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Antibacterial Activity and Chemical Composition of Clove Essential Oil

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Abstract

The essential oil derived from cloves possesses a broad spectrum of pharmacological and biological properties, making it a common ingredient in the pharmaceutical, flavoring, and fragrance industries. The main objective of this study is to analyze the chemical composition of clove essential oil and evaluate its antibacterial activity against four types of bacteria *Citrobacter.sp, Staphylococcus aureus, Escherichia.coli and Pseudomonas auruginosa*). The results of this study showed that, clove essential oil (EO) was extracted at a substantial rate of 13.004%. Through chromatographic analysis, 99.56% of its constituents were identified, with eugenol emerging as the predominant component at 92.94%, followed by eugenol acetate at 5.03%, and caryophyllene at 0.88%. Furthermore, this study demonstrated that clove EO exhibits significant inhibitory activity against the bacterial growth. All bacteria assessed (*Citrobacter spp, W. staphylococcus, and E. coli*) displayed sensitivity to Clove EO at a concentration of 1/50 < MIC < 1/200, except for *Pseudomonas auruginosa*. The essential oil of cloves showed an important inhibiting effect on the studied bacterial strain.

Keywords: Essential oil; Antibacterial activity; Clove, Extraction; Bacterial strain.

Full length article **Corresponding Author*, e-mail: alaizari2@gmail.com

1. Introduction

The antimicrobial properties of aromatic and medicinal plants have been recognized since ancient times and this interest has continued into the 20th century [1, 2]. These properties are attributed to the essential oil (EO), an aromatic substance extracted from plants. The EO has a biological and varied therapeutic activity as well; for example, it has antiviral, anti-inflammatory and anticancer activities [3]. The problem raised by the resistance of bacteria to antibiotics has led scientists, by necessity, unless mandatory, to look for other reliable and sustainable alternatives. The EO has been scientifically demonstrated to work both "in vitro" and "in vivo" as it has been empirically known for decade [4].For centuries, spices like clove, oregano, mint, thyme, and cinnamon have been utilized as both food preservatives and medicinal plants, primarily because of their antioxidant and antimicrobial qualities[5]. In contemporary times, numerous studies have substantiated the antibacterial, antifungal, antiviral, and anticarcinogenic attributes of these spice plants. Notably, clove has garnered significant attention for its exceptional antioxidant and antimicrobial properties, setting it apart from other spices[6, 7].

The clove (*Syzygium aromaticum*) belongs to the *Myrtaceae* family, which includes 3,000 species[5, 8].It originated in the small volcanic islands of the Maluku archipelago in Indonesia. It is a medium-sized perennial tree whose stem from 8 to 10 meters is divided into twigs which constitute the overall pyramidal crown shape. Large oval leaves, with opposing sides, slightly fused at the base, are whole, oval, sharp, and persistent. These pink flowers are finally grouped in corymbs. The fruit is a dry drupe. The clove tree starts to produce floral buds after 8 to 9 years of plantation[9].

Clove stands as one of the principal botanical reservoirs of phenolic compounds, including flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenyl propones. Eugenol, the primary bioactive compound in clove, is present in concentrations ranging from 9381.70 to 14650.00 mg per 100 g of fresh plant material [10, 11]. Clove EO boasts potent antibacterial properties. Numerous EO have demonstrated highly effective clinical outcomes in disinfecting dental pulp and in the treatment and prevention of cavities[12, 13]. Clove EO is recognized as an antiseptic, antispasmodic, physical and intellectual stimulant, anesthetic, and analgesic, making it particularly valuable in alleviating dental pain[14, 15].Clove EO demonstrates a broad spectrum of pharmacological and biological properties, including antioxidant, antifungal, anticarcinogenic, anesthetic, and antiprotozoal effects[5, 16, 17].Besides, some studies have reported the antibacterial activity of essential oil from clove buds against several food-borne pathogens. However, to the best of our knowledge, although the in vitro antimicrobial activity of clove EO has been reported earlier, very little is known about their antibacterial mechanism of action. *S. aureus* is renowned for its resistance to certain antibiotics and its ability to produce multiple enterotoxins responsible for various forms of enteritis and septicemia [4, 16, 18–20].

In view of the interest in EO extracted from cloves, the current work aims at testing in vitro the activity of this essential oil on certain bacterial strains after studying the characterization of its chemical composition.

2. Materials and methods

2.1. Material

The studied part of the clove is the floral part, which is composed of flower buds in the shape of hypanthias (nails) with a quadrangular part of 10 to 12 mm in length and a globular bulging head of 4 to 6 mm in diameter surrounded by four lobes, which are nested petals. These nails are marketed internationally. The clove samples were brought from a local herbalist in Keneta City, Morocco.

