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Review

Clove Essential Oil (*Syzygium aromaticum L. Myrtaceae*): extraction, chemical composition, food applications and essential bioactivities for human health

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Abstract: Clove (*Syzygium aromaticum L. Myrtaceae*) is an aromatic plant widely cultivated in tropical and subtropical countries, rich in volatile compounds and antioxidants such as eugenol, β-caryophyllene, and α-humulene. Clove essential oil has received considerable interest due to its wide application in the perfumery, cosmetic, health, medical, flavoring, and food industries. Clove essential oil has relevant biological activities to human health, including antimicrobial, antioxidant, and insecticide. This review describes the effect of the extraction method (hydrodistillation, steam distillation, ultrasound-assisted extraction, microwave-assisted extraction, cold pressing, and supercritical fluid extraction) on the chemical composition of essential oil and its correlation with their biological activities. Likewise, are summarized the main compounds and their reported biological activities.

Furthermore, the main applications in clove essential oil in the food industry are presented. Finally, this review presents the new biological activities such as anti-inflammatory, analgesic, anesthetic, antinociceptive and anticancer, which are beneficial for human health. This review aims to compile the effect of different methods of extracting clove essential oil on chemical composition, food applications, as well as a current description of biological activities of interest to human health. Biological activities have increased interest in research into this essential oil and its future applications in the food or pharmaceutical industry.

Keywords: Clove essential oil; biological activity; chemical composition, extraction.

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1. Introduction

The essential oils (EOs) are complex mixtures of volatile compounds characterized by a strong odor from secondary metabolites of aromatic plants [1,2]. The EOs are liquid, uncolored, soluble in organic solvents, and lipid-soluble, less dense than water. It is estimated that of the 3000 EOs known, only 10% of EOs are used commercially. The EOs are recognized for several biological activities (bactericidal, antiviral, and fungicidal), medicinal, and aromatic properties. Amongst their multiple uses, they are considered suitable substances to replace chemical additives in food preservation and as antimicrobial, analgesics, sedatives, anti-inflammatory drugs, spasmolytic agents, and local anesthetics [1,2]. In addition, EO and its components are used in the production of perfumes, makeup, health, dental, agricultural products, and alternative therapies. [1].

The EOs are obtained from different plant organs. The most widely used are flowers (*Jasminum spp.*, *Rosa spp.*, *Viola spp.*, *Lavandula spp.*, *S. aromaticum L.*), leaves (*T. vulgaris*, *Eucalyptus spp.*, *L. graveolens*, *M. spicata*, *O. basilicum*, *S. rosmarinus*, *C. citratus*, and *M. alternifolia*), fruits (*I. verum*, *C. sinensis*, *C. limon*), seeds (*E. cardamomum*, *C. arabica*, *P. nigrum L.*), bark (*Cinnamomum spp.*), and roots [1,3]. The EOs are very complex natural mixtures that can contain more than 20 components at different concentrations. Terpenes, terpenoids, aromatic, and aliphatic components are the main constituents. The main components regularly constitute 20-70% of the total concentration, while the rest correspond to the minority components [1,2]. The relative concentration of these principal compounds determines the biological properties of the EOs [1,2]. However, their composition and extraction yield varied according to climate, soil composition, plant organ, age, cycle stage, extraction method selected, and extraction conditions [1,3–9]. This review focuses on differences in the chemical composition of Clove essential oil (CEO) obtained through different extraction methods, the main bioactivities of interest to human health and food applications.

2. Clove essential oil (CEO)

Cloves (*S. aromaticum L.*) belong to the *Myrtaceae* family, which has more than 3000 species and 130-150 genera such as the Myrtle, eucalyptus, cloves, or guava family. Clove is an aromatic flower cultivated in Madagascar, Sri Lanka, Indonesia, and China [4,5,10]. Several reports suggest that *S. aromaticum L.* contains approximately 15-20% wt. of EO. CEO contains a high amount of phenolic compounds with several biological activities, including antibacterial, antifungal, insecticidal, and antioxidant properties [4,5,10,11]. The FDA classified CEO as generally recognized as safe (GRAS); for this reason, they are used in perfumes, cosmetics, sanitary, medicines, and foods [6,10].

2.1. CEO Composition

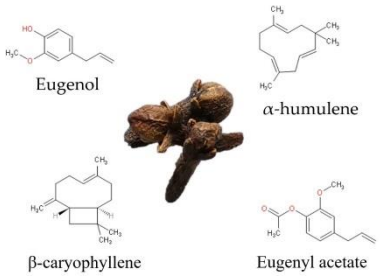

Figure 1. Chemical structure of the main compounds of the clove essential oils (*S. aromaticum L.*).

At least thirty compounds have been identified in the CEO [10]. Eugenol is the major compound in CEO, with at least 50% of CEO. The remaining 10 to 40% of CEO are eugenyl acetate, β-caryophyllene, and α-humulene (Figure 1). Less than 10% corresponds to minor or trace components such as diethyl phthalate, caryophyllene oxide, cadinene, α-copaene, 4- (2-propenyl) -phenol, α-cubebene, γ-muurolen, among others, as shown in Table 1 which shows the general composition reported by different authors [1,2].

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Table 1. Comparison of the composition of CEOs reported by different authors

	Compound	HD [10]	SD [10]	MA HD [10]	MA SD [10]	SFE 30 °C 20 MPa [5]	SFE 40 °C 30 MPa [5]	SFE 50 °C 10 MPa [5]	SO [5]	UA SFE [8]	MA SD outside [12]	MA SD inside [12]	Coaxial MA HD [13]
1	Eugenol	87.26	82.65	88.8	83.39	54.58	55.14	57.36	57.24	59.18	65.36	71.84	66.9
2	Eugenyl acetate	10.43	15.63	7.46	14.34	20.55	20.32	22.34	19.37	18.6	5.71	9.49	2.7
3	β-Caryophyllene	1.35	0.91	2.65	1.37	17.32	15.52	13.99	17.5	15.35	24.62	15.6	24.8
4	α-humulene	0.19	0.13	0.4	0.21	2.26	2.02	1.9	2.03	1.93	-	0.01	3.1
5	Diethyl Phthalate	-	-	-	-	0.35	1.43	-	-	-	-	-	-
6	Caryophyllene oxide	0.2	0.17	0.19	0.22	0.88	0.93	0.89	0.49	-	-	-	-
7	Cadinene	-	-	-	-	0.68	0.64	0.68	0.72	-	-	-	-
8	α-Copaene	-	-	-	-	0.63	0.51	0.65	0.93	-	0.01	tr	-
9	4-(2-propenyl)-Phenol	-	-	-	-	0.48	0.46	0.46	0.52	-	-	-	-
10	α-Cubebene	-	-	-	-	0.51	0.43	0.32	-	-	-	-	-
11	γ-Muurolen	-	-	-	-	0.4	0.36	0.65	-	-	-	-	-
12	Isoaromadendrene epoxide	-	-	-	-	0.25	0.25	-	-	-	-	-	-
13	Tetracyclo [6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	-	-	-	-	0.27	0.25	0.37	0.54	-	-	-	-
14	Tetratetracontane	-	-	-	-	-	0.22	–	–	-	-	-	
15	α-Muurolene	-	-	-	-	0.27	0.22	–	–	-	-	-	
16	α-Farnesene	-	-	-	-	0.23	0.2	0.2	0.16	-	-	-	
17	Chavicol	0.31	0.24	0.22	0.22	-	-	-	-	-	0.13	0.1	-
18	Methyl salicylate	0.08	0.08	0.1	0.07	-	-	-	-	-	0.1	0.08	-
19	Benzaldehyde	0.07	0.08	0.06	0.05	-	-	-	-	-	-	-	-
20	Benzyl acetate	0.05	0.04	0.06	0.05	-	-	-	-	-	0.02	0.02	
21	2-Nonanone	0.04	0.04	0.05	0.04	-	-	-	-	-	-	-	
22	Benzyl benzoate	0.02	0.03	0.01	0.02	-	-	-	-	-	-	-	
23	Ethyl benzoate	0.01	0.01	0.01	0.01	-	-	-	-	-	0.02	0.02	
24	1,8-Cineole	-	-	-	-	-	-	-	-	-	0.03	0.03	
25	1.3.8-p-Menthatriene	-	-	-	-	-	-	-	-	-	0.03	0.01	
26	2-Heptanone	-	-	-	-	-	-	-	-	-	0.01	tr	
27	2-Heptyl acetate	-	-	-	-	-	-	-	-	-	0.03	0.01	
28	2-Nonanol	-	-	-	-	-	-	-	-	-	0.01	tr	
29	6-Methyl coumarin	-	-	-	-	-	-	-	-	-	0.03	tr	
30	Acetophenone	-	-	-	-	-	-	-	-	-	0.03	0.01	
31	caryophyllene alcohol	-	-	-	-	-	-	-	-	-	0.04	tr	
32	Epizonarene	-	-	-	-	-	-	-	-	-	0.07	0.05	
33	Germacrene D	-	-	-	-	-	-	-	-	-	0.14	0.09	
34	Methyl benzoate	-	-	-	-	-	-	-	-	-	0.01	tr	
35	Methyl eugenol	-	-	-	-	-	-	-	-	-	0.04	tr	
36	Methyl undecanoate	-	-	-	-	-	-	-	-	-	0.02	tr	
37	N-butyrate de citronellyl	-	-	-	-	-	-	-	-	-	0.01	tr	
38	Viridiflorol	-	-	-	-	-	-	-	-	-	0.02	-	
39	Z-Nerolidol										0.06	0.02	

