Clove

Overview of Potential Health Benefits

Keith Singletary, PhD

Whole cloves are prepared from the dried, unopened flower buds of the tropical evergreen tree *Eugenia caryophyllata* L. Merr and Perry (Myrtaceae), also known as *Syzygium aromaticum*. Culinary uses for clove include as a flavoring addition to meats, especially ham, stewed fruits, pickles, curries, pies, salads, and spiced alcoholic beverages. It also finds application in perfumes, oral products, and soaps. In Indonesia, cloves are added to tobacco in kreteks, aromatic high-tar cigarettes. Clove owes its value to the aromatic essential oil, obtained from the steam distillation of powdered clove buds or leaves. A predominant bioactive phytochemical present is eugenol [2-methoxy-4-(2-propenyl)phenol]. Numerous research studies have attempted to characterize the potential health benefits attributed to clove and eugenol. These include antimicrobial effects, management of diabetes, and amelioration of neurological problems. This review provides a summary of some of the potential health benefits of clove and the variety of scientific research on this topic. Nutr Today. 2014;49(4):207–224

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Whole cloves are prepared from the dried, unopened flower buds of the tropical evergreen tree *Eugenia caryophyllata* L. Merr and Perry (Myrtaceae), also known as *Syzygium aromaticum* and *Eugenia aromatica*. Clove trees are indigenous to the Maluku Islands of Indonesia, although now are cultivated in such diverse areas as Madagascar, Tanzania, the West Indies, China, and Malaysia. The name for this hard, brown nail-shaped spice derives from the Latin *clavus*, meaning "nail." In traditional Chinese medicine, clove is known as ding xiang or "nail spice" and was used to treat among other things indigestion, nausea, vomiting, and infections.[1] Even today clove purportedly can be a remedy for such diverse problems as coughs and colds, diarrhea, digestive disorders, diabetes, toothaches, memory loss, erectile dysfunction, and arthritis.[2,3] Culinary uses for clove include as a flavoring addition to meats, especially ham, stewed fruits, pickles, curries, pies, salads, and spiced alcoholic beverages.[1] Clove is known to mask spoiled food smells by interfering with odor maps in the olfactory bulb of the forebrain.[4] It also finds application in perfumes, oral products, and soaps and detergents. In Indonesia, cloves are added to tobacco in kreteks, aromatic high-tar cigarettes.

Cloves owe their value to the aromatic essential oil, obtained from the steam distillation of powdered clove buds or leaves. A predominant constituent of it is the allyl chain-substituted guaiacol compound eugenol [2-methoxy-4-(2-propenyl)phenol] (Figure). Other active components include β-caryophyllene (Figure), α-humulene, isoeugenol, eugenyl acetate, flavonoids, and cinnamic acids.[5–7] The identities of the active compounds in clove have not been fully characterized, but eugenol is believed to be a main contributor to its biological actions. In fact, *E caryophyllata* is considered the main natural source of eugenol, although this compound has been identified in other spices such as nutmeg, cinnamon, and basil. The bioavailability and tissue distribution of eugenol and other clove components have been studied in animals, humans, and other organisms.[6–14] In healthy male

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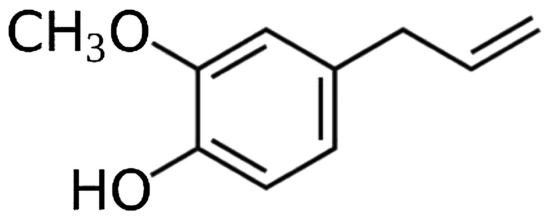
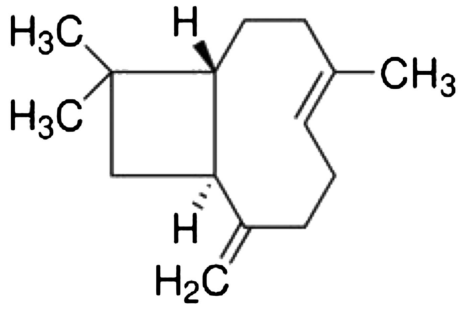


Eugenol



Beta-caryophyllene

FIGURE. Some of the bioactive compounds in cloves.

and female human volunteers, acute oral administration of eugenol is followed by rapid absorption in the digestive tract, evidence of a first-pass effect, and almost complete excretion in the urine within 24 hours.[9,10] Following an acute oral dose of 150 mg eugenol, amounts in the range of 0.02 to 100 µg/mL were detected in serum, urine, and bile. A serum concentration of 7 µg/mL (42.6 nM) was specifically reported.[9] Eugenol levels in the blood of rats peak rapidly after repeated oral administration and exhibit a mean half-life in blood of 14.0 hours.[6] It should be noted that eugenol and clove oil have been reported to improve the oral and percutaneous absorption and bioavailability of a variety of drugs.[15,16]

This discussion of the potential health benefits of clove is intended to be a general overview of the scientific literature associated with the biological actions of clove potentially impacting human health. Information is presented to introduce the reader to the variety of topics on clove and health, to highlight some topics where there is a better understanding of clove's possible benefits, and to assess the relevance of culinary use of this spice to any benefits.

METHODS

The PubMed literature database was consulted for this overview. Search terms included *S aromaticum*, flos caryophyllata, *E caryophyllata*, clove, clove oil, eugenol, and β-caryophyllene. Full reports and English abstracts of foreign-language articles from peer-reviewed journals were primary sources of information. The quality of some studies' methodologies varied, particularly in regard to adequately describing the composition of test samples. Nonetheless, these were included in this discussion so that the variety of information can be evaluated and important issues for future research can be identified. Commercial and governmental reports also were supplementary sources.

