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Clove (*Syzygium aromaticum*): a precious spice

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PEER REVIEW

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Comments
In the review, the authors demonstrated the main studies reporting the biological activities of clove (*S. aromaticum*) and eugenol. Based on the information presented, it could be concluded that clove represents a very interesting plant with an enormous potential as food preservative and as a rich source of antioxidant compounds.
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ABSTRACT

Clove (*Syzygium aromaticum*) is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes. Clove is native of Indonesia but nowadays is cultured in several parts of the world including Brazil in the state of Bahia. This plant represents one of the richest source of phenolic compounds such as eugenol, eugenol acetate and gallic acid and posses great potential for pharmaceutical, cosmetic, food and agricultural applications. This review includes the main studies reporting the biological activities of clove and eugenol. The antioxidant and antimicrobial activity of clove is higher than many fruits, vegetables and other spices and should deserve special attention. A new application of clove as larvicidal agent is an interesting strategy to combat dengue which is a serious health problem in Brazil and other tropical countries. Pharmacokinetics and toxicological studies were also mentioned. The different studies reviewed in this work confirm the traditional use of clove as food preservative and medicinal plant standing out the importance of this plant for different applications.

KEYWORDS
Spice, Clove, Aromatic plant, Volatile, Antioxidant, Dengue fever, Larvicidal

1. Introduction

Spices as clove, oregano, mint, thyme and cinnamon, have been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants. Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices[1].

Syzygium aromaticum (*S. aromaticum*) (synonym: *Eugenia cariophylata*) commonly known as clove, is an median size tree (8-12 m) from the Mirtaceae family native from the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region[2].

The clove tree is frequently cultivated in coastal areas at maximum altitudes of 200 m above the sea level. The production of flower buds, which is the commercialized part of this tree, starts after 4 years of plantation. Flower buds are collected in the maturation phase before flowering. The collection could be done manually or chemically-mediated

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using a natural phytohormone which liberates ethylene in the vegetal tissue, producing precocious maturation[3].

Nowadays, the larger producer countries of clove are Indonesia, India, Malaysia, Sri Lanka, Madagascar and Tanzania specially the Zanzibar island[2]. In Brazil, clove is cultured in the northeast region, in the state of Bahia in the regions of Valença, Ituberá, Taperoá, Camamu and Nilo Peçanha, where approximately 8 000 hectares are cultivated, producing near 2 500 tons per year[4-5].

2. Chemical compounds isolated from clove

Clove represents one of the major vegetal sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids and hidroxiphenyl propens. Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9 381.70 to 14 650.00 mg per 100 g of fresh plant material[6].

With regard to the phenolic acids, gallic acid is the compound found in higher concentration (783.50 mg/100 g fresh weight). However, other gallic acid derivates as hidrolizable tannins are present in higher concentrations (2 375.8 mg/100 g)[1]. Other phenolic acids found in clove are the caffeic, ferulic, elagic and salicylic acids. Flavonoids as kaempferol, quercetin and its derivates (glycosilated) are also found in clove in lower concentrations.

Concentrations up to 18% of essential oil can be found in the clove flower buds. Roughly, 89% of the clove essential oil is eugenol and 5% to 15% is eugenol acetate and β-cariofileno[7]. Another important compound found in the essential oil of clove in concentrations up to 2.1% is α-humulen. Other volatile compounds present in lower concentrations in clove essential oil are β-pinene, limonene, farnesol, benzaldehyde, 2-heptanone and ethyl hexanoate.

3. Biological activities

Clove is an important medicinal plant due to the wide range of pharmacological effects consolidated from traditional use for centuries and reported in literature. A review of several scientific reports of the most important biological activities of clove and eugenol is presented in the following paragraphs.

3.1. Antioxidant activity

Recently, the United States Department of Agriculture in collaboration with Universities and private companies create a database with the polyphenol content and antioxidant activity of different kind of foods. Based on this database, Pérez-Jiménez *et al*. classified the 100 richest dietary sources of polyphenols[8]. Results indicate that the spice plants are the kind of food with higher polyphenol content followed by fruits, seeds and vegetables. Among spices, clove showed the higher content of polyphenols and antioxidant compounds.