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40	α-pinene	-	-	-	-	-	-	-	-	-	tr	0.03	-
41	β-cubebene	-	-	-	-	-	-	-	-	-	0.02	tr	-
42	β-Pinene	-	-	-	-	-	-	-	-	-	tr	tr	-
43	γ-Gurjunene	-	-	-	-	-	-	-	-	-	2.35	1.65	-
44	δ-Cadinene	-	-	-	-	-	-	-	-	-	0.22	0.2	-
45	ϱ-Acoradiene	-	-	-	-	-	-	-	-	-	0.03	0.01	-
46	ϱ-Cymene	-	-	-	-	-	-	-	-	-	tr	0.07	-

Hydro Distillation HD, Steam Distillation SD, Supercritical Fluid Extraction SFE, Microwave-assisted MA, Solvent Extraction SO.

2.1.1. Eugenol

Eugenol is a phenylpropanoid compound found in *S. aromaticum L.*, *Cinnamomum spp.*, *P. nigrum*, *Z. officinale*, *O. vulgare*, and *T. vulgaris* [14]. Eugenol is a volatile compound that varies from colorless to light yellow, has low water solubility (approximately 2460 mg/L at 25 °C), a strong odor, and an intense flavor. Among the reported biological activities of eugenol is insecticide activity, antimicrobial, anti-inflammatory, wound healing, antiviral, antioxidant, and anticancer [7,8,14–18].

Banerjee and coworkers [18] observed the anti-inflammatory and wound healing ability of a clove oil emulsion in murine experiments. The eugenol-treated skins showed re-epithelialization twenty days after the wound. These results were similar to a diclofenac gel and a neomycin cream currently used to control inflammation and heal wounds [18]. Other research reported that eugenol does not modify interleukin 8 (IL-8) levels in human skin cells (HaCat), but instead targets other pro-inflammatory cytokines [18]. The inhibition of voltage-gated Na^+ channels modulates the analgesic effects of eugenol. Eugenol produced the activation of transient receptor potential cation channel V1 ($TRPV_1$), a similar effect to local anesthetics such as lidocaine [19].

Eugenol has shown potential anticancer activity against colon, gastric, breast, prostate, melanoma, leukemia, or skin cancer [14]. Eugenol inhibits tumor proliferation and formation, increases the reactive oxygen species (ROS), generates apoptosis, and has a genotoxic effect in different cancer cells [14,20–22].

2.1.2. Eugenyl acetate

Eugenyl acetate is a phenylpropanoid derivative of eugenol, which exhibits antibacterial, anticancer, antimutagenic, antioxidant, and anti-virulence activities [5,10,18,23,24]. It showed inhibition of 94.5%, 92.1%, and 100% at 200 μg/ml against *F. moniliforme, H. oryzae, and R. solani* respectively [25]. Eugenyl acetate was described as a potent antioxidant agent, it showed 90.30% DPPH free radical scavenging at 35 μg/ml, while NO free radical scavenging showed 89.30% at 60 μg/ml. On the other hand, exhibited a potential antifungal activity against *Candida spp.* and inhibited the biofilm formation capacity [24]. Pasay et al. (2010) reported high toxicity against human scabies mites [23]. Eugenyl acetate also showed 100% toxicity against *A. salina* at 0.3 μg/mL. The low lethal concentrations obtained for eugenyl acetate could also indicate toxicity to other organisms such as disease vector insect larvae [23]. Eugenyl acetate showed an LC_{50} of 0.1 mg/ml against *A. aegypti*, showing potential utility as larvicides [26]. The mechanism of larvicidal action is mainly due to interference with the octopaminergic system [27]. The beneficial antioxidant, antimicrobial, antitumor, and potential larvicidal properties have increased the demand by the food and cosmetic industries [5,10].

2.1.3. β-caryophyllene

The *β*-caryophyllene is a sesquiterpene found in clove (*S. aromaticum L.*), hemp (*C. sativa L.*), black pepper (*P. nigrum L.*), *Eugenia cuspidifolia*, *Eugenia tapacumensis,* and guava leaves (*Psidium cattleianum Sabine*) [28,29]. The *β*-caryophyllene is insoluble in water but is soluble in ethanol. The *β*-caryophyllene has demonstrated antimicrobial, anticarcinogenic, anti-inflammatory, antioxidant, anxiolytic-like and local anesthetic effects, and

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anticancer properties, including prostate, breast, pancreatic, skin, leukemia, lymphatic, cervical, and cervix cancer [5,8,12,28–32]. These studies suggested that the *β*-caryophyllene decreased the cell growth and proliferation in colon cancer, interfering with the stages of tumor development and reducing the activity of extracellular matrix metalloproteinases. The *β*-caryophyllene can act as a chemosensitizer, improving the effectiveness of drugs against tumor cells [28,29,31]. β-caryophyllene is effective against *An. subpictus* (LC_{50} = 41.66 µg/ml), followed by *Ae. albopictus* (LC_{50} = 44.77 µg/ml) and *Cx. tritaeniorhynchus* (LC_{50} = 48.17 µg/ml). Dahham et al. [32] reported that the radical scavenging ability of the *β*-caryophyllene was approximately 1.25 µM and 3.23 µM by the DPPH and FRAP scavenging methods, respectively. These results indicated the *β*-caryophyllene has high antioxidant activity.

