

Chemical Composition and Biological Activities of *Eugenia caryophyllata* Thunb Essential Oil

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Authors' contributions

This work was carried out in collaboration among all authors. Author FA designed the study, performed the statistical analysis, wrote the protocol and revised the manuscript. Authors BB, NA and BA performed the laboratory work and wrote the first draft. Author LB revised and approved the protocol. Authors SMH and RB managed the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study the antibacterial and antioxidant activities of the essential oil of *Eugenia caryophyllata* were investigated.

Study design: The study contains determination of the chemical composition of the essential oil of *E. caryophyllata* and the *in vitro* evaluation of the antibacterial and antioxidant activity of this oil.

Place and Duration of Study: The study was carried out at the laboratory of research on local animal products of Ibn-Khaldoun University, Tiaret, Algeria during the period from December 2020 to March 2021.

Methodology: The essential oil composition was characterized by gas chromatography/mass spectrophotometrical analyses. The antibacterial activity of this oil was tested against four pathogenic bacteria: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, *Bacillus cereus* ATCC 11778 by using disc diffusion method and agar incorporation method to determine the Minimum Inhibitory Concentration (MIC) of the tested oil. The antioxidant activity was evaluated by using DPPH radical scavenging, hydrogen peroxide scavenging assays and ferric reducing antioxidant power (FRAP) assay.

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Results: Our results have shown a greater antibacterial effect of *E. caryophyllata* essential oil against all the bacterial tested strains with inhibition zone diameters varied from 17.5 to 20.5 mm and minimal inhibition concentration (MIC) ranged between 0.8 μ l / ml and 4.4 μ l / ml, *B. cereus* and *S. aureus* are the most sensitive species with a MIC value of 0.8 μ l / ml, however *P. aeruginosa* is the most resistant species with a MIC value of 4.4 μ l / ml. The result of the antioxidant effect showed that the essential oil of *E. caryophyllata* is a powerful antioxidant that expresses a higher antioxidant activity than the standard antioxidants: gallic acid, vitamin C and BHT.

Conclusion: The obtained results suggest that the essential oil of *E. caryophyllata* has a strong antibacterial and antioxidant effect and it may be an alternative natural source medicine to prevent and treat many diseases caused by pathogenic bacteria and oxidative stress.

Keywords: *Eugenia caryophyllata*; Essential oil; chemical composition; antibacterial activity; Antioxidant activity.

1. INTRODUCTION

Biological redox reactions and environmental factors (pollution, smoke, sun light) generate free radicals, the excess of these substances can cause damage to living organisms and menace human health [1]. Due to the harmful effects that synthetic antioxidants may cause, such as toxicity (e.g. carcinogenicity) the interest in the discovery of natural antioxidants has increased considerably [2]. Recently, many species of bacteria have improved resistance as a consequence of excessive and improper use of antibiotics. The research to obtain new antibacterial compounds is vitally important [3] Hence, herbal medicines are considered as safe alternatives to synthetic drugs [4]. Plants have been used for medicinal purposes since ancient times and they play an important role in preventing various diseases and have received much attention from many researchers over the last few decades [5]. There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants [6]. Cloves (*Eugenia caryophyllata*) are dried aromatic unopened floral buds of an evergreen tree 10-20 m in height belonging to the family of *Myrtaceae*. Cloves have many therapeutic uses such as anti-inflammatory, antioxidant and antifungal, anti-emetic, stimulant, antifatulent and for treatment of dyspepsia. It is also used as an anodyne and antiseptic in dentistry. Essential oils from different plant sources carry out several biological activities, such as antibacterial, anticancer, anti-inflammatory, antimutagenic, antifungal, antioxidant and antiprotozoal ones [7]. The main objective of this study was to evaluate the chemical composition and the antibacterial

and antioxidant activities of *Eugenia caryophyllata* Thunb. essential oil (EO) in an attempt to contribute to the use of this oil as an alternative product.

2. MATERIALS AND METHODS

2.1 Vegetal Material

The dried buds of clove (*Eugenia caryophyllata* Thunb.) were purchased as commercial products from the local market and it was identified by a taxonomist in our institution.