In another work published by Shan *et al*.[1], the main phenolic compounds in 26 spices were identified and quantified by high performance liquid chromatography, followed by the *in vitro* antioxidant activity analysis by the ABTS method. Results showed the high correlation between the polyphenols content and the antioxidant activity. Clove (buds) was the spice presenting higher antioxidant activity and polyphenol content, (168.660±0.024) tetraethylammonium chloride (mmol of Trolox/100g dried weight) and (14.380±0.006) g of gallic acid (equivalents/100g of dried weight) respectively. The major types of phenolic compounds found were phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol) and tannins. It was highlighted the huge potential of clove as radical scavenger and as a commercial source of polyphenols.

The antioxidant activity of clove and caraway were screened using various *in vitro* models, such as b-carotene-linoleate, ferric thiocyanate, 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical, hydroxyl radical and reducing power model systems concluding that the antioxidant activity of clove and caraway is comparable with butylated hydroxytoluene (BHT), a synthetic compound commonly employed as food preservative Bamdad *et al*[9]. According to Gülçin *et al*.[10], the antioxidant activity of clove oil compared with synthetic antioxidants measured as the scavenging of the DPPH radical decreased in the following order: clove oil>BHT>alfa-tocopherol>butylated hydroxyanisole>Trolox.

The antioxidant activity of aqueous extracts of clove has been tested by different *in vitro* methods as 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), oxigen radical absorbance capacity, ferric reducing antioxidant power, xanthine oxidase and 2-deoxiguanosine. Clove and plants as pine, cinnamon, and mate proved its enormous potential as food preservative among the other 30 plants analyzed[11].

Ethanol and aqueous extracts of clove and lavender at concentrations of 20, 40 and 60 μg/mL showed inhibitions up to 95% when tested as metal quelants, superoxide radical capture and scavenging of the DPPH radical. The powerful antioxidant activity of both extracts may be attributed to the strong hydrogen donating ability, metal chelating ability and scavanging of free radicals, hydrogen peroxide and superoxide[12].

Gülcin studied the antioxidant activity of eugenol by several *in vitro* methods and discusses the structure-activity relationship[13]. Compared to butylated hydroxyanisole, BHT, Trolox and α-tocopherol, eugenol presented higher antioxidant activity in most of the methods tested, DPPH, ABTS, N,N-dimethyl-p-phenylenediamine, CUPRAC and ferric reducing assay. It was remarked that plant polyphenols are multifunctional in the sense that they can act as reducing agents, hydrogen atom donators, and singlet oxygen scavengers. Eugenol allows the donation of an hydrogen atom and subsequent stabilization of the phenoxil radical generated forming stable compounds that do not start or propagate oxidation. The eugenol molecule possesses an interesting conjugation of the carbon chain with the aromatic ring which could participate in the stabilization of the phenoxyl radical by resonance. This chromophoric system is also present in molecule of resveratrol which is another important antioxidant. It has been proposed the hypothesis that eugenol reduces two or more DPPH radicals, despite the availability of only one hydrogen from a hydroxyl group. The formation of dimers of eugenol (dehydrodieugenol) with two phenolic hidroxyl groups originated from eugenyl intermediate radicals has also been proposed as mechanism between eugenol and DPPH radicals .

In the same way, *S. aromaticum* oil and *Nigella sativa* oil significant protect male rats exposed to aflatoxins which caused hepato and nephrotoxicity and oxidative stress. Regarding to the biochemical parameters, such as alanine aminotransferase, aspartate aminotransferase, alkaline phospatase, total billirubin, urea, total protein, cholesterol, the activity of both oils were comparable with the controls[14].

Antioxidants are important compounds for treatment of memory deficits caused by oxidative stress[15]. Pretreatment with clove essential oil decreases the oxidative stress assessed by malondialdehyde and reduced glutathione levels in mice's brain. This study concluded that clove oil could revert memory and learning deficits caused by scopolamine in short and long term as a result of the reduction in the oxidative stress[16]. Memory and learning improvements of clove oil were observed in scopolamine-treated mice at doses of 0.025, 0.05, and 0.1 mL/kg when compared with saline solution control group in an elevated plus maze test. These works prove the benefits of the employment of clove as a rich source of antioxidants for the treatment of memory deficits caused by oxidative stress.

Extracts from clove buds could also be used as food antioxidants. The shelf-life and frying stability of encapsulated and un-encapsulated eugenol-rich clove extracts were tested in soybean oil[17]. Controlled release of antioxidants could be achieved by encapsulated clove powder obtained by spray drying using maltodextrin and arabic gum as wall materials.