2.1.4. *α*-humulene

The *α*-humulene is a sesquiterpene found in *S. aromaticum L.*, *Senecio brasiliensis*, *Humulus lupulus L*, or *Salvia officinalis L.* This compound has shown anti-inflammatory and antitumor activities in lung, colon, prostate, and breast cancer. Some reports suggested that the *α*-humulene demonstrated antiproliferation and alteration of the mitochondrial cell membrane in colon cancer cells [8,28,33–37]. It can also improve the antiproliferative of cytostatic drugs and other anticancer bioactivities [33,35]. Linh Thuy Nguyen et al. [37] reported that *α*-humulene inhibits the activity of the CYP3A enzyme in human and rat liver microsomes, which is a drug-metabolizing enzyme [37]. Fernandes et al. [38] reported that oral treatment with *α*-humulene and β-caryophyllene (50 mg/kg) produced comparable anti-inflammatory effects with dexamethasone treatment in model mice and rats. *α*-humulene prevents the generation of TNF*α*, while *β* -caryophyllene only decreases its release. In addition, they reduce the production of prostaglandin E_2, the inducible expression of nitric oxide synthase, and cyclooxygenase. *α*-humulene exhibited larvicidal activity against three vector mosquitoes *An. Subpictus* (LC_{50} = 10.26 µg/ml), *Ae. albopictus* (LC_{50} = 11.15 µg/ml) and *Cx. tritaeniorhynchus* (LC_{50} = 12.05 µg/ml), however, it is safe for G. affinis (LC_{50} = 1024.95 µg/ml). *α*-humulene showed larvicidal LC_{50} of 20.86 µg/ml and EC_{50} values on *H. armigera* eggs were 77.10 µg/ml. *α*-humulene has also been evaluated against beetle species that attack stored products [26,39,40]. The toxicity of *α*-humulene against *S. granaries* was LC_{50} = 4.61 µL/mL. *α*-humulene reduced the respiration rate of *S. granarius* at 1 and 3 h after exposure [41].

3. Extraction of EOs

The EOs are extracted from plant raw material by conventional methods, including hydrodistillation (HD), steam distillation (SD), solvent extraction (SO), and cold pressing; or by advanced/innovative methods, such as ultrasound-assisted extraction(UAE), microwave extraction(MAE), ohmic heating assisted extraction, and supercritical fluid extraction (SFE) (Figure 2)[3–5].

3.1. Conventional/classical extraction methods

The conventional extraction methods are based on the distillation process by heating to recover EOs from a plant matrix [3]. The extraction is done by injecting steam or water, which crosses the plant matter from the bottom up and carries the volatile materials together with the water as if they were a single component. The EOs are immiscible in the water, being easily removed by decanting [3]. The HD and SD methods are the most extensively used procedures for extracting EOs because they are easy to operate processes with high reproducibility and do not use organic solvents, making this process economical and environmentally friendly [5,10,42]. However, these methods present several drawbacks, including a long extraction time, use of large volumes of solvent and energy, the potential thermal degradation, hydrolysis, and water solubilization of some of the constituents originated by long contact with boiling water or steam overheating and loss of polar molecules in the water extraction [3]. Additionally, these conventional extraction methods

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have few adjustable parameters to control the selectivity of the extraction process, impacting the purity of EOs [8,10]

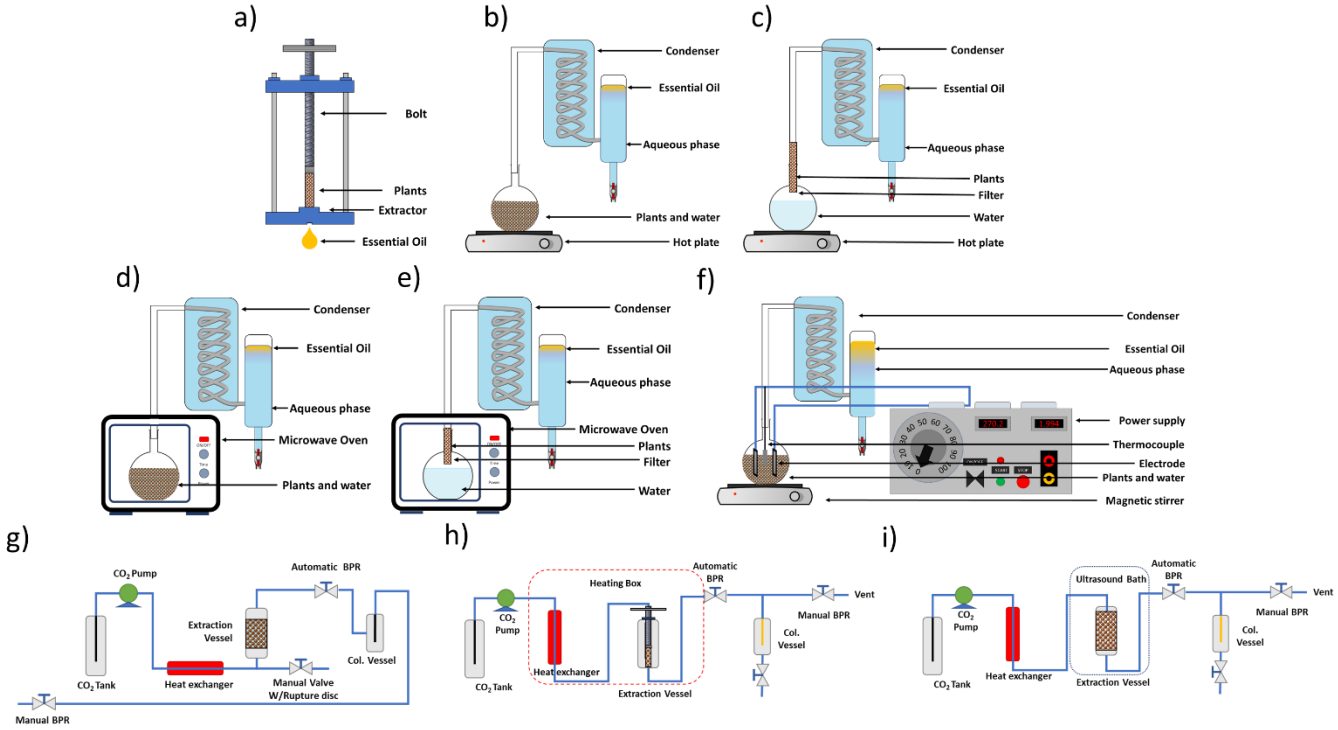

Figure 2. Main extraction methods: a) cold pressing, b) hydrodistillation, c) steam distillation, d) microwave-assisted hydrodistillation, e) microwave-assisted steam distillation, and f) ohmic heating assisted extraction, g) supercritical fluid extraction, h) supercritical fluid extraction assisted by cold pressing, and i) supercritical fluid extraction assisted by ultrasound.

3.2. *Advanced/innovative extraction methods*

Advanced extraction methods include microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), subcritical extraction fluids, and supercritical fluid extraction (SFE) enhance the extraction yield, reduce the extraction time and energy consumption, and enhancing the quality of EOs [3,8,10]. These methods improve EO extraction performance by applying microwave or ultrasonic energy capable of destroying the cell wall of the plant matrix, allowing the compounds to flow better from the biological material [3,9,12]. A. Kennouche et al. [12] carried out microwave extractions of CEO, the oils obtained contained a high percentage of eugenol (65-71%), which maintained their antimicrobial and antioxidant properties. MAE reduced energy consumption, heating time, and oil degradation [10,12,13]. The ultrasonic waves from 20 to 100 kHz can be applied by direct contact with the sample (ultrasound system coupled with a probe) or indirectly through the walls of the sample container (ultrasonic bath systems). The acoustic power and wave frequencies applied in liquid media can produce the acoustic cavitation phenomena, where bubble creation, expansion, and implosion enhance the selectivity of target molecules. UAE improves extraction performance between 1-4 times more than HD and 2 times more than SD [6,43,44].