Numerous research studies have attempted to characterize the potential health benefits attributed to clove and its constituents, particularly eugenol. These include antioxidant, anti-inflammatory, antimicrobial, cardiovascular, and neurological benefits. Table 1 provides an overview of select, potential health benefits of clove. Table 2 lists the potential mechanisms of action for those topics presented in Table 1. The author's points of view for rating of evidence in each category are based on consideration of cell culture and animal studies and on human data published primarily from the peer-reviewed scientific literature. A rating of *preliminary* indicates that the collective evidence for a specific health benefit is not conclusive in light of the limited and sometimes inconsistent data from animal studies and well-controlled human trials. A rating of *emerging* indicates that data were suggestive of health benefits based on preclinical investigations and some clinical studies. The strength of a potential relationship between clove and improved health would be improved by additional and consistent reports from larger, well-controlled human studies. A rating of *strong* is for data that are consistent among preclinical experiments and at least 2 well-conducted human trials. In addition, evidence for a plausible biological mechanism is available.

It should be noted that the referenced studies evaluate effects of diverse clove samples, including its essential oil, as well as water and alcoholic and hexane extracts. Often the composition of the sample is not reported or not quantitatively analyzed. However, general qualitative information can be used to discern major differences in composition among the samples. For example, clove oil is reported to contain 75% to 90% eugenol, followed by β-caryophyllene and lesser amounts of α-humulene and eugenyl acetate.[5–7] Ethanolic extracts contain eugenol, β-caryophyllene, and eugenol acetate as major components and possibly some flavonoids, tannins, and alkaloids.[17,18] Hexane extracts have been reported to contain eugenol, eugenol acetate, and β-caryophyllene, as well as flavonoids, phenols, saponins, alkaloids, and tannins.[19,20] Water extracts can contain eugenol, *trans*-caryophyllene, anthraquinones, saponins, flavonoids, tannins, and eugenol derivatives.[21] Eugenol, β-caryophyllene, and eugenol acetate are likely present in all types of samples, although the relative composition of components within an extract will vary considerably, depending on specific isolation protocols used by each investigator.

MISCELLANEOUS ACTIONS

There are a variety of purported actions of clove supported mainly by preliminary evidence. For example, there are

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TABLE 1 Overview of Potential Health Benefits of Clove

Scientific Evidence	Rating
Antimicrobial actions	Emerging
A variety of herbs and spices including clove have been studied for their ability to inhibit the growth of diverse microorganisms.[22–24]	
1. Antibacterial effects	
1a. Effects of clove extracts	
The growth of a variety of organisms associated with human infections such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Shigella* species, *Salmonella enterica* serovar *typhi*, and *Pseudomonas aeruginosa* is suppressed in vitro by aqueous and alcoholic extracts of clove with minimum inhibitory concentration (MIC) ranges of 11 to 250 μg/mL.[25–28] An ethanolic extract was moderately effective in suppressing several multidrug-resistant bacteria with MIC of 195–1560 μg/mL.[29,30] Without knowledge of the composition of the alcoholic extracts in these 6 studies, it is difficult to determine why there is variation in antibacterial potency. Growth of *Helicobacter pylori*, the gram-negative bacterium associated with human gastritis and gastric ulcer, was suppressed following treatment with hydroalcoholic extracts of clove with MIC values of 50–60 μg/mL.[31,32] In addition, a methanol extract of clove was found to be the most effective growth inhibitor among 9 plant extracts tested and also produced synergistic inhibitory actions when combined with such antibiotics as tetracycline, erythromycin, ampillicin, cefoxitin, and cotrimoxazole.[33] Petroleum ether and chloroform extracts of clove demonstrated antibacterial activity against 40 clinical isolates of pathogenic bacteria including drug-resistant strains of *Shigella* and *Vibrio cholerae*, but were not effective against *P aeruginosa*.[34] It is of interest that extracts of clove have demonstrated growth-inhibitory properties against causal cariogenic pathogens, such as *Streptococcus mutans*, as well as periodontopathogens causing oral infections.[20,35–39] In a growth-inhibitory assay with cultures of anaerobic gram-negative bacteria, the bioassay-directed fractionation of a methanol extract of clove identified 8 active compounds. Two flavones, kaempferol and myricetin, and gallic acid showed potent inhibitory activity toward the anaerobic periodontal pathogens *Porphyromonas gingivalis* and *Prevotella intermedia*.[35] The authors suggested that select flavonoids from clove may have potential use as natural antiplaque or antigingivitis agents. In a recent study, human saliva samples were obtained from 25 periodontically healthy subjects and 25 subjects with chronic periodontitis.[40] When samples were plated and incubated with clove extract–impregnated discs, only modest evidence of inhibition was found. Because the composition of this extract is not known, it cannot be determined how the profile of individual constituents might have contributed to this lower potency. A randomized double-blind, placebo-controlled human study evaluating a mixture of 9 traditional Indian herbs (including *Syzygium aromaticum*) found that plaque, gingival bleeding, and growth of anaerobic bacteria significantly decreased compared with controls.[41] The individual sontribution of clove cannot be ascertained, however, and deserves further scrutiny. A review of patents obtained for herbal remedies of periodontal diseases has been published.[42]	
In light of the diverse extracts reported to have antibacterial potency, the report by Cai and Wu[35] underscores the need to identify specific effective phytochemicals within extracts by bioactivity-directed fractionation. This may be especially helpful in identifying new inhibitors of *P aeruginosa*, because hydroalcoholic but not chloroform/ether extracts appear to suppress this pathogen.	
1b. Effect of clove oil	
Clove oil was reported to be a potent antimicrobial agent in vitro against 4 oral *Streptococcus* pathogens, as well as *S aureus* and *Bacillus cereus*,[36,38,39] with MIC values of 155–310 μg/mL. It also was active against *Escherichia coli* O157:H7, *Propionibacterium acnes*, and *Listeria monocytogenes* but less active against *P aeruginosa*[43–47] and showed considerable efficacy against multidrug-resistant hospital-acquired bacterial strains.[48] When evaluated in vitro against 11 oral bacteria strains, clove oil and eugenol were much more effective than β-caryophyllene. Moreover, combinations of clove oil or eugenol synergized with ampillicin and gentamicin in suppressing these oral bacteria.[37] In 1 study, clove oil (0.01%) added to cosuspensions of *E coli* and human intestinal Caco-2 cells reduced the cytotoxic effect of *E coli* on the Caco-2 cells, even though the growth of *E coli* was only modestly inhibited.[44] This suggests that clove oil can lessen toxic insults to the intestinal environment, even though pathogen colonization is not eliminated.	
In vivo demonstrations of antibacterial efficacy are limited. In 1 study, mice were fed clove oil (0.5 mL of 1.0% wt/vol suspension) for 15- and 30-day periods prior to intranasal instillation with *Klebsiella pneumoniae* to induce acute pneumonia. Clove oil–fed animals exhibited a significant decrease in *K pneumoniae* colonization and tissue damage in the respiratory tract compared with controls.[49] Although a small increase in serum tumor necrosis factor α levels accompanied the administration of clove oil, possible mechanisms for this beneficial effect need to be explored further.	