3.2. Antimicrobial activity

The antimicrobial activities of clove have been proved against several bacteria and fungal strains. Sofia *et al.* tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove[18]. The only sampled that showed complete bactericidal effect against all the food-borne pathogens tested *Escherichia coli* (*E. coli*), *Staphylococcus aureus* and *Bacillus cereus* was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action.

In another work published by Dorman and Deans[19], the antibacterial activity of black pepper, geranium, nutmeg, oregano, thyme and clove was tested against 25 strains of Gram positive and Gram negative bacteria. The oils with the widest spectrum of activity were thyme, oregano and clove respectively.

The antibacterial activity of clove, oregano (*Origanum vulgare*), bay (*Pimenta racemosa*) and thyme (*Thymus vulgaris*) essential oil was tested against *E. coli* O157:H7 showing the different grades of inhibition of these essential oils[20]. Likewise formulations containing eugenol and carvacrol encapsulated in a non ionic surfactant were tested against four strains of two important foodborne pathogens, *E. coli* O157:H7 and *Listeria monocitogenes*, results reinforces the employment of eugenol to inhibit the growth of these microorganisms in surfaces in contact with food[21].

Rana *et al.* determined the antifungic activity of clove oil in different strains and reported this scale of sensibility *Mucor* sp.>*Microsporum gypseum*>*Fusarium monoliforme* NCIM 1100>*Trichophytum rubrum*>*Aspergillus* sp.>*Fusarium oxysporum* MTCC 284[22]. The chromatographic analyses showed that eugenol was the main compound responsible for the antifungic activity due to lysis of the spores and micelles. A similar mechanism of action of membrane disruption and deformation of macromolecules produced by eugenol was reported by Devi *et al*[23].

The activities of clove oil against different dermatophytes as *Microsporum canis* (KCTC 6591), *Trichophyton mentagrophytes* (KCTC 6077), *Trichophyton rubrum* (KCCM 60443), *Epidermophyton floccosum* (KCCM 11667) and *Microsporum gypseum* were tested and results indicate a maximum activity at concentration of 0.2 mg/mL with an effectiveness of up to 60%[24].

Pure clove oil or mixes with rosemary (*Rosmarinus officinalis* spp.) oil were tested against *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, E. coli, Proteus vulgaris, Pseudomonas aeruginosa* and results showed minimum inhibitory concentrations between 0.062% and 0.500% (v/v) which is promising as antiinfenccious agents or as food preservative[25].

The anticandidal activity of eugenol and carvacrol was tested in a vaginal candidiasis model, microbial and histological techniques were employed to compare the samples with the controls. The results suggest that eugenol and carvacrol could be a promising antifungal agent for treatment and prophylaxis of vaginal candidiasis[26].

In addition to the wide spectrum of activity of eugenol against bacteria, a study showed that eugenol and cinnamaldehyde at 2 μg/mL inhibited the growth of 31 strains of *Helicobacter pylori*, after 9 h and 12 h of incubation, respectively, being more potent that amoxicillin and without developing resistance. The activity and stability of those compounds was checked at low pH values since *Helicobacter pylori* resides in the stomach[27].

Solid lipid nanoparticles containing eugenol were prepared employing stearic acid, caprylic triglyceride and Poloxamer 188 in different concentrations by a modified hot homogenization ultrasonication method. The particles formed were characterized by the particle size, polydispersitivity index, morphology, zeta potential, crystalline state and encapsulation efficiency. The antifungal activity of solid lipid nanoparticles was tested *in vivo* by using a model of oral candidiasis (*Candida albicans*) in immunosupressed rats. The results showed the increase in the therapeutic effectiveness of eugenol and the modification of the release when administrated as solid lipid nanoparticles[28].

Beta-cyclodextrin inclusion complexes containing eugenol and clove bud extracts were tested against two common foodborne pathogens, *Salmonella enterica* serovar Typhimurium LT2 and *Listeria innocua*[29]. Clove products have a great potential as food additives since they are very effective and for being natural products are preferred for consumers. Moreover, the solubility and the delivery are improved with the encapsulation process.