2.2 Essential Oil Extraction

The flower buds of clove were dried at room temperature, hydrodistilled for 3 h using a Clevenger type apparatus (British Pharmacopoeia, 1998). The oil was dried over anhydrous sodium sulfate and stored in the dark at 2- 4°C. The hydrodistillation was done several times to have a sufficient volume of the essential oil to carry out the various tests of our study. The study was carried out at the laboratory of research on local animal products of Ibn-Khaldoun University, Tiaret, Algeria during the period from December 2020 to March 2021.

2.3 Essential Oil Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of *Eugenia caryophyllata* essential oil was carried out using a Shimadzu 2010 Plus gas chromatography coupled to a Shimadzu QP2010 Ultra mass selective detector. The separation was performed by means of a Restek Rxi-5MS capillary column, 60 m length, 0.25 mm i.d. and a 0.25 μ m phase thickness. The split mode was used. The oven was programmed as

follows: Initial temperature was 60 °C for 2 min, which was increased to 240 °C at 3 °C min⁻¹, 250 °C was maintained for 4 min. Helium (99.999%) was used as carrier gas with a constant flow-rate of 1 mL min⁻¹. Detection was carried out in electronic impact mode (EI); ionization voltage was fixed to 70 eV. Scan mode (40-450 *m/z*) was used for mass acquisition. The volatile compounds were identified by comparison of their retention indices (relative to C7-C30 alkane standards), and matching mass spectral data with those held in FFNSC1.2 and W9N11 library of mass spectra and literature comparison [8]. This part of the study has been performed in the department of chemistry, Faculty of Sciences, Karadeniz Technical University, Trabzon Turkey.

2.4 Evaluation of the Antibacterial Activity

2.4.1 Bacterial strains and inoculums standardization

Reference bacterial strains of *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 33862, *Bacillus cereus* ATCC 11778 were kindly provided by the university hospital center of Mustapha Pasha, Algiers (Algeria). Prior to the experiment the strain was maintained by subculture in the specific media (King A, Chapman, Mac conkey and Mossel) ; the inoculums suspensions were obtained by taking five colonies from 24 hours culture from each bacterial strain , then suspended in 5 ml of sterile saline (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to 1×10^8 cfu/mL).

2.4.2 Disc diffusion method

Diffusion method using filter paper disk was used for the screening of essential oil antibacterial activity. For this, sterile filter paper discs (6 mm in diameter) were impregnated with 10 µL of *E. caryophyllata* essential oil and then placed on the surface of Mueller Hinton plates previously inoculated with a bacterial suspension (10^8 CFU/mL) using a sterile cotton swab. Petri dishes were incubated for 2 h at 4°C to permit the diffusion of the essential oil from disk to medium. After incubation at $37 \pm 1^\circ\text{C}$ for 18-24 h the antibacterial effect was evaluated by measuring the diameter of the growth inhibition zone around

the discs with a ruler or a caliper and expressed in millimeters [9].

2.4.3 Minimum Inhibitory Concentration Measurement (MIC)

The MIC value of *Eugenia caryophyllata* Thunb.essential oil has been determined by using an agar incorporation technique method. The essential oil was incorporated into Mueller-Hinton media with increasing concentrations. The mixture was shaken moderately and poured into plates, then standard inoculums of 0.5 McFarland of each bacterial strain was inoculated and the plates were incubated at 37°C for 24 hours. The MIC was determined based on the lowest concentration of *E. caryophyllata* essential oil that inhibited the growth of the tested bacterial strains.

2.5 Evaluation of the Antioxidant Activity

2.5.1 Test of Ferric Reduction Antioxidant Power (FRAP test)

The ferric reducing power of the tested EO was determined according to the method of Oyaizu [10]. One ml of ethanolic solutions of EO at different concentrations (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 µg/ml) and the standards butylated hydroxytoluene (BHT), Vitamin C and gallic acid (100 to 200 µg/ml) as positive control were applied to 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 min. Then 2.5 ml trichloro acetic acid (TCA) (10%) was added to the mixture and it was centrifuged at 3000 rpm for 10 min. Then 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (1%). The absorbance was measured at 700 nm after allowing the solution to stand for 30 min at room temperature. The reducing potential of essential oil and standards (gallic acid, vitamin C and BHT) is expressed by values of 50% effective concentrations (EC₅₀) that correspond to the concentration of sample necessary to give an absorbance equal to 0.5 at 700 nm.