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TABLE 1 Overview of Potential Health Benefits of Clove, Continued

Scientific Evidence	Rating
1c. Effect of eugenol	
Several studies provide evidence that the individual clove constituent eugenol showed bacteriostatic and bactericidal activity against *E coli* and *S aureus* (MIC of 0.05% vol/vol) as well as toward several bacterial mastitis pathogens, but not against *P aeruginosa*.[44,50,51] Eugenol was reported to be effective against susceptible and drug-resistant strains of *Neisseria gonorrhoeae* too.[52] It also suppressed the production of verocytotoxin by *E coli*.[53] In contrast, eugenol was less effective against the beneficial human intestinal bacterium *Bifidobacterium bifidum* and the food-borne pathogen *Campylobacter jejuni*.[46,54,55] The reasons for the differential sensitivity of these bacteria to eugenol is not known. In another report, eugenol was studied in combination with 10 different hydrophilic and hydrophobic antibiotics against 5 different gram-negative bacteria. The MIC of the eugenol-antibiotic combinations was observed to decrease by 5- to 1000-fold compared with the MIC values of eugenol and antibiotic alone.[56] The authors pointed out that these findings could lead to new combination strategies for treating gram-negative bacteria, but cautioned that additional pharmacokinetic and pharmacodynamic data in vivo would be needed.	
As far as mechanisms of antibacterial action are concerned, clove oil and eugenol have been reported to inhibit quorum sensing and biofilm formation, disrupt cellular membranes, and reduce the expression of virulence-related exoproteins.[57–62] The hydrophobic behavior of eugenol allows it to penetrate and disrupt the lipopolysaccharide cell membrane of gram-negative bacteria.[56] In eugenol-antibiotic combinations, it was suggested that eugenol damages the bacterial membrane, which then allows enhanced penetration of the antibiotics or other agents into the microbe.	
2. Antifungal effects	
2a. Effect of clove extracts	
Several studies have been conducted to assess in vivo antifungal efficacy. A hot water extract of clove provided in the drinking water decreased symptoms of oral candidiasis in mice orally infected with *Candida albicans*.[63] The chemical composition of this extract was not reported. In another experiment in which mice were orally infected with *C albicans*, administration of an undefined clove preparation (10.4 mg) into the oral cavity decreased macroscopic lesions on the lingual surface and retarded invasion of *Candida* mycelia into the lingual tissue.[64] This dose of preparation was determined to contain the equivalent of 0.23 mg of eugenol. Intragastric dosing of these mice with this clove extract (41.5 mg) decreased viable *Candida* cell numbers in the stomach and feces. Despite the lack of data on extract composition, these studies do, as the authors suggested, provide evidence that oral intake of clove as an herbal food may be helpful in suppressing the colonization of *C albicans* in the alimentary tract. It would be of interest to determine whether the supplementation of a mouse diet with clove would have similar antifungal actions.	
2b. Effect of clove oil	
The essential oil of clove has been reported to effectively inhibit various fungi and yeast in vitro. For example, fungi isolated from onchomycosis, such as *Candida* species and *Trichophyton* species, were inhibited by essential oil of clove (2%) as measured in a disc diffusion method.[65] Similarly, in disc diffusion assays and growth studies, clove oil showed considerable potency in inhibiting clinical *Candida* species as well as *Aspergillus*, *Saccharomyces*, *Microsporum*, and *Trichophyton* species.[66–71] Clove inhibited 38 *Candida* species isolated from denture wearers.[72,73] Regarding fungal skin infections, in an in vitro model using human hair samples, clove oil was found to fully inhibit hair penetration by *Microsporum gypseum* and *Trichophyton vanbreuseghemii*.[74]	
In a vaginal disease model, female mice were infected intravaginally with *C albicans*. Clove oil emulsions (36% eugenol, 8% β-caryophyllene, 1.6% eugenyl acetate, 1.6% α-bergamotene) delivered subcutaneously or by topical vaginal treatment substantially reduced the *Candida* titer in vaginal lavage samples.[75] When clove oil was delivered topically to mice in liposomes, it reduced candidiasis more substantially than topical application of the clove oil emulsion.	
2c. Effect of eugenol	
The phytochemical eugenol has been observed to exhibit in vitro inhibitory actions against various fungi.[65,76–80]	

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TABLE 1 Overview of Potential Health Benefits of Clove, Continued

