrations.

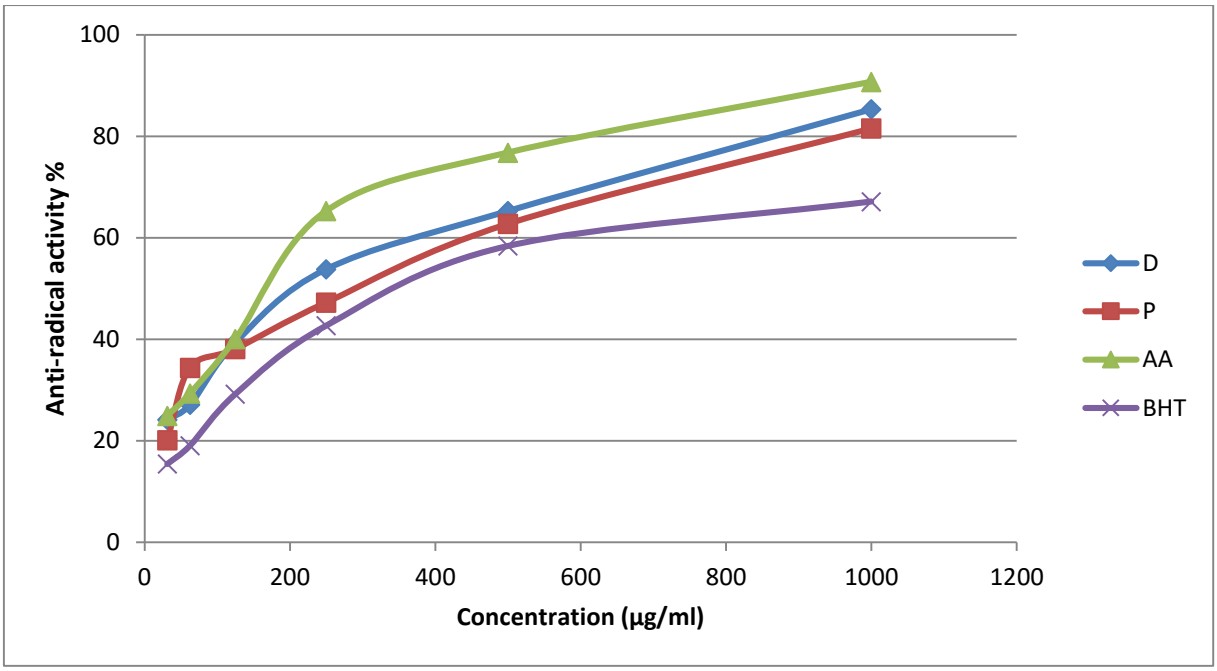


Figure 3.1: Free radical scavenging percentages of cardamom extract compared to purchased oil, ascorbic acid, and BHT.

AA: Ascorbic Acid, BHT: butylated hydroxytoluene, D: EO distilled, P: EO purchased

A positive correlation was observed between the concentration of the tested EOs and the percentage of the Radical Scavenging Activity (RSA %). The percentage of the RSA increases with the increased concentration of the tested EOs.

Table 3.1 shows Free radical scavenging ability of cardamom extract compared to purchased oil, ascorbic acid, and BHT at different concentrations.

Table 3.1 : Free Radical Scavenging Ability of EO tested at 1mg/ml concentration.

	IC_{50} value (µg/ml)	**% RSA at 1mg/ml**
D	184.86±2.78 c	85.36±3.37 ab
P	207.02±6.20 b	81.58±0.40 b
AA	141.66±3.49 d	90.75±1.98 a
BHT	357.63±5.16 a	67.16±1.51 c

Data are presented as means±SD obtained from triplicate (n=3).

AA: Ascorbic Acid, BHT: butylated hydroxytoluene, D: EO distilled, P: EO purchased

Statistical data showed significant differences ($p<0.05$) in RSA % between the different tested compounds (Table 3.1). AA had the highest free radical scavenging activity (90.75 %), while BHT showed the lowest RSA % (67.16 %). No significant difference ($p>0.05$) was observed between

AA and distilled EO (90.75 and 85.36 %, respectively). Also, no significant difference ($p>0.05$) was marked between distilled and purchased EOs (85.36 and 81.58 %, respectively). However, a significant difference ($p<0.05$) was noticed between AA and purchased EOs (90.75 and 81.58 %, respectively).