On the other hand, SFE is used to selectively remove chemical compounds using a solvent in its supercritical state, typically carbon dioxide [45]. Also, cosolvents such as methanol, ethanol, or water change the density, viscosity, and solvation power of the supercritical solvent, promoting the extraction of specific compounds [3,8]. SFE process reduces the undesirable organic pollutants, toxins, and pesticide residues present in the biological material. The EOs extracted by innovative methods have superior quality relative to those obtained using the conventional extraction methods. The selectivity can be adjusted by changing the extraction conditions to obtain fractions with desirable compounds [8]. However, these technologies require the acquisition of expensive equipment, highly trained operators, and high operation and maintenance costs [3].

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3.3. *Effect of the extraction method in CEO composition.*

It is well known that the difference in the composition of EOs depends on variety, agroecological conditions, pretreatments, processing conditions, and extraction methods. Table 2 shows a summary of the impact of different extraction methods and conditions on the composition of CEOs.

Table 2. Effect of the extraction method in the CEOs' composition.

Method	Extraction Conditions	Eugenol	β-Caryophyllene	α-Humulene	Eugenyl acetate	Final EO yield (%)	Visual appearance
HD [13]	360 min 100 °C Clove:Water 1:5	87.10	5.10	0.60	6.40	5.90	-
HD [46]	Comercial	85.50	7.40	1.50	2.7	-	-
HD [25]	240 min 100 °C Clove:Water 1:10	69.68	12.23	1.50	14.38	12.30	pale yellow
HD [12]	150 min 100 °C Clove:Water 1:2	64.91	22.01	-	6.31	7.42	
HD [10]	240 min 100 °C Clove:Water 1:10	87.26	1.35	0.19	10.43	12.98	Pale yellow
HD [5]	360 min 100 °C Clove:Water 1:5	58.20	20.59	2.61	13.84	11.50	Pale yellow
Microwave-assisted Extraction [12]	30 min 850 W 100 °C Clove:Water 1:5	69.52	17.20	0.01	9.11	10.88	-
Microwave-assisted HD [10]	80 min 1000 W 100 °C Clove:Water 1:10	88.80	2.65	0.40	7.46	13.94	Pale yellow
Microwave-assisted HD Coaxial [13]	120 min 300 W 100 °C Clove:Water 1:5	66.90	24.80	3.10	2.70	9.70	
Microwave-assisted SD [10]	80 min 1000 W 100 °C Clove:Water 1:10	83.39	1.34	0.21	14.34	12.71	Pale yellow
Microwave-assisted SD Inside [12]	10 min 500 W 100 °C Clove:Water 1:5	67.54	18.33	0.02	10.59	5.97	-
Microwave-assisted SD Outside [12]	10 min 500 W 100 °C Clove:Water 1:5	56.06	34.15	-	4.69	16.25	-
SD [10]	240 min 100 °C Clove:Water 1:10	82.65	0.91	0.13	15.63	11.54	Pale yellow
SD [5]	600 min 100 °C Clove:Water 1:5	48.82	36.94	4.41	3.89	10.10	Pale yellow
SFE [8]	170 min SC–CO_2 40 °C 20 MPa	55.63	14.48	1.81	17.15	19.06	-
SFE [11]	14 min SC–CO_2 40 °C 15 MPa	55,44	7.77	0,86	12.53	-	-
SFE [5]	120 min SC–CO_2 30 °C 20 MPa	54.58	17.32	2.26	20.55	19.43	Pale yellow
SFE [5]	120 min SC–CO_2 40 °C 30 MPa	55.14	15.52	2.02	20.32	20.43	Pale yellow
SFE [5]	120 min SC–CO_2 50 °C 10 MPa	57.36	13.99	1.90	22.34	19.56	Pale yellow
SFE assisted by cold pressing [11]	15 min SC–CO_2 40 °C 15 MPa 40 N.m	57,69	8.33	0,92	12,61	-	-
SFE assisted by cold pressing [11]	15 min SC–CO_2 40 °C 15 MPa 80 N.m	54,85	7,94	0,88	12.12	-	-
Soxhlet Extraction [8]	720 min 69 °C Clove:Hexane 1:20	34.03	9.12	1.04	10.50	17.46	-

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Soxhlet Extraction [5]	360 min 100 °C Clove:Ethanol 1:8	57.24	1.75	2.03	19.37	41.80	Brown ointment
Ultrasound-assisted SFE [8]	115 min SC–CO_2 40 °C 15 MPa	59.18	15.35	1.93	18.60	22.04	-

Hydro Distillation HD, Steam Distillation SD, Supercritical Fluid Extraction SFE.

The main compounds of the CEOs obtained by the different extraction methods were similar; however, the concentration of each compound was different. Besides, the general characteristics of the CEOs obtained by the processes were of high quality with a characteristic pale-yellow color. However, only the Soxhlet extraction (SO) can produce brown oils due to impurities, waxes, and organic waste [5,10]. UAE, MAE, and SFE methods reduce extraction time from 10 to 2 hours and at least double the extraction yield [5,10,25]. Golmakani et al. reported that the extraction performance in MA HD after 60 min was like the final performance in HD after 240 min. Similarly, the MA SD operated almost 4.8 times faster than SD. This behavior occurs because MAE reaching the extraction temperature faster than conventional methods [6,8,10].

Tekin et al. [44] reported that the CEO obtained from the clove by UAE (53 kHz) to had a significant content of eugenol, α-caryophyllene, and eugenyl acetate [44]. Ghule and Desai [47] used ultrasound-assisted hydrotropic extraction to selective isolation of eugenol and eugenyl acetate from clove buds. The extraction yield was approximately 20% applying 158 W sonication power (26 kHz with a 7 mm diameter probe) into 8.2 g grounded clove buds in 150 ml of sodium cumene sulfonate 1.04 M for 30 min at 38 °C [47].

The main factors in the extraction of the CEO by SFE are particle size, temperature, pressure, and extraction time. Extraction performance increases by decreasing the crushed particle size of the clove because the diffusion paths are shorter and result in less resistance to diffusion between particles. Temperature and pressure of extraction modify the CO_2 density. Thus, the extraction performance is higher due to the increased solubility of clove components. However, there is a risk that high molecular weight compounds may also be extracted in the CEO (fatty acids, fatty acid methyl esters, sterols, etc.) [5,6,8]. The SFE offers substantial advantages over other traditional methods, including higher extraction yield, a higher percentage of active antioxidant ingredients, shorter extraction time, etc. Therefore, SFE is considered an appropriate process for obtaining high-quality CEO [5,6,8].

4. Food applications

In recent years, food industries have faced great challenges in producing safe foods with a longer shelf life, preserving nutritional values and sensory characteristics. The deterioration of food causes significant economic losses. It is also harmful to human health due to the toxic secondary metabolites produced. Growing consumer demand for natural alternatives to synthetic chemical preservatives in food has made EOs one of the natural substitutes due to their antioxidant, antibacterial, and antifungal [48–53]. However, the main challenge of EOs as food preservatives lies in maintaining their functional properties without changing the taste of food and increasing the appetite of the consumers (Table 3) [48,53].

Generally, complex food matrices require higher EOs concentrations than those used in *in vitro* tests. For example, foods with high protein content can produce protein-phenolic EOs complexes reducing the EOs effectiveness [48–50,54]. Also, the lipid fraction of food can absorb the antimicrobial agent lessening its bactericidal action. Likewise, reducing water content in food could hinder the transfer of antimicrobial agents to the active site in the microbial cell [48–50,54]. Furthermore, external factors such as storage temperature, packaging, initial concentration, application method, and the type of microorganism can interfere with the effectiveness of the CEO [49,55].

Table 3. CEO main food applications reported.