Scientific Evidence	Rating
In an in vivo experiment in which the oral cavities of immunosuppressed rats were infected with *Candida* and then treated with eugenol (0.5 mL) for 7 d, the number of animals with an infection decreased by 72%, and the number of colony-forming units sampled from the rats was significantly reduced, compared with controls. Histologically, following eugenol treatment, considerably fewer zones of hyphal colonization on the dorsal surface of the tongue were detected.[81] It is interesting that, in contrast to treatment of the rats with the drug nystatin, following oral eugenol administration, no hyphae invading the folds of the tongue were observed. These data provide evidence that eugenol (and clove oil in which it is a major constituent) can be considered a strong antifungal agent for potential therapy of oral candidiasis.[73] Of related interest, controlled-release eugenol-containing mucoadhesive tablets have been developed to improve oral efficacy against fungal infections.[82] In an immunocompromised rat model of vaginal candidiasis, intravaginal treatment with eugenol (500 μL in 0.8% agar solution) led to elimination of *Candida* from the lumina of the vagina.[83] The authors stated that daily dosing caused no vaginal toxicity and that the volatile nature of eugenol likely allowed it to more readily penetrate inaccessible areas. A recent randomized human study with more than 450 female patients was conducted to evaluate a commercial vaginal douche (Saugella gel containing thymol and eugenol from thyme and clove oils as active ingredients) against vaginal candidiasis and bacterial vaginosis in comparison to econazole and metronidazole suppositories.[84] For bacterial vaginosis, there was similar symptom reduction from the douche and the metronidazole vaginal suppository. Likewise, the econazole suppository and the commercial douche suppressed vaginal candidiasis. From this study, however, the individual potency of eugenol cannot be determined. In a skin model, eugenol was tested in guinea pigs in which hairless skin areas were infected with *Microsporum gypseum* conidia, and a eugenol gel (10%) was applied for 3 wk beginning 5 d after inoculation. Eugenol induced significant clinical lesion remission during early stages of healing and led to a lower degree of hyperkeratosis and inflammatory cell infiltration compared with controls.[85] The authors suggested that eugenol should be considered as a supplemental topical antifungal agent for clinical remission of dermatophytosis.	
Various mechanisms of antifungal action have been identified and include disrupting the architecture of cell envelopes, arresting the cell cycle, preventing biofilm formation, and suppressing virulence factors.[66–70,77–80]	
3. Antiviral effects	
3a. Effect of clove extracts	
Extracts of clove and individual clove constituents have been tested for in vitro antiviral activity. For example, a crude extract of *Eugenia caryophyllata* exhibited anti–herpes simplex virus (HSV) activity,[86,87] and a water extract of *S aromaticum* was effective against hepatitis C virus at a dose of 100 μg/mL.[88]	
Three investigations examined the ability of clove extracts to act in vivo. In 1 study, immunosuppressed mice were infected intraperitoneally with murine cytomegalovirus. Oral dosing of mice with a hot water extract of clove (250 mg/kg) for 3 d beginning 1 d prior to infection significantly decreased the viral yield in lungs compared with controls.[89,90] A hot water extract of clove also was studied in mice intradermally infected with HSV-1. Oral dosing (750 mg/kg per day) of this extract for 10 d arrested the progression of recurrent HSV-1 disease, reduced the prevalence of severe erythema, and/or shortened the period of severe recurrent lesions compared with controls.[91] The authors pointed out that the doses used correspond to conventional doses of dried herbal medicines used in traditional therapy in China and Japan. A related study was conducted in mice subcutaneously infected with HSV-1 and treated orally with hot water extracts of clove (250 mg/kg) for 7 d along with a subclinical dose of the antiherpetic drug acyclovir. Combined treatment more substantially delayed skin lesion development and/or prolonged mean survival times compared with acyclovir alone and extract alone.[86] In addition, a combination of extract with acyclovir substantially suppressed virus yield in skin and brain samples compared with drug and extract alone.	
These in vivo studies underscore that oral delivery of clove phytochemicals can lead to their systemic bioavailability as evidenced by amelioration of infections in distant tissues such as the lung and skin. This justifies further studies to examine whether dietary administration of clove has any similar benefits in alleviating viral infections.	
3b. Effect of eugenol and other constituents	
Eugenol was observed to inhibit HSV in vitro in several reports.[87,92,93] In addition, the clove constituents eugeniin, the tannin tellimagrandin, and β-caryophyllene were identified as having antiviral actions.[92,94,95]	

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TABLE 1 Overview of Potential Health Benefits of Clove, Continued

Scientific Evidence	Rating
In vivo efficacy of eugenol has been tested in mice subjected to corneal injection of HSV-1. Mice were treated ocularly with eugenol (0.5–1.9 mg/mL) for 5 d, with only the highest dose of eugenol significantly suppressing the development of herpes virus-induced keratitis.[93]	
The efficacy of eugenol as an antibacterial and antifungal agent has led to its incorporation in nanoparticles and polymeric derivatives for improved biomedical outcomes.[96–98] Also clove oil and eugenol are used for preservation of postharvest food materials and prepared and processed foods and even for maintaining the health of animals used for food production.[99–111]	
Collectively, there is an emerging body of evidence supporting the antibacterial, and particularly the antifungal, actions of clove. It is likely that eugenol is the primary antifungal agent in clove oil, a conclusion that is supported by the considerable in vivo antifungal data for eugenol. Yet, the oral efficacy of water extracts against *Candida* and viruses suggests additional clove components may have appreciable activity. Bioactivity-directed fractionation of such samples could identify other effective constituents. The diversity of clove samples used and the lack of additional composition data are a general limitation of the evidence base that needs to be addressed in future studies. This hinders making meaningful comparisons of potency among extracts and oils. Much of the data regarding antimicrobial effects of clove and constituents were generated at doses that are not relevant to typical culinary use of clove. In light of this, it would be important to determine in appropriate animal models and possibly in humans how diets containing multiple, culinary-relevant levels of well-characterized clove can mitigate infections. For example, disease events such as plaque development, growth of cariogenic bacteria, antimicrobial potency of saliva, and mitigation of respiratory infections could be examined. Likewise, the in vivo impact of such diets on the microbial population of the gastrointestinal tract, both beneficial and detrimental, is of interest. Based on human consumption estimates from India,[112] doses of powdered clove at ≤1.0 mg/kg and clove oil at ≤0.1 mg/kg could be considered for studies evaluating culinary or nutritional relevance.	
Diabetes	Emerging
a. Effect of clove extracts	
In cultures of hepatoma cells and rat hepatocytes, addition of an uncharacterized water extract of clove (150 μg) acted like insulin by reducing phosphoenolpyruvate carboxykinase and glucose-6-phosphatase gene expression,[113] indicating a potential role for clove-derived compounds as insulin-mimetic agents.	
In in vivo studies phytochemical constituents in clove exhibited hypoglycemic effects. Specifically, in type 2 diabetic KK-Ay mice, an ethanol extract of clove administered in the diet (0.5 g/100 g diet) for 3 wk significantly suppressed blood glucose levels compared with control animals in which blood glucose had increased 3-fold during that same period.[114] Subsequent in vitro bioassay-directed fractionations in this report identified the neolignan derivative of eugenol, dehydrodieugenol, and oleanolic acid as contributors to this action, in part via peroxisome proliferator-activated receptor γ (PPAR-γ) activation. This is relevant because PPAR-γ activators are in use as insulin sensitizers as part of therapies for type 2 diabetes and the metabolic syndrome.[115]	
b. Effect of eugenol and other constituents	
In in vitro studies with 3T3-L1 adipocytes and L6 myotubes, eugenol (2.5–50 μM) was able to increase 2-deoxyglucose (2DG) uptake.[116,117] Furthermore, eugenol synergized with oral hypoglycemic drugs (OHDs) in stimulating 2DG uptake. In 3T3-L1 adipocytes, 10 μM eugenol could reduce by one-fourth the dose of metformin that was needed to achieve a nearly 3-fold increase in 2DG uptake. It was also noted that in these cells eugenol was able to stimulate gene expression of GLUT4, PPAR-γ, and PI3K while suppressing expression of fatty acid synthase and HMG CoA reductase. It was also observed that eugenol significantly reduced triglyceride levels in L6 myotubes. The authors suggested that eugenol and other hydrocinnamic acid derivatives may be beneficial for treating diabetes and may be able to lower secondary complications from OHDs.[118] They also concluded that such phytochemicals could partly replace commercial drugs for stimulating glucose uptake.	