2.2. Bacteria tested

Bacterial activity was assessed on four bacteria; *Escherichia coli, Staphylococcus aureus, Cirobacter spp, Psedomonas aeruginosa*. The selected bacteria are pathogenic of human origin and have at least a resistance to an antibiotic. The bacterial strains are maintained by subculture on an agaric nutrient medium available for their growth for 24 hours at 37 ° C.

2.3. Extraction by hydro distillation Clevenger

The extraction of the clove essential oil was carried out by hydro distillation in a device called Clevenger-type distillation invented in 1928 [21]. At least three distillations were made by boiling; during each test, 100 g of raw material was treated with 1 L of distilled water in a 2 liter flask surmounted by a 60 cm-long column connected to a condenser. The distillation time is of the order of 3 hours. The preservation of the essential oil requires certain essential safety measures. That is why essential clove oil was kept in a dark, sterile glass vial at a temperature close to 4 °C [22].

2.4. The yield

According to the AFNOR (1986) standard, the income of essential oil (Yd) is defined as the ratio between the mass of the essential oil obtained after extraction (M ') and the mass of the plantused material (M). The formula for calculating the yield (Yd) based on the used material (M) is expressed as follows:

$$Yd = \frac{M`(g)}{M\,(g)\,x\,100} \quad (1)$$

Yd represents the essential oil income as a percentage (%), where M' denotes the mass of the obtained essential oil in grams (g), and M represents the mass of the dry plant material used in grams (g).

2.5. Chromatographic analyzes

The chromatographic analysis was conducted using an electronically controlled gas chromatograph of Hewlett Packard type (HP 6890 series). The apparatus was equipped with a capillary column, HP-5 (composed of 5% diphenyl and 95% dimethylpolysiloxane), measuring 30 m in length and 0.25 mm in diameter, with a film thickness of 0.25 µm. The Flame Ionization Detector (FID) was set to 260 °C and operated with a mixture of H2/Air gas, while the split-splitless injector was maintained at 275 °C. The injection volume was set at 1 µl. The injection is performed in split mode (leakage ratio: 1/50, flow: 66 ml/min), utilizing nitrogen gas at a flow rate of 1.7 ml/min. The column temperature is programmed to increase from 50 to 250 °C at a rate of 4 °C/min, maintaining a 5-minute period at the final temperature. The detection limit is below 1 ppm. The entire system is computer-controlled using the 'HP ChemStation' software, overseeing device operations and facilitating the progression of chromatographic [23].

2.6. Antibacterial effect of EO

The microbiological part was evaluated by applying by disk method targets the sensitivity of bacterial strains by means of the essential oil tested and allows for the determination of the diameter of the zone of inhibition. The minimum inhibitory concentration (MIC) determines the minimum concentration of the essential oil that inhibits growth or kills bacterial strains. The disc method is a method of diffusion of the products being tested on a paper disc, which makes it possible to qualitatively measure the sensitivity of the strains to the antimicrobial effects of the essential oils [24, 25].

The minimum inhibitory concentrations (MIC) of the essential oils were fixed according to the method reported by[26]. Because essential oils are immiscible in water and, consequently, in the culture medium, an emulsification process was conducted using a 0.2% agar solution. This method enables achieving a uniform distribution of essential

oils in the medium, optimizing the contact between the oils and microorganisms. Dilutions were prepared at ratios of 1/10, 1/25, 1/50, 1/100, 1/200, 1/300, and 1/500 using the agar solution. Subsequently, 1.5 ml of each dilution was aseptically added to test tubes containing 13.5 ml of Tryptic Soy Agar (TSA) solid medium, which had been sterilized by autoclaving for 20 minutes at 121 °C and then cooled to 45 °C. Dilutions were performed to achieve final concentrations of 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000, and 1/5000 (v/v). To ensure the proper dispersion of the essential oil in the culture medium, the tubes were agitated before being transferred into the petri dishes. Additionally, control samples consisting of the culture medium and the 0.2% agar solution alone were prepared. Inoculation was carried out by streaking with a calibrated platinum loop to ensure uniform inoculum collection. The inoculum was obtained from a 24-hour culture broth. Incubation took place at 37°C for 24 hours, and each experiment was conducted in triplicate[27].