2.5.2 Test of DPPH • (2,2-diphenyl-1-picrylhydrazyl) scavenging activity

The scavenging activity of *Eugenia caryophyllata* Thunb essential oil for radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described by Tien, *et la* [11] with some modifications; 1.5mL of various dilutions of the

essential oil of *E. caryophyllata* prepared in ethanol solution were mixed with 1.5 mL of a 0.2mM ethanolic DPPH solution. After an incubation period of 30 min at 25 C°, the absorbance was determined at 517 nm, the wavelength of maximum absorbance of DPPH was recorded as a (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material (EO) and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = 100 (A (\text{blank}) - A (\text{sample}) / A (\text{blank}))$$

Antioxidant activity of *Eugenia caryophyllata* Thunb essential oil was expressed as IC₅₀, defined as the concentration of the tested material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid, gallic acid and BHT were used as a standard. All measurements were performed in triplicate.

2.5.3 Test of hydrogen peroxide scavenging capacity

The ability of the *E.caryophyllata* essential oil to scavenge hydrogen peroxide was determined according to the method of Kumar, et al [12]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH7.4). 01 ml of solution of essential oil at different concentration (01µg/mL to 0.06 µg/mL) in DMSO was added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *E.caryophyllata* essential oil and standard compounds were calculated:

$$\% \text{ Scavenged } [H_2O_2] = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of *E. caryophyllata* essential oil or standards.

2.6 Statistical Analysis

Results are expressed as mean ± SD of three individual experiments. Standard deviation (SD) was calculated using Microsoft excel.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of *Eugenia caryophyllata* Thunb Essential Oil

The essential oil obtained from *Eugenia caryophyllata* Thunb gave an average yield of 08.91 ± 0.02% w/w (from three independent hydrodistillation experiments). Our result is close to those obtained by Boukraa, et al [13] and Alzahrani, et al [14] where they reported a yield of 7.45%. The constituents of essential oils are important, as their qualitative and quantitative composition determines the characteristics of the oils and subsequent effect on their antimicrobial and antioxidant potential. The result of Gas chromatography-mass spectrometry (GC-MS) analysis indicated that fifteen components were identified in our essential oil according to their retention indices (RI) (Table 1). The main constituents of our oil were Benzylidene acetaldehyde (57.75%), 10, 12-Tricosadiynoic acid methyl ester (23.33%), followed by other molecules in low concentrations: Alloaromadendrene (7.99%), Pentacosane (4.03%), and Eugenol (2.34%). Our results are quite different from those obtained by other studies. Jirovetz, et al [15] reported that clove essential oil comprises in total 23 identified constituents, among them eugenol (76.8%) followed by β-caryophyllene (17.4%), α-humulene (2.1%), and eugenyl acetate (1.2%) as the main components. Alma, et al [16] found in their study that the major compounds of the essential oil from clove cultivated in mediterranean region of turkey were eugenol (87%), eugenyl acetate (8.01%) and β caryophyllene (3.56%). In a study done by Chaieb, et al [17] they found that the major components present in the clove bund oil were eugenol (88.6%), eugenyl acetate (5.6%), β-caryophyllene (1.4%). and 2-heptanone (0.9%). Saeed and Shahwar [18] found that eugenol (80.0%) is the major constituent of the essential oil of *Eugenia caryophyllata* followed by caryophyllene (10.27%) and eugenol acetate (5.03%). In a study done by Xu, et al [19] twenty-two components in essential oil from clove buds were identified and eugenol (76.23%) was the major component of the essential oil. The specific and quantitative composition of buds clove oil varies according to the genotype, growing location, climatic conditions and morphological characteristics. In addition, the chemical composition of essential oils depends on seasonal and geographic conditions, harvest

period and distillation technique [20]. The volatile components of a plant differ in their constituents and concentration, even between different cultivars of the same plant [21].

3.2 Antibacterial Activity

The essential oil of *E. caryophyllata* was evaluated for its antibacterial activity against four pathogenic bacteria. This oil was able to totally inhibit the growth of all the tested strains as illustrated in Table 2.