Food category	Food	Application form	Dose	Results	Reference

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Baked foods	Cake, bread, green bean cake, and finger citron crisp cake*	Storage conditions	1 %	Extend shelf life up to 2-12 days	[48]
	Bread*	Storage conditions	250 mg/g	Extend shelf life up to 15 days	[56]
	Refrigerated steamed buns*	Coating	0-1.2 %	Extend shelf life up to 10 days, but the volatile components evaporate during the re-steaming process	[55]
Dairy products	Fresh soft cheese*	Fortification	0.01%	Extend shelf life up to 3 weeks	[55]
Meat, Poultry and Marine Products	Fresh rainbow trout*+	Coating		Extend shelf life up to 5-12 days	[57]
	Chicken breast meat*	Coating		Extend shelf life up to 12 days	[50]
	Beef sucuks*+	Coating	1.50 %	Extend shelf life up to 45 days	[58]
	Beef cutlets*+	Coating	2 mg/g	Extend shelf life up to 12 days	[51]
	Seabream*+	Storage conditions	10-15 mg/kg	Extend shelf life up to 15 days	[59]
	Bluefin tuna*+	Coating	0.5 ml	Extend shelf life up to 14 days	[60]
	Ground beef meat*+	Fortification	10 %	Extend shelf life up to 7 days at refrigeration and chilling temperatures 60 days. Can produce unpleasant flavors	[53]
	Gelatin–chitosan film, Cod fillets*	Coating	15 %	Extend shelf life up to 12 days, improved the mechanical, structural, and barrier properties	[61]
	Raw grass carp fillets+	Coating	0.1-1.0 %	Reduced the content of off-odor volatiles for 12 days	[62]
	White shrimp*+	Coating	0.25-0.5 %	Extend shelf life up to 15 days and inhibit melanosis	[63]
	Salmon Burgers*+	Fortification	0.005-0.01 %	Extend shelf life up to 14 days and inhibit melanosis	[64]
	Chicken patties*+	Coating	0.50 %	Extend shelf life up to 35 days and inhibit melanosis	[65]
	Chicken breast*+	Storage conditions	0.2-0.5 %	Extend shelf life up to 15 days and inhibit melanosis	[66]
Packaging material	Mechanically deboned chicken meat protein films*+	Fortification	1 %	Improvement of antioxidant and antimicrobial properties	[67]
	Poly (lactic acid) biocomposite food packaging film*	Fortification	3 %	Improvement of antimicrobial properties	[68]
	Polylactide/poly(ε-caprolactone)/zinc oxide/CEO and Scrambled eggs*	Fortification	25 %	Extend shelf life up to 21 days, improved the mechanical, structural, and barrier properties	[69]
	Chitosan-gum arabic film*	Fortification	5 %		[70]
	Citrus pectin films*+	Fortification	0.5-1.5 %	Improve the barrier, mechanical, antioxidant, and antimicrobial properties of pectin films.	[71]
	Chicken eggs*	Storage conditions	10–80 µg/g	Extend shelf life up to 30 days, less weight reduction	[72]
Processed food	Ketchup*	Fortification	500 ppm		[54]
	Sausages*	Fortification	2000 mg/L	prolong the shelf life 14 days	[52]
Vegetables	Mango (cv. Banganapalli and cv. Totapuri) *+	Storage conditions	106 µL	Extend shelf life up to 20-21 days	[73]

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Persimmon*	Storage conditions	1.56 %	inhibition of mold growth on the persimmon fruits by 28 days	[74]
Pak choi*	Storage conditions	0.02 %	Extend shelf life up to 17 days	[75]
Avocado*	Coating	0.20 %	Extend shelf life up to 7 days	[76]

Bioactivity: * Antimicrobial, + Antioxidant

4.1. *Baked food*

The baked food industry emphasizes preventing mold growth and maintaining safety and nutrition [48,55,56]. The preservatives methods in baked foods include modified atmospheres stored, irradiation, aseptic packaging, and preservative acids. However, the use of organic acids (propionic acid, benzoic acid, and sorbic acid) has been restricted in several countries due to their negative impacts on human health [48,55,56]. The eugenol attributed to the CEO a broad spectrum against pathogenic foodborne microorganisms and deterioration as *Penicillium spp., Aspergillus spp., E. coli,* and *S. aureus*. Their addition in baked foods can extend the shelf life without influencing their original taste, flavor, texture, appearance, or sensory acceptability [48,55,56].

4.2. *Dairy products*

Consumption of dairy products such as cheese has been responsible for various outbreaks of foodborne [49,61]. Ahmed et al. [49] applied approximately 1 kg of CEO per 200 liters of raw milk as an antimicrobial agent for cheese production. The CEO demonstrated significant antimicrobial action without affecting organoleptic properties during 1 month at 4 °C, showing a potentially cost-effective use [49]

4.3. *Processed food*

In recent years, the market for ready-to-cook processed foods (pre-cooked foods) has expanded due to lifestyle changes and the development of refrigerated distribution networks. The microbial deterioration of processed foods causes unpleasant odors, discoloration, stickiness, sediments, gases, and a decrease in pH, reducing the quality of the food product and putting the products' health at risk. The CEO's addition of 5% w/w to processed foods has shown a negative impact on the organoleptic properties of foods, so its application has been focused on as a flavoring component with antimicrobial and antioxidant properties [52–54].

4.4. *Meat, Poultry and Marine Products*

The application of CEO in animal food products reduces undesirable reactions that involve the deterioration of the taste, smell, color, texture, and sensory properties [50,53,57,58,61,63]. Their antimicrobial activity generates a decrease in the bacterial count, decreases the deamination capacity of non-protein nitrogenous compounds, and reduces hydroperoxide formation due to their antioxidant properties. Mechanisms behind the antioxidant properties of CEO include transition metal binding, inhibition of chain reactions, breakdown of hydroperoxides, and interaction with free radicals. The CEO has been applied in white shrimp, salmon burgers, fish fillets, ground beef, chicken patties, and chicken breast meat during storage in refrigeration or freezing [50,53,57,60,63–65]. Films fortified with CEO can reduce the loss of weight, water activity, lipid oxidation, color change, and microorganisms growth for up to 45 days heat-treated and 12 days in refrigerated foods of animal origin [51,58,60].

4.5. *Vegetables*

Post-harvest vegetable deterioration during transport and storage present significant economic losses along the supply chain [73,74,76]. The antimicrobial properties of CEO can prevent fungal spoilage in vegetables and adverse health effects, serving as a potential alternative to chemical fungicides. The antimicrobial activity can be improved when is combined with UV-C light treatment or modified atmosphere packaging. These processes allow effective post-harvest decomposition control and preserve the physicochemical

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quality of vegetables, prolonging their life useful without affecting organoleptic properties [73–76]. CEO is added in the wash treatment of fresh-cut vegetables as an alternative to acetic acid, sodium bicarbonate, and chlorine-based disinfectants, reducing microbiological hazards and extending shelf-life. Also, CEO wash does not impacts color attributes, bioactive content, composition, and sensory attributes. Therefore, CEO application in conjunction with cold storage is an excellent ecological substitute that could be further improved for commercial applications to bring vegetables to market with better and longer-lasting post-harvest quality with greater acceptance by the consumer [75,76].

4.6. Packaging materials

Recently, new biodegradable packaging materials have been developed from natural polymers (polysaccharides, lipids, proteins). Their antioxidant and antimicrobial properties can be enhanced by incorporating essential oils to extend shelf life and reduce or inhibit foodborne pathogens [61,70]. The incorporation of EO in films for coating has aimed to modify the functional properties, such as water vapor permeability and the antimicrobial and antioxidant properties [58,67–69,71]. The antimicrobial properties of the CEO fortified films showed antibacterial and growth inactivation for up to 21 days. due to penetration and destruction of cell structure by CEO compounds [69]. The CEO addition can modify the packaging materials' moisture content, improving spatial distance within the film matrix, resulting in thicker films [67–71].