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3. Results and Discussions

3.1. Yield

The essential oil of cloves is yellow, limpid, and turns brown if exposed to air. Its smell is spicy. The yield of the essential oil of clove is close to 13.004%. This result is in agreement with the data in the literature. Cloves from Comoros and Zanzibar yielded an essential oil yield of 13.4%. The essential oil content of cloves of Madagascar origin is 14.3%, while the essential oil clove content from Indonesia registered only 4.1%. This result is probably due to the great fragility of the buds coming from Indonesia [28].The difference in yield is influenced by several factors, mainly the origin, species, harvest period, drying time and extraction technique of essential oils [29–32].

3.2. Chemical composition

Clove essential oil is analyzed by gas chromatography (Figure 1). The obtained results allowed the identification of four constituents representing 99.56% of the essential oil in our clove sample: Eugenol, eugenol acetate, caryophyllene and D-Limonene (Table 1) represents these function constituents according to their percentages.

The chromatogram of the clove essential oil sample shows the dominance of eugenol as a major constituent with 92.94%, followed by eugenol acetate, which represents 5.03%, caryophyllene with 0.88%, and D-limonene with 0.71%. According to Bruneton (1995), essential oils are complex and variable mixtures of components belonging to two groups: the first group of terpenoids and the second group of aromatic compounds derived from phenylpropane [33]. It can be pointed out that our sample of EO has two components belonging to the class of terpenes, which are: eugenol acetate and D-limonene; a component of sesquiterpenes, caryophyllene; and a phenolic component, eugenol. A comparative study of the constituents of the essential oil cloves according to their origin country (Comoros, Madacascar, Zanzibar, and Indonesia) showed a greater presence of the major components (eugenol and eugenyl acetate) and other minority components:*Heptanone-2, -Copene, C-ylangene, β-Caryophyllene, α-Humulene, δ-Cadinene, Cubenene* and *Calamenene*[34]. The presence of these minority constituents is the result of a study carried out by C.P.V.-S.M. However, these clove constituents are present only in very low percentages (in the form of traces). Another study on EO extracted from leaves and clove buds from Cuba showed the presence of eugenol with 69.88%, eugenol acetate with 16.16%, and caryophyllene with 13% in EO buds. while the EO of clove leaves has only 78.1% eugenol and 20% caryophyllene [35]. Further research on the EO of clove leaves from India ensured the dominance of eugenol with 94.4 and β-caryophyllene with 2.9% [29]. The number of chemical molecules present in an essential oil can vary; alongside the predominant components, which typically range from 2 to 6, there are also minor compounds as well as a number of constituents present in trace amounts. The variability of the chemical components of essential oils is explained by the influence of several factors, which can be grouped into two categories: Intrinsic factors related to the species, the type of clone, the concerned organ, the interaction with the environment (type of soil or climate, etc.), and the degree of maturity of the concerned plant, or see the harvesting moments during the day[36]. Extrinsic factors are related to the extraction method[36]. The vegetative stage at the time of harvest remains a determining factor for the yield and constituents of the EO plant [37]. The storage of the raw material before distillation also influences the component and the crop of the EO. The storage time of essential oils can change their components or decrease the properties of the EO. Other works have highlighted the influence of the geographical origin of the raw material [38].

3.3. Sensitivity of bacteria to essential clove oil

Previous works on essential oils have shown good results on microorganisms. Essential oils are known for their inhibitory activities against bacteria, yeasts, and molds. In the current work, the antibacterial activity has been evaluated by the disc method was used to qualitatively determine the effect of essential clove oil on the tested bacteria.The methods of diffusion and dilution on Muller Hinton agar described by Remmal (1993) allow the quantitative evaluation of the antibacterial effect of the essential oil. The result of the first test was positive for the 4 strains; essential oil clove showed a significant inhibitory effect of the 3 bacteria tested (*E. coli*, *Staphylococcus aureus and Citrobacter spp* against *Pseudomonas aeruginosa* which showed a significant resistance to the product (Table 2). Table 3 shows that *Staphylococcus aueus* is the most sensitive, with an inhibition zone of 22 mm. This could be explained by the fact that G+ve bacteria have structural features that make them more susceptible to essential oils (Abdul Rahman et al., 2010). In contrast to Escherichia coli and Citrobacter s p, which are less sensitive, Sensitive strains of an essential oil can be identified based on the diameter of the inhibition zone, typically falling within the range of 8 to 13 mm [39].