The best RSA percentage had the lower IC50. Significant differences ($p<0.05$) in IC50 values were noted between the different tested compounds AA, distilled EO, purchased EO, and BHT of 141.66, 184.86, 207.02, and 357.63 µg/ml, respectively. BHT exhibited the highest IC50 value of 357.63 µg/ml, while distilled EO showed an IC50 value of 184.86 µg/ml.

Therefore, our results 184,86 µg/ml, concord with those made by Padma Kumari Amma et al. (2010) who reported an IC_{50} of 186 µg/ml, we also find that the value is less than that we obtained Al-Zereini et al. (2022) an IC_{50} 63.60 Aftab Alam et al. (2021) while gets a result that represents more value than us an IC_{50} of 588,60 µg/ml.

3.2.2 Ferric ion Reducing Antioxidant Power (FRAP) assay

The reducing power of purchased cardamom EO as well as that obtained by Clevenger distillation was estimated by the FRAP test using AA and BHT as standards (Figure 3.2).

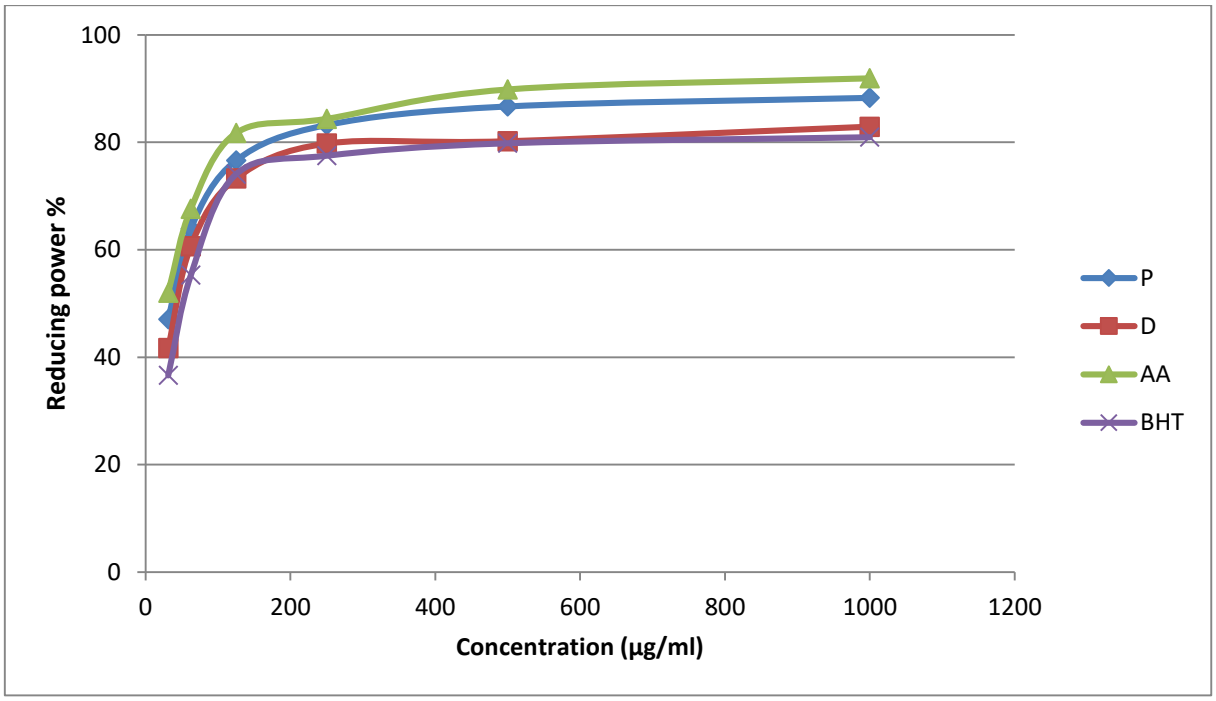


Figure 3.2 : Ferric-Reducing Antioxidant Power of tested EO.

AA: Ascorbic Acid, BHT: butylated hydroxytoluene, D: EO distilled, P: EO purchased

We notice a positive correlation between the concentration of the tested EOs and the RP %, the more the concentration of the EOs tested increases, the more the percentage of the RP increases.

Table 3.2 : Ferric-Reducing Antioxidant Power of EO tested at 1mg/ml concentration.