The results of the antibacterial activity of *Eugenia caryophyllata* Thunb essential oil extracted by hydrodistillation method are gathered in Table 02. The results obtained from the disc diffusion method, indicated that the studied oil exhibited a stronger antibacterial effect against all the tested

strains with inhibition zone diameters ranged between 17.5 and 20.5 mm. *Staphylococcus aureus* showed a strong sensibility with inhibition zone diameters of 20.5 mm. In contrast, the lowest antibacterial effect was obtained against *P. aeruginosa* with an inhibition zone diameter of 17.5 mm. The antibacterial effects of clove essential oil have been described in literature. Our results of inhibition zone diameter (IZD) of clove essential oil against *E. coli* (18.5±0.70) were greater than those noted by Oulkheir, et al [22] (16 mm), Prabuseenivasan, et al [23] (17 mm) and Bartkiene, et al [22] (11 mm). The IZD of clove essential oil against *S. aureus* (20mm) noted in this study was greater than those described by Prabuseenivasan, et al [23] (16 mm) and Bartkiene, et al [24] (16 mm). According to the obtained results, it was found that *Eugenia caryophyllata* essential oil has an important

Table 1. Chemical composition of *Eugenia caryophyllata* essential oil

No	Compound	RI	%
1	α -Pinene	944	0.27
2	Benzaldehyde	971	0.97
3	Trimethylbenzene	1003	0.45
4	Eucalyptol	1041	0.42
5	Benzenepropanol	1170	0.88
6	Endo-Borneol	1175	0.13
7	Benzylidene malonaldehyde	1229	0.37
8	Benzylidene acetaldehyde	1279	57.75
9	Bornyl acetate	1294	0.25
10	Eugenol	1365	2.34
11	<i>E</i> -caryophyllene	1434	0.17
12	Alloaromadendrene	1762	7.99
13	β -Cedrene	1772	0.63
14	Pentacosane	2506	4.03
15	10, 12-Tricosadiynoic acid methyl ester	2609	23.33

RI: retention indices (relative to C7-C30 alkane standards)

Table 2. The antibacterial potency of *Eugenia caryophyllata* Thunb essential oil against the tested strains expressed by the diameter inhibition zones and MIC

Bacterial strains	Means of clear zone (mm) \pm SD			MIC Values (μ l/ml)
	<i>E. caryophyllata</i> EO (10 μ L/dis)	Tetracycline (30 μ g/dis)	Amikacin (30 μ g/dis)	
<i>Bacillus cereus</i> ATCC 11778	20 \pm 0.81	12.34 \pm 0.43	20.06 \pm 0.28	0.8
<i>Staphylococcus aureus</i> ATCC 33862	20.5 \pm 0.57	17.12 \pm 0.16	26.69 \pm 0.11	0.8
<i>Escherichia coli</i> ATCC 25922	18.5 \pm 0.70	14.29 \pm 0.035	23.07 \pm 0.87	1.4
<i>Pseudomonas aeruginosa</i> ATCC 27853	17.5 \pm 0.70	10.61 \pm 0.65	20.70 \pm 0.049	4.4

MIC: minimum inhibitory concentration. Values given as μ L/mL

antibacterial effect against all the tested strains evaluated by incorporation technic method, Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* are the most sensitive species with a MIC value of 0.8 µl / ml while the Gram-negative bacteria *Pseudomonas aeruginosa* is the most resistant species with a MIC value of 4.4 µl / ml. This may be due to the nature of the Gram-negative bacteria wall which is formed mainly of lipoprotein, lipopolysaccharide and lipid. These compounds act as a barrier and limit the penetration of antimicrobial agents through the bacterial wall, unlike *S. aureus* and *B.cereus*, which have Gram-positive walls, free of these compounds [25]. The antibacterial activity of the essential oil of *Eugenia caryophyllata* is mainly related to the action of the constituents of this oil, eugenol is the main phenolic compound found in the oil of *E. caryophyllata*. This compound causes inhibition of extracellular enzyme synthesis and disruption of cell wall structure of bacteria. It also causes granulation and hyperacidity of the cytoplasm and depletion of intracellular ATP, oxygenated and hydrocarbon terpenes present in the oil accumulate in the microbial membrane causing loss of membrane integrity, leakage of cytoplasmic content, and cell lysis [26]. Several scientific researchers have investigated the antibacterial activity of clove oil. Smith-Palmer, et al [27] found in their study that the essential oil of *Eugenia caryophyllata* has an important antibacterial effect against the pathogenic strains involved in food contamination (*L. monocytogenes*, *E. coli* and *S. aureus*). A study done by Kim, et al [28] showed that eugenol, the main compound of clove essential oil, has an interesting antibacterial activity against *E. coli*, *S.typhimurium* and *L.monocytogenes*. Dormans and Deans [29] evaluated the antibacterial activity of six essential oils against 25 different genera of bacteria; all bacteria had a degree of sensitivity to the tested essential oils. Oils with higher activity were thyme, oregano and cloves. The results of a study done by Burst and Reinders [30] showed that the essential oil of *E. caryophyllata* is effective against non-toxicogenic strains of *E. coli* O157: H7. Similarly, in another study conducted by Lopez, et al [31] clove essential oil was found to be active against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative bacteria (*E. coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *P.aeruginosa*). Sabahat and Perween [32] found that clove essential oil has a strong antibacterial activity