In the current work, the Gram Pseudomonas aeruginosa bacterium is probably the most resistant; it shows a 7 mm inhibition zone. The work of Derwich et al. (2010) and Bari et al. (2010) prove that G-ve bacteria are highly resistant compared to G+ve[40–42]. This is explained by the presence of a layer of lipopolysaccharide (LPS) in G-bacteria that functions as an effective barrier against any biomolecule entering and also by the action of certain volatile components of the studied essential oil [43].*E. coli and Citrobacter sp* have been shown to be sensitive, even though they are negative gram. This observation is because of the different components of essential oils that have different degrees of

activity on G-ve and G+ve bacteria and also because of the variation of the chemical composition of essential oils according to several intrinsic and extrinsic factors [44–46]. Furthermore, the bacterial species do not have the same sensitivity to an antibacterial agent. In the same bacterial population, the individual sensitivity varies from one bacterium to another. Then the antibacterial effect is sometimes partial, and when the number of bacteria decreases, there is a resumption of bacterial growth. The composition of the culture medium also affects the antibacterial activity [44]. The antimicrobial activity of all essential oils is related to terpenoids and phenolic components [26, 47]. .

Table 1: Chemical composition of the essential oil cloves identified by (GPC).

Tr	Area	%	Name
6.415	256307	0.71	*D-Limonene*
12.664	318661	0.88	*Caryophyllene*
12.875	33520063	92.94	*Eugenol*
15.390	1814662	5.03	*Eugenol acetate*

Table 2: Anti-bacterial activity of the essential oil of cloves on the 4 strains tested

Tested Strains	Gram stain	Inhibition diameters Of essential clove oil (mm)
Pseudomonas aeruginosa	G-ve	06
Escherichia coli	G-ve	14
Staphylococcus aueus	G+ve	22
Citrobacter spp	G-ve	13 ,5
Cultures performed on Muller Hinton medium at 37 ° C for 24 hours		

Table 3: MIC of clove buds on the four studied bacteria in solid medium.

Petri dish	T	1	2	3	4	5	6	7
concentration of Clove EO on Medium (TSA)	+	1/500	1/300	1/200	1/100	1/50	1/25	1/10
Citrobacter spp	+	+	+	+	-	-	-	-
S*taphylococcus aureus*	+	+	+	+	-	-	-	-
Escherichia coli	+	+	+	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	+	-	-	-
(+) = Resistance. (-) = Sensitive								