	EC_{50} value (µg/ml)	% RPA at 1mg/ml
D	30.13±3.56 b	82.90±1.34 b
P	21.64±4.82 c	88.29±1.13 a
AA	14.21±7.19 c	91.93±2.46 a
BHT	43.10±2.41 a	80.95±1.28 b

Data are presented as means±SD obtained from triplicate (n=3).
AA: Ascorbic Acid, BHT: butylated hydroxytoluene, D: EO distilled, P: EO purchased

Statistical analysis revealed notable disparities ($p<0.05$) in the percentage among the various compounds that were examined (Table 3.2). No significant difference ($p>0.05$) was marked between AA and the purchased EO and both have the highest reducing power activity (91.93 and 88.29 %, respectively). However, distilled EO and BHT didn't differ significantly ($p>0.05$) and had the lowest RPA % (82.90 and 80.95 %, respectively).

The best RP percentage had the lower EC_{50}. Significant differences ($p<0.05$) in EC_{50} values were noted between the different tested compounds, except for AA and purchased EO didn't differ significantly ($p>0.05$) and presented fewer EC_{50} values of 14.21 and 21.64 µg/ml, respectively. BHT exhibited the highest EC_{50} value of 43.10 µg/ml, while distilled EO showed an EC_{50} value of 30.13 µg/ml.

Our EC_{50} values of 30,13 µg/ml were less valuable compared to the value obtained by Agnieszka Joanna Brodowska et al., (2014), which is 613,64 µg/ml. While, there was a value less than our value, it was obtained by (Sowmya K M et al., (2022), which was EC_{50} 3,43 µg/ml.

3.2.3 Total Antioxidant Capacity (TAC) assay

The TAC of the different tested EOs was estimated using 2,6-di-tert-butyl-4-methylphenol butyl hydroxytoluene, (BHT) and Ascorbic Acid (AA) as a reference. The TAC expressed as a percentage, of the different EOs at different concentrations is represented in Figure 3.3.

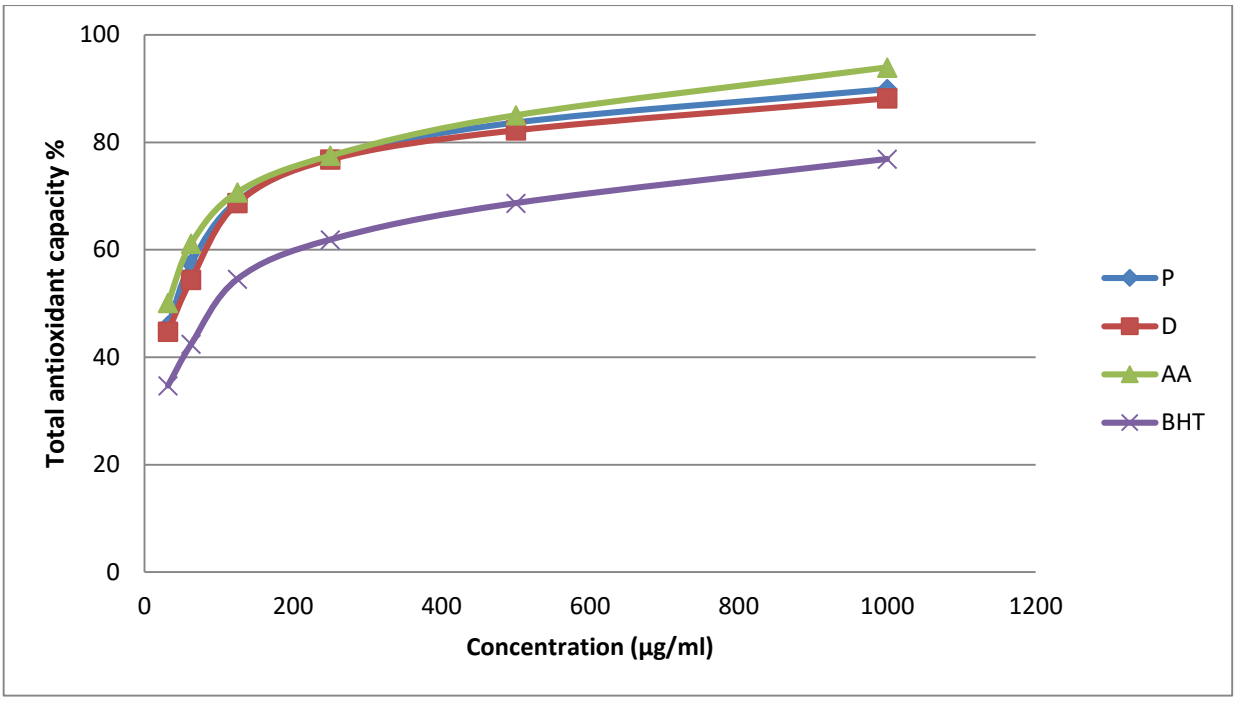


Figure 3.3 : The Total Antioxidant Capacity (TAC) of EO tested.