The optical properties of films impact the appearance and quality of foods. The application of coating also reduces the rate of lipid oxidation [67,71]. In these senses, the CEO's different coloring components can change the color of the films [67,71]. Its incorporation increases opacity values due to an increase in light scattering caused by oil droplets in the film network. These reduce transparency, which generates an advantage over photosensitive food [67].

The incorporation of CEO in a film network partially replaces the stronger polymer-polymer interactions with weaker interactions (polymer-oil) This generates a more heterogeneous network and a discontinuous microstructure by rearrangement of the polymers. Likewise, the incorporation of the CEO has a plasticizing effect, decreasing the glass transition temperature and the elastic modulus of the films [67–71]. F. T. Sarıcaoglu and S. Turhan [67] observed a decrease in elastic modulus and tensile strength with added CEO in mechanically deboned chicken meat protein films. However, the tensile strength value was kept above 3.5 MPa, a recommended value for coating film on food [67,69–71]. These changes in the structure due to the incorporation of the CEO, also produce rougher and more porous films [67,68,71].

5. Biological activities of CEO

The CEO has been shown to have different health benefits, mainly principally to eugenol. However, the rest of the compounds have had various health benefits too. The CEO principal biological activities are shown in Table 4 [77].

Table 4. The CEO principal biological activities.

Pharmaceutical form	Bioactivities	Mechanism	Model	Dose	Reference
Clove essential oils C, HD, SD	Analgesic	Mediation through opioidergic and cholinergic systems. Inhibition of voltage-gated Na^+ channels and activation of $TRPV_1$.	Adult male Wistar rats [19] Yellowtail clownfish *Amphiprion clarkia* [78]	40-500 µL/L	[19,78]
	Anesthetic	Inhibition of voltage-gated Na^+ channels and activation of $TRPV_1$. Losses in the power of contraction of the dorsal muscle.	Wistar rats [19] Cardinal tetra *Parachei-rodon axelrodi* Angelfish *Pterophyllum scalare* [79]	50-500 µl/L	[19,79–81]

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		Cherax quadricarinatus [80] Adult male Tilapia del Nilo *Oreochromis niloticus* [81]		
Anticancer	Decreases the levels of inflammatory biomarkers. Inhibit tissue remodeling in protein molecules. Inhibit pro-inflammatory genes and proteins such as pro-inflammatory cytokines. Cytotoxic. Genotoxic. Induction of apoptosis. Antiproliferative activities. Growth inhibition. Changes in the polarization of cancer cells. Inhibits proton pumps and ATP production.	Human dermal fibroblast[77], Cancer cell lines (cervical, liver, breast, prostate, colon, erythroleukemia, and lung) [82–87]	13-127 µg/mL	[77,82–87]
Anticoagulant	Time delay of blood coagulation.	Swiss male mice (Mus musculus) [88]	0.0625-4 mg/mL	[88]
Antidiarrheal Activity	Ability to balance the gut microbiota. Helps intestinal motility. Potentiates the digestive process due to its ability to increase enzyme activity and nitrogen absorption. Regulates neurotransmitters, such as histamine and dopamine. Ca_2^+ activated Cl channel inhibitor TMEM16A, causing reduced intestinal motility in mice.	Swiss male mice (Mus musculus) [88]	50-100 mg/kg	[88]
Anti-inflammatory	Inhibition of the release or synthesis of some inflammation mediating compounds. Decreased levels of inflammatory biomarkers. Inhibition of tissue remodeling proteins. Inhibition at the level of expression of genes and proteins, pro-inflammatory proteins such as cytokines. Inhibition of prostaglandin synthesis and neutrophil chemotaxis. Inhibition of factor NF-kB in the activation of tumor necrosis factor-α (TNF-α). Inhibits the expression of cyclooxygenase (COX-2).	Rats [89] Human dermal fibroblast [77] BALB/c mice [17]	100-250 mg/kg	[17,77,89]
Antimicrobial	Inhibit growth. Destabilization of membrane permeability and integrity.	*Candida albicans, Clebsiella sp., Escherichia coli, Proteus sp., Pseudomonas aeruginosa.,*	1.25-6.25 mg/m	[82,86,87,89,90]

		Rupture of the phospholipid membrane, resulting in electron transport inhibition, protein translocation, phosphorylation, and other enzymatic activities, leads to cell death.	*Agrobacterium tumefaciens, Erwinia sp, Staphylococcus aureus, Listeria innocua, B. subtilis, B. cereus, L. monocytogenes, S. typhimurium, L. acidophilus, L. reuteri, L. casei, L. rhamnosus, Aspergillus niger, and T. pyriformis* [82,86,87,89,90]		
	Antinociceptive	Inhibition of COX-2 and transient vanilloid receptor potential (TRPV) by high-voltage inhibition of Ca_2^+ currents in primary neurons.	Female Wistar rats [91]	100 µg/kg	[91]
	Antioxidant	Radical scavenging activity Inhibition of lipid peroxidation Transfer of electrons or hydrogen atoms to neutralize free radicals and block oxidative processes. Protective effect on ROS-induced biochemical changes and histopathological damage, the balance between oxidant/antioxidant ratio	DPPH, *β*-carotene-linoleate, ABTS, FRAP, Folin-Ciocalteu, flavones and flavonols, flavonoids, TAC [83,87,89,90,92]. Wistar rats/blood, histopathological study [92]	30-600 µg/mL	[83,87,89,90,92]
	Antipyretic activity	Reduces chemotaxis. Inhibition of COX-1 and COX-2.	Swiss male mice (Mus musculus) [88]	50-100 mg/kg	[88]
	Hemolytic	Interaction with the cell membrane	Swiss male mice (Mus musculus) [88]	0.625-2.5 mg/m	[88]
	Insecticide Contact toxicity Repellency Larval toxicity Oviposition deterrence	Life cycle inhibition. Developmental inhibition. It attacks three possible molecular targets (i.e., transient receptor potential (TRP) channels, octopamine receptors, and gamma-aminobutyric acid (GABA) receptors). Neurotoxic action. Increased permeability in the cell membrane, breaking the cytoplasmic membrane and interacting with proteins. The hydroxyl group present in eugenol binds to proteins and affects their properties. Inhibition of the enzymes ATPase, histidine decarboxylase, amylase, and protease. CEOs absorption by cuticular lipids and then entering the hemocoel and nervous system, or the tracheal system absorb it.	*C. felis, Rhopalosiphum maidis Coccinellidae, Coleomegilla maculate, C. pipiens, Blattella germanica, and Ae. j. japonicus* [93–97]	4 ml/cm 5-80 mg/L	[93–97]
Microemulsion^{SD} 303 nm Montanov 202™ Phase inversion method	Anti-inflammatory	Re-epithelialization and formation of dermis and epidermis. Increases collagen synthesis.	Cell linem5S Male Wistar rats [18]	0.2 g	[18]

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Nanoemulsion [C] 6–27 nm Tween 20 and 80 Spontaneous self-emulsification	Antimicrobial	Destabilization of membrane permeability.	*S. aureus* [98]	19-24 µg/m	[98]
	Anticancer	Antiproliferative effect. Cytotoxic activity. Induces necrosis.	Thyroid cancer cell line [98]	19-24 µg/mL	[98]
Nanoemulsion [C] 29.1 nm Tween-80 Spontaneous self-emulsification	Wound healing	Reduces the epithelialization period of the wound. Increases leucine content. Increases collagen content. Induces neovascularization.	Female Albino Wistar rats [99]	0.61 mg/g	[99]