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TABLE 1 Overview of Potential Health Benefits of Clove, Continued

Scientific Evidence	Rating
In streptozotocin (STZ)-induced diabetic rats and nondiabetic rats, oral oleanolic acid (40–120 mg/kg) that had been isolated from clove, was effective, like insulin-treated controls, in decreasing blood glucose concentrations following an oral dose of glucose. When combined together in STZ-induced rats, oleanolic acid and insulin created a synergistic antihyperglycemic response, even causing blood glucose concentrations to decrease to hypoglycemic levels. Oleanolic acid did not significantly alter insulin secretion in either rat model.[118] Moreover, in contrast to controls, extended (5 wk) oleanolic acid treatment of STZ-induced diabetic rats not only lowered blood glucose levels but also restored hepatic and muscle glycogen levels to near-normal levels. In a subsequent follow-up animal study, the authors reported that oral oleanolic acid (80 mg/kg) restored hepatic and muscle glycogen concentrations to near normalcy, in part by increasing the activities of the liver and muscle glycogenic enzymes glucokinase and hexokinase that were lowered in STZ-induced diabetic rat controls.[119] Similar hypoglycemic effects were reported in Dahl Salt Sensitive hypertensive rats administered oleanolic acid (60 mg/kg per day, intraperitoneally) for 6 wk.[120]	
Taken together, there is an emerging body of evidence in support of the antidiabetic actions of individual clove constituents particularly in light of the in vivo hypoglycemic effects of eugenol, dehydrodieugenol, and oleanolic acid. However, these studies do not necessarily mean that similar responses could be expected from the culinary use of clove. Therefore, culinary-relevant doses of well-defined samples of clove need to be evaluated in appropriate animal models for an impact on glucose and insulin homeostasis. Besides the models reported here, expanding studies to include other animal models such as nonobese diabetic mice, high-fat-fed C57BL/6 rats, and Zucker diabetic fatty rats would provide valuable insights into both obese and nonobese models and highlight differences in efficacy under diverse diabetic conditions.[121] Such investigations would provide important insights prior to measuring similar end points in human pilot studies of diets supplemented with clove.	
Neurological effects	Emerging
Clove and its constituents have been reported to elicit a variety of complex actions on the nervous system. Eugenol in particular is known to cause general central nervous system responses in mammals, such as anesthetic, myorelaxant and anticonvulsant actions.[122]	
1. Anesthetic and analgesic actions	
1a. Effect of clove extracts	
An ethanol extract of clove when injected into mice (50–200 mg/kg, intraperitoneally) significantly decreased pain in 2 models of chemically induced algesia.[18] The extract was reported to generally contain flavonoids, resins, glucosides, tannins, saponin, and alkaloids. In a human study,[123] a chemically-undefined clove gel preparation was applied to the buccal mucosa of 73 volunteers prior to needle sticks superior to the mucogingival border. Clove treatment was equivalent to benzocaine in significantly lowering pain scores compared with controls. A limited number of participants experienced small ulcers at the site of the gel application, most likely due to eugenol-induced irritation.	
1b. Effect of clove oil	
Clove bud oil administered to mice (0.025–0.1 mL/kg, intraperitoneally) significantly reduced pain responses to 2 nociceptive stimuli.[123] The composition of the clove oil was reported to be 87% eugenol, 5.2% eugenyl acetate, and 2% β-caryophyllene.	
1c. Effect of eugenol	
In rats and mice, eugenol demonstrated antinociceptive activity in several animal pain models[18,122,124–133] when administered by a variety of routes and doses such as by the oral route (1–10 and 20–40 mg/kg), by subcutaneous injection (1–10 μg and 50–200 μg), by intravenous injection (5–60 mg/kg), by intraperitoneal injection (1.2–2.4mg/kg), and by intrathecal injection (12.5–50 μg). A prospective, randomized human study assessed the efficacy of a eugenol-loaded gauze strip compared with a thermosetting gel containing prilocaine and lidocaine for relief from postextraction alveolar osteitis.[134] Both treatments significantly reduced pain.	