AA: Ascorbic Acid, BHT: butylated hydroxytoluene, D: EO distilled, P: EO purchased

The results show the presence of a positive correlation between the concentration of the tested EOs and the TAC %, the more the concentration of the EOs tested increases, the more the percentage of the TAC increases.

Table 3.3 : The Total antioxidant capacity of EO tested at 1mg/ml concentration.

	Valeur IC_{50} (µg/ml)	% TAC à 1mg/ml
D	39.15±3.00 b	89.92±1.30 a
P	34.91±1.21 b	88.20±3.16 a
AA	26.96±2.07 c	93.94±2.52 a
BHT	103.84 ±4.11 a	76.88±1.94 b

Data are presented as means±SD obtained from triplicate (n=3).
AA: Ascorbic Acid, BHT: butylated hydroxytoluene, D: EO distilled, P: EO purchased

From Table 3.3, distilled and purchased EOs and AA showed no significant differences and presented high TAC values of (93.94, 89.92, and 88.20 %, respectively), while BHT presented a significant difference with all tested compounds and had the lowest TAC value of 76.88 %.

In addition, significant differences ($p<0.05$) in IC_{50} values were marked between the different tested compounds, AA presented the lowest IC_{50} value of 26.96 µg/ml. Contrariwise, BHT

was the highest one with a value of 103.84 µg/ml. Purchased and distilled EOs have no significant differences ($p>0.05$) and showed close IC_{50} values of 34.91 and 39.15 µg/ml, respectively. After conducting research, we found that El Bouzidi et al. (2023) obtained a higher IC_{50} value of 165 µg/ml.

the observed variations in the extraction yield and the antioxidant activities present in the investigated plant EO may be attributed to two main factors:

Firstly, studying the extrinsic factors of the plant itself, such as its origin, species, and organs (Valnet, 1980). while environmental and climatic conditions, the stage of growth and harvest, the vegetative cycle, as well as the techniques and duration of preservation, have also been reported by Smallfield (2001) and Bruneton (1993) to have an impact on both the extraction yield and the antioxidant activities.

Secondly, the extraction procedures employed also contribute to the observed differences. Factors such as particle size, choice and concentration of solvent, ratio, extraction time, temperature, pressure, and the extraction method utilized (e.g., maceration, decoction, infusion, hydro distillation, Clevenger, steam distillation, etc.) have been identified by several authors as influential in determining the extraction yield and the content of compounds (Silva et al., 2007; Min and Chun-Zhao, 2005; Naczk and Shahidi, 2004; Zhou and Yu, 2004).

Conclusion

Conclusion

Medicinal plants will always remain a reliable source of bioactive compounds of therapeutic interest.

The objective of this study falls within the framework of the valorization of natural resources in Algeria. For this, we first carried out the extraction by hydro distillation of the essential oils from the seeds of *Elettaria cardamomum* using the Clevenger apparatus. Then we evaluated the antioxidant activity efficacy of this EO by three methods: DPPH, FRAP, and TAC assays.

From the obtained results in the present study, we can conclude that the distilled cardamom EO exhibits a strong potential free radical scavenging ability, while the purchased cardamom EO has a strong Ferric-Reducing Antioxidant Power and lower Anti-radical scavenging activity. Moreover, both distilled and purchased cardamom EO have a good Total Antioxidant Capacity.

Our study suggested that the assessment of antioxidant activity of EO should be evaluated using several assays.

This study suggests prospects for application in the food, cosmetics, and pharmaceutical industries. The results obtained could contribute to the valorization of these aromatic and medicinal plants. More in-depth investigations on the chemical composition by Gas chromatography/mass spectrometry (GC/MS), biological activities, including antioxidant activities such as radical trapping (ABTS), Iron chelating power, hydroxyl radical trapping, Thiobarbituric Acid Reactive Substances (TBARS), Liposomes, Hydrogen peroxide radical trapping, Oxygen Radical Antioxidant Capacity (ORAC), and microbiological activity, anti-inflammatory activity, anti-diabetic, antihemorrhagic, hypertensive, etc. Without ignoring the toxicity of this plant, it will be required to demonstrate its medical potential or limits of use.

In this respect, it is predicted that it is possible to develop new and natural antioxidative drug formulations from the cardamom plant, which has a high antioxidant potential, as an alternative to synthetic antioxidants in the future.