Source: [C] Commercial CEO, [SD] Steam distillation, [HD] Hydrodistillation

5.1. *Antimicrobial*

The CEO has shown broad-spectrum inhibitory activity against pathogens. The antibacterial mechanism of the CEO has been related to the -OH groups located at the meta and ortho positions in the CEO main chemical composition, respectively. These functional groups can interact with the cytoplasmic membrane of microbial cells [82,86,87,89,90]. The CEO is permeable through the cell membrane due to its lipophilic properties. The interaction of CEO with polysaccharides, fatty acids, and phospholipids causes loss of cellular membrane integrity, leakage of cellular contents, interference in proton pump activity, and conducts to cell death [82,86,87,90,100]. The CEO can inhibit Gram-negative bacteria like (*E. coli, Salmonella, K. pneumoniae, E. carotovora, Agrobacterium,* and *P. aeruginosa)* and Gram-positive bacteria as *(S. aureus, Streptococcus,* and *L. monocytogenes), Aspergillus* (*A. flavus, A. parasiticus,* and *A. ochraceus), Penicillium, C. albicans* , and yeast [82,86,87,90]. The CEO inhibited Gram-positive bacteria to a greater extent than Gram-negative bacteria. This behavior was attributed to a diffusible mucopeptid layer in Gram-positive bacteria that makes them susceptible to antimicrobial agents. In contrast, the complex layer of lipopolysaccharide in the outer cell membrane of Gram-negative bacteria can significantly reduce the diffusion rate of lipophilic antibacterial compounds through the cell membrane [87]. Likewise, food-related pathogens have shown greater sensitivity to CEO than probiotics and fungi [90].

5.2. *Antioxidant*

The CEO has antioxidant compounds like eugenol, eugenyl acetate, β-caryophyllene, and α-humulene, which protect cells from free radical oxidation (ROS). Diseases such as cancer, arteriosclerosis, Alzheimer's disease, and Parkinson's disease are related to the presence of ROS compounds [100]. The CEO had shown scavenging activity on radicals and inhibition of lipid peroxidation [32,83]. The hydroxyl group available in eugenol on the aromatic ring is responsible for the antioxidant activities [89]. The phenolic compounds transfer electrons or hydrogen atoms and neutralize them to free radicals, resulting in a blocked oxidative process [90]

The CEO has a protective effect on biochemical changes and histopathological injuries in the kidney, liver, and brain induced by ROS. The main ROS changes inhibited were increased in the lipid parameters (HDL-C, TC, LDL-C, and VLDL), blood electrolyte (Na^+, K^+, Cl^-) and creatinine levels liver, hepatic enzymes, blood urea, increase in the liver, and kidney weight, increase in the serum creatinine and decrease in the total protein and albumin [92]. Marmouzi et al. [89] reported that CEO antioxidant activity in the three test methods reported was: 150 mg TE/g EO for DPPH, 110mg TE/g EO for $ABTS^+$, and 34 mg AAE/g EO for FRAP.

5.3. *Insecticide*

Insect-borne diseases are an ongoing challenge to public health. Some species are crucial invasive urban pests, transmitting numerous pathogenic microorganisms, including allergic reactions and asthma, to young and older people. Commonly used insecticides

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cause significant health problems and have long-lasting adverse effects on the environment. Besides, an increase in resistance against insecticides has been reported. Due to this, different investigations have focused on the development of natural insecticides, using EO as a basis for the control of agricultural and urban pests [95–97]. However, their high volatility decreases the time in which EOs remain in the human body, so sometimes several applications are required in a day

The CEO has shown a high level of repellency and fumigant toxicity on flea, aphids, nymphal instars, mites, red imported fire ant, *C. pipiens*, and American and German cockroach [93,95,96]. The oviposition-deterrent activity of CEO could be found in other mosquito species (*An. stephensi, An. subpictus, Ae. aegypti, Cx. pipiens, Ae. albopictus, Cx. quinquefasciatus, Cx. tritaeniorhynchus*) [95,97]. The CEO targets the egg stage as oviposition-deterrent and the larval stages as a larvicide against *Ae. japonicus*, *Ae. aegypti* and *Cx. quinquefasciatus*. The CEO was showed repellent action in the laboratory and field settings against adults *Ae. aegypti, Ae. cinereus,* and *Ae. communis* [95,97].

The primary target of CEO and other EOs are Octopamine and gamma-aminobutyric acid (GABA) receptors, transient receptor potential (TRP) channels [94]. The CEO dose-response ratio showed an increase in the mortality rate, increasing concentration [95]. The CEO increased the permeability activity on the cell membrane, disrupted the cytoplasmic membrane, and interacted with proteins ATPase, histidine decarboxylase, amylase, and protease enzymes, each also inhibited.

Lambert et al. [93] evaluated against adult fleas and the development of eggs of *Ctenocephalides felis felis.* The CEO's LC 50 against adult fleas was 5.70 µg/cm^2 and 0.30 g/cm^2 against flea eggs, however, the insecticidal activity of eugenol was 3 times higher [93]. P. F.S. Toledo et al. [94] reported and its effect against aphids, but also the non-efficacy against ladybugs. They reported an LC95 of 0.17 µL/cm^2 for aphids, while under the same dose it only had a lethality of less than 18% for *C. maculata*. The ladybugs that were exposed to CEO did not exhibit impaired locomotion abilities. Therefore, they conclude that the CEO application represents an alternative to control aphid infestations [94]. E. Elzayyat et al. [95] evaluated the insecticidal activities against adults and larvae of *C. pipiens*, reported an LC_{50}: 0.374% and 0.036%, respectively [95]. Neupane et al. [96] observed that CEO, eugenol, and eugenyl acetate applied at 4.0 ml/cm^2 provided 95%, 85%, and 87% mortality of German cockroaches. they also reported a repellency for 30 min by applying 80% CEO. F. Reuss et al. [97] observed that CEO functions as an oviposition repellent and as a larvicide with an LC 50 of 17 mg/L.

The CEO and their main constituents are products with low toxicity to mammals, and residual concentrations are zero. Their application is limited to plage insect control, which is essential to prevent infestations in the environment [93].

5.4. Antiviral

The CEO has shown antiviral activity against Ebola [101], influenza A virus [102], herpes simplex virus type 1, and type 2 [101]. Recent studies by de Oliveira et al. showed that eugenol derivatives could inhibit the activity of the West Nile Virus, providing a promising compound against flaviviruses, such as dengue virus, Zika virus, and yellow fever virus [15]. Eugenol has also been studied as a possible inhibitor of the initial stage of infection by the HIV-1 virus because it can reduce virus replication. Likewise, eugenol can increase lymphocyte production; therefore, the lymphocyte proliferation capacity of eugenol may be responsible for anti-HIV-1 activity [16].

The CEO has demonstrated antiviral activity against human norovirus, substitutes for the feline calicivirus of human norovirus. for this reason, the application of the CEO in the processes of washing fruits and vegetables eliminates the viral load that may exist. In addition, the application of CEO in cleaning wipes allows the decontamination of surfaces. [103]. Furthermore, CEO has been shown to increase the resistance of tomato plants to tomato yellow leaf curl virus more than moroxydine hydrochloride. [104].

5.5. Antinociceptive

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Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs to treat inflammatory nociceptive pain. Their principal mechanism is the cyclooxygenase (COX) inhibition, decreasing the prostaglandins that cause nociceptive pain. The antinociceptive and anti-inflammatory eugenol activities are related to the COX-2 inhibition and vanilloid transient receptor potential (TRPV) by Ca^{2+} currents high-voltage inhibition in neurons primary afferent [91]. This antinociceptive response is related to opioid, cholinergic, and α2-adrenergic receptors, but not serotoninergic receptors. The antinociceptive effect of eugenol probably is related to gamma-aminobutyric acid (GABA) receptor modulation due to eugenol administration inhibits GABA receptor currents in trigeminal ganglion neurons and inhibits GABA $\alpha 1\beta 2\gamma 2$, expressed in these neurons [19,91].