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TABLE 1 Overview of Potential Health Benefits of Clove, Continued

Scientific Evidence	Rating
The mechanisms of action of clove and eugenol in ameliorating pain are not completely understood, nor is it clear whether other constituents of clove may be contributing to any benefits. β-Caryophyllene, for example, may be an additional antinociceptive phytochemical.[135] Based on these in vivo studies as well as numerous in vitro investigations,[136–144] it appears that multiple biochemical processes are mediating changes in neural function. Examples include alteration of α2-adrenergic and opioidergic receptor activities, inhibition of voltage-gated sodium and calcium channels, modulation of vanilloid receptors, inhibition of hyperpolarization-activated cyclic-nucleotide-gated channels, and dysregulation of neuropeptides in the spinal column. The specific mechanisms identified depend on the neural tissue studied, pain stimuli used, and doses of eugenol or clove-derived samples selected.	
2. Neuroprotective effects	
Spices have been sources of numerous phytochemicals exhibiting protection against seizures and neurodegenerative diseases.[145]	
2a. Effect of clove extracts	
In a recent in vitro study, the effect of clove on an enzyme responsible for α-amyloid production in the brain, β-secretase, was evaluated.[146] Specifically, a dried, water extract of clove consistently inhibited β-secretase (EC50 = 64 μg/mL). But, interestingly, a separate evaluation of eugenol (0.1–100 μg/mL) did not account for this inhibitory potency of the water extract. The composition of this extract was not reported, and determining the bioactive constituent(s) would be informative.	
In 2 animal studies, mice were orally administered a traditional Chinese medicine, Shitei-To. This is a mixture historically used for the treatment of hiccups in Japan and China that contains extracts from 3 herbs including *S aromaticum*. Repeated dosing of Shitei-To at 3 g/kg protected against the development of chemically induced seizures and convulsions.[147,148] However, the specific contribution of clove extract cannot be determined.	
2b. Effect of clove oil	
Injection of mice with clove oil (0.025–0.1 mg/kg, intraperitoneally) resulted in anticonvulsant activity against chemically induced seizures.[149]	
2c. Effect of eugenol	
In experiments using cell preparations and rat brain slices, eugenol was able to block Na^+ currents and could also suppress epileptiform field potentials in part by inhibiting synaptic function.[150–152] The authors suggested that eugenol has potential for use in treatment of epilepsy and migraines. In primary cortical cell cultures, eugenol (100–300 μM) protected against excitotoxic and oxidative injury due to oxygen-glucose deprivation, reactive oxygen-induced neurotoxicity, and *N*-methyl-d-aspartate exposure.[153]	
Neuroprotective benefits of eugenol were also demonstrated in several animal models. For example, gerbils that had been injected with eugenol (50–200 mg/kg, intraperitoneally) after occlusion of the carotid arteries showed delayed ischemia-induced neuronal cell death in the brain compared with controls,[154] possibly due to a hypothermic effect of eugenol. In a STZ-induced diabetic rat model, eugenol was evaluated for its capacity to improve nerve function. Following 6 wk of untreated diabetes, a 2-wk treatment with eugenol (200 mg/kg) corrected deficits in sciatic nerve endoneurial blood flow and in dysfunctions of gastric fundus nitrergic nerve-mediated relaxation.[155] The authors suggested that eugenol could have potential therapeutic benefit for diabetic neuropathy and vasculopathy. However, eugenol's reported actions are not always beneficial. For example, contradictory actions of eugenol toward pathologies associated with Parkinson disease (PD) are evident in 2 in vivo studies. Injection of mice with eugenol (0.1–1.0 μmol/kg) for 3 d prior to and 7 d after an intracerebroventricular injection of 6-hydroxydopamine (6-OHDA) prevented the reduction of striatal dopamine and its metabolites, compared with controls.[156] This pretreatment with eugenol also was associated with decreased lipid peroxidation and increased glutathione in the striatum. These findings are relevant in light of the progressive deterioration of dopaminergic neurons in the nigrostriatal system and dopamine decreases in the striatum characteristic of PD. In a subsequent study, a different dosing schedule was used. Mice were first injected with 6-OHDA (intracerebroventricular) and then given daily doses of the PD therapeutic medicine L-DOPA, and 10 μmol eugenol/kg per day in the drinking water for 4 wk. In this scenario, administering eugenol after 6-OHDA actually exacerbated the striatal depletion of dopamine.[157] Thus, eugenol elicited both beneficial and adverse effects depending on the timing of its administration within the progression of the brain changes induced by 6-OHDA. Also, the method of eugenol administration may have contributed to these differences. This highlights issues that need to be further explored in future animal studies.	

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TABLE 1 Overview of Potential Health Benefits of Clove, Continued