3.3. Antinociceptive

The employment of clove as analgesic have been reported since the 13th century, for toothache, join pain and antispasmodic, being the eugenol the main compound responsable for this activity. The mechanism evolved has been attributed to the activation of calcium and chloride channels in ganglionar cells[30]. The voltage dependant effects of eugenol in sodium and calcium channels and in receptors expressed in the trigeminal ganglio also contributed to the analgesic effect of clove[31]. Other results show that the analgesic effect of clove is due to the action as capsaicin agonist[32]. The peripheral antinociceptive activity of eugenol was reported by Daniel *et al*. showing significant activity at doses of 50, 75 and 100 mg/kg[33].

3.4. Antiviral

The antiviral activity of eugeniin, a compound isolated from *S. aromaticum* and from *Geum japonicum*, was tested against herpes virus strains being effective at 5 μg/mL, and it was deducted that one of the major targets of eugeniin is the viral DNA synthesis by the inhibition of the viral DNA polymerase [34].

In another study, aqueous extracts of *S. aromaticum* (L.) Merr. et Perry and other plants as *Geum japonicum* Thunb., *Rhus javanica* L., and *Terminalia chebula* Retzus among others showed strong antiherpes simplex virus type 1 (HSV-1) activity when combined with acyclovir. This synergic activity was stronger in the brain that in the skin and it was also proved that those combinations were not toxic to mice[35].

3.5. Citotoxicity of eugenol

After several years of intensive research, various molecular targets for the prevention and treatment of cancer have been identified. Eugenol was selected as a potential molecule that can interfere with several cell-signaling pathways, specifically the nuclear factor kappa B (NF-KB). This factor is activated by free radicals and results in the expression of gens that suppress apoptosis and induce cellular transformation, proliferation, invasion, metastasis among others[36].

The anti-oxidative, citotoxic and genotoxic effects of eugenol and borneol were tested as the ability to modulate resistance against the damaging effects of H_2O_2 on DNA of different strains of human cells: malignan HepG2 hepatome cells, malignan Caco-2 colon cells and non malignan human VH10 fibroblast. Results showed that eugenol presented a notable anti-oxidative potential at all the concentrations tested. It was also evidenced that the citotoxic effects of eugenol were stronger than those of borneol. With regard to toxicity, eugenol presented strong genotoxic effects (DNA-damaging) on human VH10 fibroblast, medium genotoxic effects on Caco-2 colon cells and non DNA-damaging effects on HepG2 hepatome cells[37]. Nevertheless the National Toxicology Program based on several long term carcinogenicity studies concluded that eugenol was not carcinogenic to rats[38].

In another study, the eugenol suppressed the growth of the malign melanoma WM1205Lu of both anchorage-dependent and anchorage-independent growth, decreases size of tumors and inhibits melanoma invasion and metastasis by the inhibition of the two transition factors of the E2F family[38].

Although there are many reports of the antioxidant activity of eugenol, at high concentration eugenol could be prooxidant. The cytotoxicity, reactive oxygen species (ROS) production, and intracellular glutathione levels in a human submandibular cell line (HSG cells) of eugenol and isoeugenol was studied by Atsumi *et al*[39]. It was found that in the absence of oxidative stress eugenol acts as an antioxidant at low concentrations but acts as a prooxidant at high concentrations. In the presence of oxidative stress eugenol increased ROS levels at low concentrations (5-10 μmol/L), but decreased them at high concentrations (500 μmol/L). Therefore, it can be concluded that the cytotoxicity of eugenol occurs in a ROS-independent manner in the presence of oxidative stress. In another work, it was reported that eugenol inhibits the enzyme MMP-9 which is related to metastasis in human fibrosarcoma cells suggesting its application for prevention of metastasis related to oxidative stress[40].

4. Toxicity and pharmacokinetics

The clove essential oil is generally recognized as safe substance when consumed in concentrations lower than 1 500 mg/kg. On the other hand, the World Health Organization (WHO) established that the daily quantity acceptable of clove per day is of 2.5 mg/kg of weight in humans[10]. The toxicity of clove oil was tested in two aquarium fish species, *Danio rerio* and *Poecilia reticulata* the medium lethal concentrations (LD_{50}) at 96 h were (18.2±5.52) mg/mL in *Danio rerio* and (21.7±0.8) mg/mL in *Poecilia reticulata*[41].

Eugenol is easily absorbed when administrated by oral route reaching rapidly plasma and blood with mean half-lives of 14.0 h and 18.3 h, respectively. A cumulative effect has been hypothesized and associated to relieve of neuropathic pain after repeated daily administrations[42].