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Abstract

Cardamom is considered as one of the most famous spices with impressive medicinal properties and has been used in traditional medicine for centuries. In this study, the essential oils (EO) of cardamom collected from the local market were extracted using the Clevenger apparatus, followed by identifying the antioxidant activity using three assays: DPPH, FRAP, and TAC.

The results showed that the extraction yield of cardamom EO obtained by Clevenger apparatus at a ratio of 1/10 of distilled water, for 3 h at 100 °C temperature was 5.34%. Distilled cardamom EO exhibited a strong antioxidant activity (IC_{50} value: 184.86 µg/ml), while the purchased EO have a strong Ferric-Reducing Antioxidant Power and lower Anti-radical scavenging activity (EC_{50} value of 21.64 µg/ml and IC_{50} value of 207.02 µg/ml). Moreover, both of them distilled and purchased cardamom EOs have a good Total Antioxidant Capacity (IC_{50} value of 39.15 and 34.91 µg/ml, respectively). This result provides the basis for further studies to evaluate the potential use of EO from cardamom for antioxidant activities.

Key words: Cardamom, Clevenger, Essential Oil, antioxidant activity, DPPH, FRAP, TAC.

Résumé

La cardamome est considérée comme l'une des épices les plus célèbres, dotée de propriétés médicinales impressionnantes et utilisée en médecine traditionnelle depuis des siècles. Dans cette étude, les huiles essentielles (HE) de cardamome collectée sur le marché local ont été extraites à l'aide de l'appareil Clevenger, par la suite l'activité antioxydante a été identifiée par trois tests : DPPH, FRAP et TAC.

Les résultats ont montré que le rendement d'extraction de la cardamome obtenue par l'appareil Clevenger dans un rapport de 1/10 d'eau distillée, pendant 3 h à une température de 100°C était de 5,34%. L'HE de cardamum distillée a présenté une forte activité antioxydante (valeur IC_{50} de 184,86µg/ml), tandis que l'HE achetée a un fort pouvoir antioxydant réducteur ferrique et un piégeage antiradicalaire inférieur (valeur EC_{50} de 21.64 µg/ml et valeur IC_{50} de 207.02 µg/ml). De plus, les deux HE de cardamome distillées et achetées ont une bonne capacité antioxydante totale (valeur IC_{50} de 39.15 et 34.91 µg/ml, respectivement). Ce résultat constitue la base d'études ultérieures visant à évaluer l'utilisation potentielle des huiles essentielles de cardamome pour leurs activités antioxydantes.

Mots clés : Cardamom, Clevenger, Huile essentielle, activité antioxidant, DPPH, FRAP, TAC.

الملخص

يعتبر الهيل من أشهر التوابل، حيث يتميز بخصائص طبية مثيرة للإعجاب، وقد تم استخدامه في الطب التقليدي لعدة قرون . في هذه الدراسة، تم استخلاص زيوت الهيل العطرية التي تم جمعها من السوق المحلي باستخدام جهاز كليفنجر، تم بعد ذلك تحديد النشاط المضاد للأكسدة من خلال ثلاث اختبارات: DPPH، FRAP و TAC.

أظهرت النتائج أن مردودية مستخلص الهيل الذي تم الحصول عليه بواسطة جهاز كليفنجر بنسبة 1/10 ماء مقطر لمدة 3 ساعات عند درجة حرارة 100 درجة مئوية كانت 5.34% و نشاطًا قويًا مضادًا للأكسدة (بقيمة IC_{50}: 184.86 ميكروجرام/مل)، في حين أن الزيت العطري الذي تم شراؤه يحتوي على قوة ارجاع الحديد قوية تقلل من قوة مضادات الأكسدة وتقلل من الكسح المضاد للجذور (EC_{50} 21.64 ميكروجرام/مل و قيمة IC_{50} 207.02 ميكروجرام/مل)، علاوة على ذلك، تحتوي كل من زيوت الهيل الأساسية المقطرة والمشتراة على قدرة إجمالية جيدة لمضادات الأكسدة (قيمة IC_{50}: 39.15 ميكروجرام/مل و34.91 ميكروجرام/مل على التوالي). تضع هذه النتيجة الأسس للمزيد من الدراسات التي تهدف إلى تقييم الاستخدام المحتمل لزيوت الهيل الأساسية لأنشطتها المضادة للأكسدة.

الكلمات المفتاحية: الهيل، كليفنجر، الزيوت الأساسية، نشاط مضاد للأكسدة، DPPH، FRAP، TAC.