against 100 Gram-negative bacilli belonging to 10 different species, namely *E. coli* (36 strains), *P. mirabilis* (6 strains), *P. aeruginosa* (10 strains), *E. aerogenes* (5 strains), *K. ozaenae* (2 strains), *K. pneumoniae* (24 strains), *S. marcescens* (4 strains), *S. typhi* (3 strains), *S. dysenteriae* (5strains), and *V. cholerae* (5strains).Boukraâ, et al [13] demonstrated that the essential oil of *Eugenia caryophyllata* has an antibacterial effect against *Pseudomonas aeruginosa* ATCC 27853 with a MIC value of 2.2 µl/ml. In a similar study Alzahrani, et al [14] found that the essential oil of *Eugenia caryophyllata* was the most effective against *Aspergillus flavus* and *Aspergillus niger* than the other tested essential oils: *Thymus vulgaris*, *Thymus fontanesii*, *Origanum vulgare* and *Geranium*. Singh, et al [33] demonstrated in their study that *Eugenia caryophyllata* essential oil has antibacterial activity against Gram-negative bacteria: *Escherichia coli* (MCCB 0016), *Klebsiella pneumoniae* (MTCC 2405) and *Pseudomonas aeruginosa* (ATCC 27853).Xu, et al [19] found in their study that the essential oil of *E. caryophyllata* exhibited strong antibacterial activity against *Staphylococcus aureus* ATCC 25923 with a minimum inhibitory concentration (MIC) of 0.625 mg/mL and the antibacterial effects depended on its concentration and action time. Oulkheir, et al [34] have shown that the essential oil of *Eugenia caryophyllata* has antibacterial activity against four Gram-negative bacteria [*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella. spp* and two Gram-positive bacteria (Group D *Streptococcus* and *Staphylococcus aureus* (ATCC25923).In a study done by Atanasova-Pancevska, et al [35] they evaluated the antimicrobial activity of freshly isolated essential oil, and commercial essential oil of *E. caryophyllata* and they were compared to pure eugenol the most active and important component. They were found that eugenol had the strongest antimicrobial effect against the tested microorganisms, with the exception of *E. coli*, where the freshly isolated essential oil had the strongest bactericide effect. The commercial essential oil had the weakest action against all the tested microorganisms. Hsaine, et al [36] reported that *E. caryophyllata* essential oil was active against *S. oralis* and they suggested that this oil may be used as an alternative to synthetic antibiotics. Sohilaït, et al [37] investigated the antibacterial activity of essential oils isolated from various parts (buds, leaves and stems) of *Eugenia caryophyllata* against Gram-positive

bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*). The result of this study showed that clove essential oil inhibited bacterial growth but their effectiveness varied and the oil from the stem of clove exhibited the strongest activity against the selected bacterial strains. This showed that the antibacterial activity related to the components of eugenol contained in the cloves where the stem contains the highest compared to the leaf and bud.