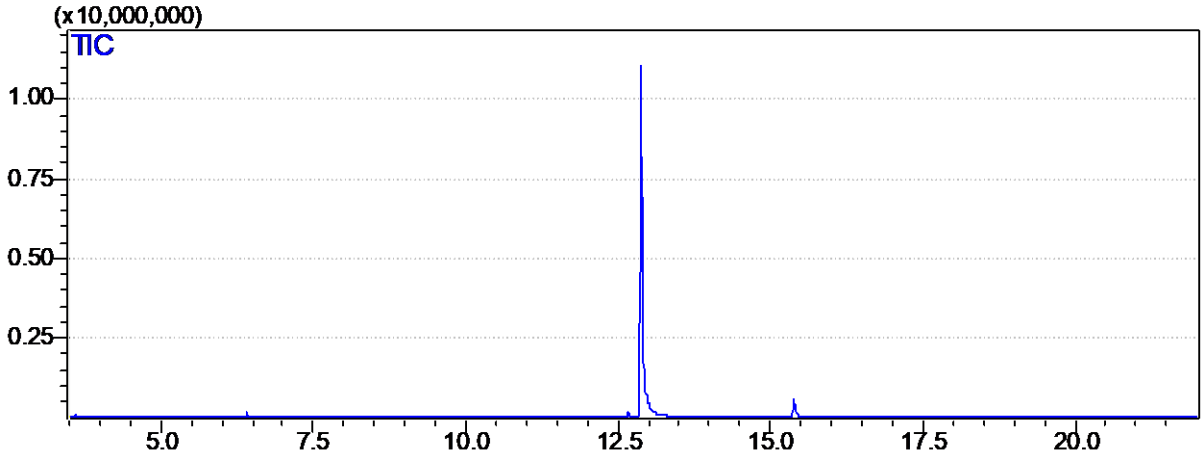

Figure 1: Chromatogram of EO clove fraction

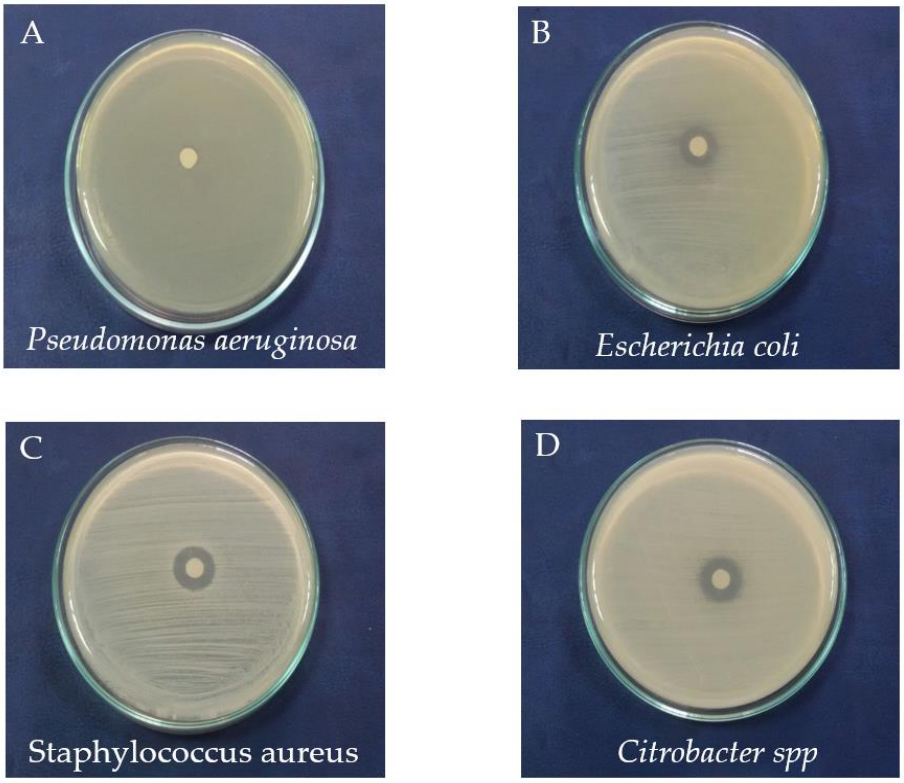

Figure 2: Growth inhibtion effect against bacteria to clove EO. **A**: *Pseudomonas aeruginosa showed resistance effect of cloves EO*. **B,C** and **D**: showed the sinsitive effecte of clove EO aginst *E. coli, Staphylococcus aureus and Citrobacter spp*.

Clove EO contains eugenol as a phenolic compound with a significant percentage of 92.94% in addition to 2 terpenoid components, which are: D-limonene with (0.71%) and caryophyllene as a sesquiterpene component with (0.88%), which interprets the sensitivity of *Staphylococcus aureus*, *E. coli* and *Citrobacter spp*. The biological activity of an essential oil also depends on the completeness of all the constituents of the EO and not only on its major constituents [48].The mode of action of the EO depends in the first phase on the type and characteristics of the active components, in particular their hydrophobic nature, which allows them to penetrate into the phospholipidic double layer of the membrane of the bacterial cell. This can induce a change in the conformation of the membrane. EO has a variety of toxic actions on bacteria, such as disruption of the cytoplasmic membrane, disruption of the proton motive force, electron leakage, and coagulation of the protein content of cells [49].It recalls that the method used to determine the minimum inhibitory concentrations (MIC) is that of dilution in Mueller-Hinton broth.

Macroscopic readings are done after 24 hours of incubation at 37 °C. The MIC is the lowest concentration of essential oil inhibiting bacterial growth, which is visible to the naked eye. All bacteria, with the exception of *Pseudomonas aeruginosa*, are sensitive to clove EO 1/50 <MIC<1 / 200.Table 3 summarizes the MIC of essential clove oil on the studied bacteria. For Clove EO, three trends emerge: When the MIC is less than 1/300, we are in the presence of *E. coli*. It is the most sensitive bacterium to essential clove oil (MIC <1/300). For the MIC below 1:200, we have *Citrobacte sp r*and *staphylococcus aureus*. These two bacteria are less sensitive than *E. coli*. The antibacterial effect of the studied EO decreases (MIC <1/50) when it concerns *Pseudomonas aeruginosa.*

4. Conclusions

Clove yielded an essential oil with a substantial yield of 13.004%. Chromatographic anal-ysis enabled the identification of 99.56% of its constituents. The primary component is eugenol, accounting for 92.94%, followed by eugenol acetate at 5.03% and caryophyllene at 0.88%. The findings from this study reveal that essential clove oil exhibits significant inhibitory activity against the tested bacterial strains. Citrobacter spp. Staphylococcus aureus, and E. coli showed sensitivity to clove essential oil at a concentration of 1/50 < MIC < 1/200, Pseudomonas aeruginosa showed resistance. Essential clove oil, with its remarkable anti-bacterial properties, holds potential applications as a food preservative and in various industries such as phytosanitary, cosmetics, and pharmaceuticals.

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