5. Agricultural and larvicidal uses

The clove essencial oil may also be employed as insecticide. Park and Shin reported the possibility of employment of clove essential oil to control the japonesse terminte *Reticulitermes speratus* Kolbe[43]. In the same way, Eamsobhana *et al*. found that clove essencial oil at 5% posses 100% of repellent activity against the chigger *Leptotrombidium imphalu* which could be a safer and cheaper alternative to synthetic repelents commonly associated to harmful side effects[44].

A formulation containing 10% of clove essential oil was effective against the bit of *Aedes aegypti* (L.) and *Anopheles dirus* Peyton and Harrion with a protection time of (80.33±10.56) and (60.00±10.00) respectively, soy bean oil was employed as control[45]. In a recent work, the structure-activity relationship of the main clove oil constituents and synthetic derivatives of eugenol against *Aedes aegypti* (Diptera: Culicidae) larvae were studied. The larvicidal methods are one of the most effective strategies to combat dengue, since there is not drug for treatment or a vaccine. Eugenol exhibited interesting results and could be a promising alternative to common insecticide[46].

Eugenol, eugenol acetate and beta-caryophyllene were effective in repellency of red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae), being eugenol the fastest acting compound[47]. Clove oil was also effective spatial repellent for pestiferous social wasps *Vespula pensylvanica* (Saussure) and paper wasps mainly *Polistes dominulus* (Christ)[48].

Clove oil can also serve as an anesthesia for a variety of fish. However, lengthy exposures can cause mortality and sub-acute morbidity[49]. The most appropriate dose to anesthetize the angelfish was determined by Hekimoglu and Ergun[50]. This study will help in the transportation and handing of this fish which is one of the most stressful aquarium species.

Clove oil could be employed as suppressor of potato tuber germination by affecting the lipid peroxidation and the enzymes activities of catalase, glutathione-S-transferase, peroxidase, polyphenol oxidase and superoxide dismutase[51].

6. Conclusion

Based on the information presented, it could be concluded that clove represents a very interesting plant with an enormous potential as food preservative and as a rich source of antioxidant compounds. It's proved biological activities suggest the development of medicinal products for human and animals uses and confirm why this plant has been employed for centuries.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Clove (*S. aromaticum*) is one of the most valuable spices that have been used for centuries as food preservative and for many medicinal purposes. Clove is native of Indonesia but nowadays is cultured in several parts of the world including Brazil in the state of Bahia. This plant represents one of the richest source of phenolic compounds such as eugenol, eugenol acetate and gallic acid and posses great potential for pharmaceutical, cosmetic, food and agricultural applications.

Research frontiers

This review includes the main studies reporting the biological activities of clove (*S. aromaticum*) and eugenol. The antioxidant and antimicrobial activity of clove is higher than many fruits, vegetables and other spices and should deserve special attention. A new application of clove as larvicidal agent is an interesting strategy to combat dengue which is a serious health problem in Brazil and other tropical countries. Pharmacokinetics and toxicological studies were also mentioned. The different studies reviewed in this work confirm the traditional use of clove as food preservative and medicinal plant standing out the importance of this plant for different applications.

Related reports

This work is a review documenting the main studies reporting the biological activities of clove (*S. aromaticum*) and eugenol.

Innovations and breakthroughs

This review includes the main studies reporting the biological activities of clove and eugenol. Based on the information presented, it could be concluded that clove represents a very interesting plant with an enormous potential as food preservative and as a rich source of antioxidant compounds. It's proved biological activities suggest the development of medicinal products for human and animals uses and confirm why this plant has been employed for centuries.

Applications

S. aromaticum (Myrtaceae) commonly known as clove, is an median size tree (8-12 m) native from the Maluku islands in east Indonesia. The clove tree is frequently cultivated in coastal areas at maximum altitudes of 200 m above the sea level. The production of flower buds, which is the commercialized part of this tree, starts after four years of plantation. The collection could be done manually or chemically-mediated using a natural phytohormone which liberates ethylene in the vegetal tissue, producing precocious maturation.

Peer review

In the review, the authors demonstrated the main studies reporting the biological activities of clove (*S. aromaticum*) and eugenol. Based on the information presented, it could be concluded that clove represents a very interesting plant with an enormous potential as food preservative and as a rich source of antioxidant compounds. It's proved biological activities suggest the development of medicinal products for human and animals uses.

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