5.6. *Anti-inflammatory and wound healing*

Oxidative stress and inflammation are near-related processes with many pathophysiological conditions such as diabetes, hypertension, cardiovascular and neurodegenerative diseases [89]. The anti-inflammatory properties of CEO and eugenol, it is comparable to diclofenac gel., reducing inflammation by 60% to 20% after 3 hours. Likewise, the induced wounds in rats treated with CEO showed a contraction more significant than 95% in the first 15 days. These results demonstrated that animals treated with CEO showed similar healing to those treated with neomycin, which is currently used to control inflammation and wound healing. Therefore, the chronic and acute side effects of synthetic antibiotics can be avoided, especially if they are given frequently [18]. The CEO inhibited important antiproliferative biomarkers, whose activity depends on the concentration. The CEO decreases the levels of inflammatory biomarkers such as VCAM-1, IP-10, I-TAC, and MIG. In addition to inhibiting the tissue remodeling protein molecules, collagen I, collagen III, M-CSF, and TIMP-1.[17,77,99]. The application of CEO can reduce the epidermal thickness, the number of inflammatory cells expressing COX-2 without affecting COX-1. The eugenol mechanism as an anti-inflammatory inhibits the expression of COX-2 and reduces the production mediators of inflammation [17,77]. Eugenol has also been reported not to alter IL-8 levels in human skin keratinocytes but to target other pro-inflammatory cytokines in pre-inflamed human dermal cells [18]. These results suggest that the CEO possesses anti-inflammatory activity and that it favors wound healing.

5.7. *Analgesic*

Headaches, joint pain, toothaches, and oral antiseptic have traditionally been treated with aromatherapy and CEO. The CEO and eugenol are safe, effective, and inexpensive analgesics. Also, the analgesic effect of eugenol in different models of pain has been well documented [19]. Khalilzadeh et al. [19] reported that the analgesic effect of CEO is mediated by the opioidergic and cholinergic systems. The analgesia produced by CEO in acute corneal pain appears to depend on their cholinergic activity. Furthermore, the analgesic and local anesthetic effects of eugenol can be modulated by its inhibitory effect on voltage-gated channels (Na^+ and Ca^{2+}) and the activation of TRPV1. Whose analgesic effects of CEO and eugenol are very similar to the effects of lidocaine. Correia et al. [78]. demonstrated the CEO analgesic efficacy in fish. When the CEO was used in concentrations between 40 to 80 µL L-1 on invasive procedures or those that can cause pain, an analgesic effect in animals was reported, minimizing the effect of harmful stimuli, avoiding stress and its negative consequences. The CEO has a potential use when performing painful procedures, minimize the effect of harmful stimuli for ethical reasons, and ensure the welfare of the animal, avoiding stress and its negative consequences [19,78,105].

5.8. *Anesthetic*

The CEO is recognized as an anesthetic in vertebrates and invertebrates without side effects using low concentration (50-500µl/L). CEO induce anesthesia faster, a brief reflex recovery, and show a low mortality rate without affecting external stimulus-response [78,80,81]. Recent studies showed that topical CEO and eugenol application reduces corneal sensitivity in rats such as lidocaine [19]. Topical application of CEO, eugenol, and

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lidocaine reduces corneal sensitivity. However, to achieve maximum anesthesia and duration, the concentration and time of exposure differ between chemicals. The CEO efficiently induces anesthesia in Nile tilapia, cardinal tetra, ringed cichlids, and angelfish, affecting swimming ability, loss of balance, and decreasing response to external stimuli until complete immobilization. The concentration of the dose decreases the time to achieve full anesthesia. Furthermore, there are no side effects of concentration and time of exposure to CEO to achieve normality after anesthesia [19,79,81]. The CEO is an effective anesthetic for red claw crayfish and other crustaceans, including Nephrops norvegicus and grass shrimp. Induction and recovery times increase with in-creased crayfish size as they are related to oxygen demand. The absorption and elimination of the CEO are observed by the oxygen consumption rate, the relationship between the body and the grill surface, and the gills infusion rate. Size is inversely related to anesthetic efficacy [80]. For invasive and painful procedures, the use of the CEO is recommended due to its better anesthetic efficacy [78].

5.9. Anticancer

The eugenol, α-humulene, and β-caryophyllene components of CEO have been used for the treatment of cancer because they have cytotoxic and antitumor activities. serving as an alternative in the prevention and co-treatment of cancer. Some reports suggested that EOs reduce the side effects of chemotherapy, which include nausea, vomiting, loss of appetite, and weight loss [100,106,107]. Its anticancer activity is mainly attributed to its antioxidant and anti-inflammatory activity since the production of ROS specifically activates signaling pathways and contributes to the development of tumors by regulating cell proliferation, angiogenesis, and metastasis [100,106]. The CEO have been tested against different cancer types, such as colon [32,83], lung [83,85], breast [85], pancreas [32], leukemia [32,84], breast [32], cervix [32], and prostate [32,83].

The CEO anticancer properties are due to the following mechanisms: activation of detoxifying enzymes, destruction of DNA by oxidative stress, antimetastatic, cytotoxicity, decreased viability, cell cycle arrest or apoptosis, elimination of the expression levels of phosphate-Akt, and MMP-2 and protein leakage [77,82,85,98]. CEO showed a low cytotoxic effect in normal cells, improving their antiproliferative activity [77,85,98].

5.10. Other bioactivities

Several authors have mentioned that CEO acts as antiseptic [6,19,90], natural stimulant [90], carminative [5,6,90,108], anticoagulant [14,88], antihelmintic [90], antivomiting [5], antiantidiarrheal activity [6,14,88], antispasmodic [5,6], hepatoprotective [108], spasmolytic [108], antimutagenic [12,14,22], anticonvulsant [12], antidepressant [109], renal reinforcement [5,14], antipyretic [18,88], neuroprotective[14] and antistress, allergies [5,6,19], antidiabetic [14,87], and hypocholesterolemia effects [14]. However, to our knowledge, these bioactivities have not been completely studied, being these new research opportunities for CEO.

6. Conclusions and future perspectives

The CEO is a food additive generally recognized as safe by the FDA. Extracted using classical and innovative methods, which directly influence its chemical composition, which is mainly responsible for its health benefits. The main biological activities are antioxidants, antimicrobial, anti-inflammatory, analgesic, antiviral and anticancer. These biological properties are attributed to eugenol, β-caryophyllene, α-humulene, and eugenyl acetate; however, no studies were found that defined the role of the major components in the overall biological activity of the CEO. The application of the CEO in food has been carried out in meat, poultry, and marine products, vegetables, dairy products, or coating films.

Even though CEO is widely consumed and applied, there are still potential areas of investigation. More studies are needed to define the role of the main components in each of the biological activities for their application in the treatment of different diseases. In

addition, it is necessary to determine if there is synergy or antagonism between the components of the CEO. Likewise, it is necessary to study the application in the food industry, mainly its use as an antioxidant or antimicrobial without negatively affecting the color, taste, smell, and texture. Few reports were found on CEO encapsulation and its effects on the main physicochemical and biological properties. More research is still required to determine the effect of encapsulation systems on solubility, absorption, and bioavailability, shelf life by avoiding degradation (photo, oxidative or thermal), as well as its effect on organoleptic properties.

Despite all the studies carried out, there are still different properties and applications that have not been thoroughly investigated, which is why a field is opened in the investigation of CEO against other diseases and their future application in various industries such as pharmaceuticals, food, cosmetic, dental, agricultural and others.

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