Scientific Evidence	Rating
3. Neurobehavioral actions	
3a. Effect of clove extracts	
A mouse model of Alzheimer disease (AD)–associated memory impairment was used to evaluate a formulation of Chinese traditional medicine that contained an ethanol extract of clove buds along with extracts from 8 other herbs.[158] Feeding this formulation for 3 mo was found to significantly improve amyloid β–induced memory deficits. However, the individual contribution of clove within this complex phytochemical mix cannot be determined. It would be of considerable value to confirm in this mouse model any improvements in memory deficits if diets are supplemented with clove alone. Oral administration of a hydroalcoholic extract of clove to rats (100–200 mg/kg) for 14 d diminished the stress responses to anoxia, cold-restraint, and sound.[159] The phytochemical analysis of this extract was reported as containing alkaloids, carbohydrates, steroids, tannins, and flavonoids. These doses of extract were based on a preliminary determination that a 2000-mg/kg dose of this extract was without toxicity. In 2 studies, enhanced sexual behavior of male mice was evident after oral administration of an ethanol extract of clove (100–500 mg/kg) for 7 d.[160,161] The extract was reported to simply contain sterols and phenols. The authors suggested that these results confirm claims by the traditional Unani System of Medicine that clove is clinically useful as a sexual invigorator in males. In a clinical trial of a topical cream, containing extracts of clove along with that of 8 other herbs, promising results in the treatment of lifelong, premature ejaculation among the 106 male participants was reported.[162] The individual effect of clove toward these benefits cannot be determined, however. Eugenol, as one of the active ingredients in this cream, was presumed to contribute to local desensitizing actions.	
3b. Effect of clove oil	
In 1 study, mice were treated with scopolamine to induce learning and memory deficits. Pretreatment of mice with clove oil (0.025–0.1 mL/kg, intraperitoneally) for 3 wk significantly reversed the progressive impairments in memory retention and learning acquisition induced by scopolamine.[163] This effect was partly attributed to decreases in oxidative stress markers in the brain. In a subsequent, similar mouse study, clove oil (0.025–0.1 mL/kg, intraperitoneally) significantly improved scopolamine-induced memory impairment at all doses.[124]	
3c. Effect of eugenol and other constituents	
Several in vivo investigations indicated that eugenol can elicit antistress and antidepressant-like actions. In 1 study, rats were orally administered eugenol (25–100 mg/kg) for 7 d prior to stress screening determinations. Eugenol treatment was found to suppress stress-induced ulcer scores, although a U-shaped dose-response curve was evident. Eugenol also reduced stress-induced activation of the hypothalamic-pituitary-adrenal axis and modulated the brain monoaminergic system.[164] An antistress response to eugenol in rats has been reported by others as well.[165]	
Regarding antidepressant actions of eugenol in vivo, mice were provided eugenol in drinking water (100 mg/kg) for 14 d and exposed to a forced-swim test. Eugenol exhibited antidepressant-like activity comparable to the antidepression drug imipramine.[166] Furthermore, both eugenol and imipramine induced brain-derived neurotrophic factor in the brain. In a subsequent, similar study by these authors, eugenol in the drinking water (0.17 mmol/kg per day) for 14 d significantly lessened the depressive response to 2 screening tests, an action suggested to be due in part to inhibition of monoamine oxidase A activity.[167] Based on the authors' work in both AD and stress models, they hypothesized that eugenol could have dual functions (as anti–amyloid-β agent and antidepressant) mediated by broader and possibly unrelated mechanisms of action. It is noteworthy that another constituent of clove, *bis*-eugenol (dihydroeugenol), when injected into mice (10–50 mg/kg, intraperitoneally) elicited antidepressant-like effects in the forced-swim test.[168] This was attributed to *bis*-eugenol's altering of dopaminergic, serotonergic, and noradrenergic systems' functions. In addition, β-caryophyllene administration to mice (100–6400 mg/kg, intraperitoneally) significantly increased their ambulatory activity and underscored the psychoactive property of this phytochemical constituent of clove.[169]	

(continues)

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TABLE 1 Overview of Potential Health Benefits of Clove, Continued

Scientific Evidence	Rating
Considered together, the consistent findings from several animal models provide an emerging body of evidence for a role of clove, and particularly eugenol, in mediating several neurological benefits. In considering studies evaluating the effects of clove oil and clove extracts, it is likely that eugenol is the main contributor to their neurological effects, although other constituents alone or in combination with eugenol cannot be ruled out. However, several issues hinder understanding any nutritional or culinary benefits of clove in humans. Without well-characterized, clove-derived samples used in in vivo studies, it is difficult to identify bioactives in addition to eugenol that may be impacting neural processes and their mechanisms of action and to make meaningful comparisons between studies. Also, in order to establish any potential relevance to typical use of clove as a food spice, studies are clearly needed to investigate culinary-relevant dietary doses of clove on established animal models of neurodegenerative disease such as those related to the pathogenesis of PD and AD.[170–172] The preclinical evidence to date that oral administration of eugenol can lead to behavioral, neurological, and biochemical changes suggests that determining the effects of typical use of clove as a food spice is warranted. For example, meals containing defined amounts of clove as a food additive could be used in evaluating quantitative measures of depression/anxiety symptom relief, perception of well-being, and other behavioral improvements in subjects. Combining these measurements with a bioavailability evaluation of clove sample constituents would be important to more clearly determine whether consumption of clove can benefit this area of health.	

numerous reports identifying extracts of clove, clove essential oil, and eugenol with antioxidant activity in vitro[173–185] using a variety of assays. Considered collectively, these extracts and clove oil were effective in demonstrating antioxidant efficacy against such end points as inhibiting lipid peroxidation and malonaldehyde formation and in scavenging superoxide anions. The method of clove extraction varied considerably and included such diverse procedures as hot water extraction, extraction with 80% ethanol, and steam distillation with dichloromethane extraction. Thus, the chemical composition of extracts likely varied widely in content of flavonoids, phenolics, and hydrolyzable tannins. Often, the composition of the extracts was not well documented.

In vivo support for the antioxidant action of clove and eugenol are limited. For example, in vivo treatment of rats with clove oil (5 mg/kg body weight per day for 30 days) reduced free-radical damage associated with aflatoxin toxicity.[186] Oral eugenol dosing (0.2–25 mg/kg body weight) inhibited lipid peroxidation–mediated liver necrosis following CCl_4 administration to rats, in part due to its apparent ability to intercept secondary radicals from endoplasmic reticulum–derived lipids.[187] In another study, when mice were treated with eugenol (75–300 mg/kg, intragastric) prior to exposure to γ-irradiation, oxidation-induced genetic damage in bone marrow was significantly reduced.[188] Oral dosing of rats with eugenol (1000 mg/kg) for 15 and 90 days led to increased intestinal activity of glutathione-S-transferase, an enzyme that plays an important role in controlling oxidative stress in cells.[189] Despite these studies, there is limited in vivo evidence that dietary clove can suppress established oxidative stress biomarkers associated with human chronic conditions.