3.3 The Result of the Antioxidant Effect

3.3.1 The result of the antioxidant effect evaluated by FRAP test

The antioxidant activity of *E. caryophyllata* essential oil evaluated by the ferric reducing antioxidant power test (FRAP) revealed that this oil has an important antioxidant activity (Fig. 1). This reducing power is much higher than those of the standards antioxidants, gallic acid, vitamin C and BHT respectively a similar result has been found by Singh, et al [33] and Ghadermazi, et al [38] they found in their study that clove EO showed the highest ferric reducing capacity at all concentrations than BHT.

In a study done by Viuda-Martos, et al [39] they found that clove EO, showed the highest ferric reducing capacity in terms of Trolox concentrations. Gülçin, et al [40] showed that reducing power of clove EO and standard antioxidants (at 15-45 µg/ml) in the following order: Clove oil > BHA ≈ BHT > α-tocopherol > trolox. The strong activity of clove EO can be due to the presence of eugenol which is known to have antioxidant activity [41].

3.3.2 The result of the antioxidant activity evaluated by the DPPH test

Fig. 2 represents the IC₅₀ values of *Eugenia caryophyllata* Thunb essential oil and standard antioxidants, BHT, vitamin C and gallic acid.

The results showed that the essential oil of *Eugenia caryophyllata* Thunb has a very important antioxidant effect. This activity is much higher than those of standards; gallic acid, vitamin C and BHT this result is similar to that obtained by Viuda-Martos, et al [39] they found that *E.caryophyllata* essential oil has a scavenger effect of the radical DPPH better than those of the standard antioxidants vitamin C and

BHT. The results of a study done by Chaieb, et al [17] showed that clove essential oil has a very strong antiradical activity evaluated by the DPPH test compared to synthetic antioxidant butylated hydroxytoluene. Some studies have shown that the antioxidant capacity of *Eugenia caryophyllata* essential oil could be attributed to the presence of some potentially active phenolic compounds such as eugenol. The result of DPPH test of a study done by Gülçin [42] showed that eugenol has a very high antioxidant activity (IC₅₀ = 16.06 µg / ml) in comparison with the standard antioxidants butylated hydroxyanisole (BHA) (IC₅₀ = 25.51 µg / ml), BHT (IC₅₀ = 34.01 µg / ml), α-tocopherol (IC₅₀ = 33.85 µg / ml), and Trolox (IC₅₀ = 86.93 µg / ml). In a study by Jirovetz, et al [15] the antioxidant activity of a commercial rectified clove leaf essential oil (*Eugenia caryophyllata* Thunb.) and its main constituent eugenol was tested. The result of this study revealed that the essential oil from clove demonstrated scavenging activity against the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical at concentrations lower than the concentrations of eugenol, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA). This essential oil also showed a significant inhibitory effect against hydroxyl radicals and acted as an iron chelator. With respect to lipid peroxidation, the inhibitory activity of clove oil determined using a linoleic acid emulsion system indicated a higher antioxidant activity than the standard BHT. In a study done by Mahboubi and Mahboubi [43] the Antioxidant activity of *E. caryophyllata* essential oil was evaluated by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene/linoleic acid system and strong activity were found for this oil. Raiesi, et al [44] reported that clove EO showed a remarkable capacity in scavenging of radicals, which was highly comparable with BHT. Ghadermazi, et al [38] found that clove EO showed higher DPPH radical scavenging than BHT at the same dose. This important antioxidant capacity of *Eugenia caryophyllata* Thunb. EO could be attributed to higher content of phenolic components such as eugenol and eugenyl acetate and their hydrogen donating ability by which they are considered to be potent free radical scavengers.

3.3.3 The result of the test of Hydrogen Peroxide scavenging capacity

The result of hydrogen peroxide scavenging capacity of *Eugenia caryophyllata* Thunb essential oil and standard antioxidants, vitamin C

and gallic acid was illustrated in the following figure:

Biological systems can generate hydrogen peroxide by many oxidizing enzymes such as superoxide dismutase. It is known that H₂O₂ is toxic and induces cell death *in vitro* it can attack many cellular energy producing systems [45]. It acts as a toxicant to the cell by converting itself into hydroxyl radical in the presence of metal ions and superoxide anion and also produces singlet oxygen through reaction with superoxide

anion or chloramines in living systems. Hydrogen peroxide can degrade heme proteins (any protein containing an iron-porphyrin (heme) prosthetic group resembling that of hemoglobin), such as hemoglobin, to release Fe ions [46]. Thus, the removing of H₂O₂ is very important for antioxidant defense in cell or food systems [47]. In our study the result of scavenging activity of hydrogen peroxide (Fig. 3) showed that *Eugenia caryophyllata* essential oil showed the highest hydrogen peroxide scavenging capacity compared with the standards. The hydrogen peroxide scavenging effect of clove oil and

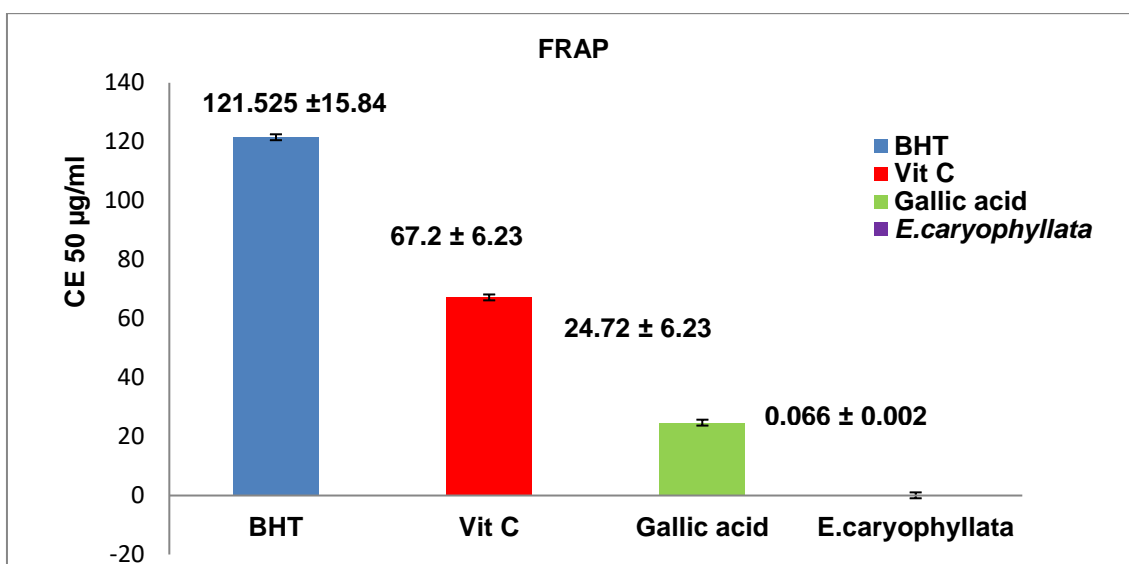


Fig. 1. Reducing power of *E. caryophyllata* and standard antioxidants

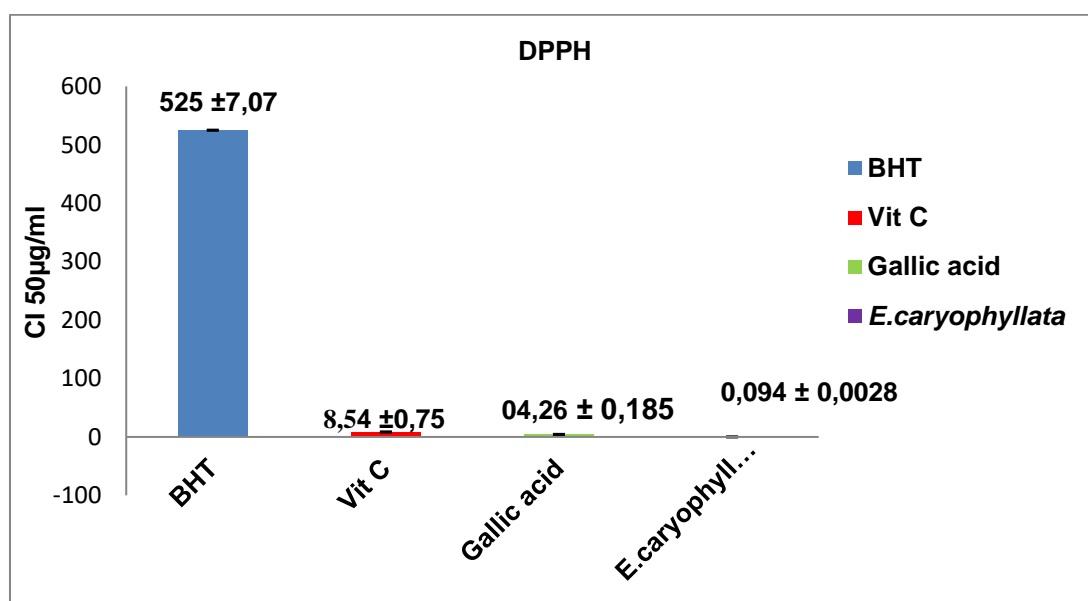


Fig. 2. IC50 of *E. caryophyllata* and standard antioxidants

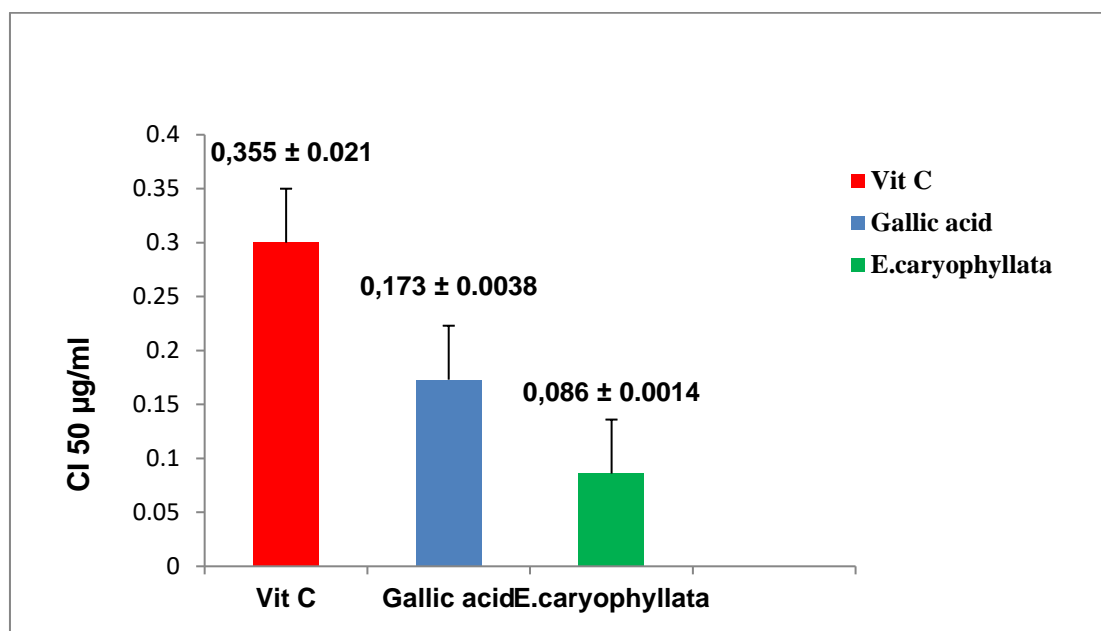


Fig. 3. Concentrations responsible for 50% of inhibition of H₂O₂ (IC₅₀)

standards compounds decreased in the following order *E.caryophyllata* > gallic acid > vitamine C > BHT, similar result has been obtained by Gulcin, et al [40] they reported that clove essential oil has an important and greater hydrogen peroxide scavenging activity compared to the standards BHT, BHA and α -tocopherol. Clove essential oil has been reported in previous studies as one of the strongest antioxidants, even higher than some synthetic antioxidants like BHT [48-49]. Other study done by Abozid and Sayed [50] they found that acetone extract of clove and clove essential oil have a liver and kidney protective effects against oxidative damage induced by H₂O₂. They suggested that this activity might be due to the effect of active compounds which found in essential oil and plant extract.

4. CONCLUSION

The results obtained in this study showed that the essential oil of *Eugenia caryophyllata* Thunb has a strong antibacterial activity against all the bacterial tested strains and this oil is a powerful antioxidant that expresses a higher antioxidant activity than the standard antioxidants: vitamin C, BHT and gallic acid. These results suggest that *Eugenia caryophyllata* Thunb essential oil could be alternative natural source medicine to prevent and treat many diseases caused by pathogenic bacteria and oxidative stress.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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