There are several potential mechanisms by which clove might counteract cardiovascular disease processes. For example, clove oil, eugenol, acetyl eugenol, and clove polysaccharides were effective as in vitro inhibitors of platelet aggregation. In 2 of these reports, eugenol exhibited

TABLE 2 Possible Mechanisms of Action of Clove and Constituents

Potential Health Benefits	Possible Mechanisms of Action
Antimicrobial effects	Disrupt cellular membranes
	Inhibit biofilm formation
	Suppress quorum sensing
	Decrease virulence factors
	Arrest cell cycle
Hypoglycemic activity in diabetes	Mimic insulin
	Sensitize to insulin
	Stimulate glucose uptake
	Increase glycogenesis
	Repress hepatic gluconeogenesis
Neurobehavioral benefits	Alter nerve transmission
	Improve nerve function
	Reduce oxidative stress
	Decrease stress-induced hormones/growth factors
	Modulate brain neurotransmitters and neuropeptides

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potency similar to aspirin.[190–195] The clove constituents eugenol and β-caryophyllene oxide were reported, mostly in in vitro or ex vivo studies, to cause vasodilation of blood vessels and relaxation of vascular smooth muscle cells, an effect that could not be completely explained by calcium-channel blockade activity. Eugenol also showed vasodilator activity by inhibiting $[K^+]_o$-induced aorta contractions.[195–205] In 1 dog and 1 rodent experiment, intravenous administration of eugenol (1–10 mg/kg) was observed to elicit hypotensive effects in part by causing a transient reduction in blood pressure and myocardial contractile force without changing the heart rate.[206,207] Inhibiting the oxidation of low-density lipoproteins (LDLs) also may elicit antiatherosclerotic consequences. Eugenol inhibited oxidation of LDL in plasma samples[208–210] and changed the affinity of LDL particles for the LDL receptor in vitro.[211] In plasma samples isolated from non–insulin-dependent diabetic patients, eugenol suppressed oxidation of LDL and very-low-density lipoprotein in a manner similar to the synthetic antioxidant butylated hydroxytoluene.[212] Eugenol also protected against oxidized LDL-induced dysfunction in endothelial cell cultures,[213] and humulene and caryophyllene were able to attenuate endothelial cell damage associated with bacterial infections.[214] In the hypercholesterolemic zebrafish model, in vivo administration of an aqueous extract of clove substantially decreased serum cholesterol and triglyceride levels, apparently due to suppression of oxidative stress, prevention of LDL phagocytosis, and inhibition of cholesteryl ester transfer protein function.[12] Determination of clove's in vivo actions toward cardiovascular end points is clearly needed.

Clove and its constituents exhibit multiple actions in modulating inflammation and immune responses in vitro. Clove and eugenol were reported to inhibit cyclooxygenase 2 and nitric oxide synthase in a variety of cell lines, in part by modulating signaling pathways and by inhibiting oxygen radical generation and the production of proinflammatory mediators.[215–227] Eugenol and, to a lesser extent, β-caryophyllene and isoeugenol demonstrated anti-inflammatory potency in several animal models of animal inflammation.[228–235] Clove oil and eugenol have been shown to produce diverse immunomodulatory activities including anaphylaxis suppression, improved humoral- and cell-mediated immunity, and inhibition of immediate hypersensitivity.[236–244] It should be kept in mind, however, that clove oil and its constituents may have complex effects on immune-responsive cells. Depending on the dose, target tissue, and method of administration, clove and its constituents may act as contact sensitizers or have anti-inflammatory actions, as well as elicit apparently opposing effects on intracellular signaling pathways and immune system behavior. It is interesting that eugenol is being developed as part of a prodrug to treat inflammation. When combined with aspirin into an aspirin-eugenol-ester compound, adverse effects of each individual compound were reduced, and agent stabilization and overall therapeutic efficacy were enhanced.[245]

HUMAN CONSUMPTION OF CLOVE

In 1 report, human consumption of clove in India was estimated to be 0.43 mg/kg per day and that of clove oil to be 0.045 mg/kg per day.[112] When used as intended as a food additive, clove is considered GRAS (generally recognized as safe) by the US Food and Drug Administration. The US Food and Drug Administration has approved clove oil for use as an analgesic in dentistry, for use as a flavoring additive in foods, and as a fragrance component in personal care and aromatherapy products. Risk of food allergy from clove oil appears to be small.[246] Although clove oil is considered safe for use as a food additive in small quantities (<1500 ppm), when taken acutely at high levels, far greater than amounts occurring in foods, it can cause systemic poisoning and severe complications including respiratory distress, rapid heartbeat, liver failure, and nervous system depression and seizures.[247–251] Human per-capita consumption of eugenol is estimated to be 0.6 mg/d.[3] The World Health Organization has established an Acceptable Daily Intake level for eugenol of 2.5 mg/kg per day based on a NOAEL (no observed adverse effect level) of 250 mg/kg per day in rats.[3,251–254] The European Food Safety Authority has concluded that eugenol is "unlikely to be genotoxic at exposures that do not result in cytotoxicity and saturation of conjugation pathways." The European Food Safety Authority also concluded that based on available data eugenol is not carcinogenic and did not exhibit teratogenic or neurotoxic effects.[255,256] Following short-term (7 days) dietary administration of eugenol (150 mg/d) to humans, no biochemical or cytogenetic evidence of toxicity was observed.[257]

There are substantial health hazards associated with inhaling clove cigarette smoke, including severe lung injury to individuals with prodromal respiratory infections and aspiration pneumonitis in some individuals with normal respiratory tracts.[3,258–260] Clove cigarettes or kreteks are more potent than regular cigarettes in delivering carbon monoxide, tar, and nicotine.

SUMMARY

The scientific literature provides some support mostly from preclinical studies that clove or individual constituents can have health benefits toward suppression of microbial growth, improvement of diabetes symptoms, and improvement of neurological problems. Beyond this, there is preliminary evidence that clove and its constituents can contribute to inhibiting oxidative stress, counteracting inflammation, and opposing processes associated with cardiovascular disease. Besides eugenol, other constituents

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of clove are likely making important contributions to these actions

A number of general research issues need to be addressed in appropriate animal models and eventually in human studies as to whether clove can have any impact on health when used as a spice in food. A considerable limitation of the evidence base is the lack of information on the chemical composition of clove samples used for both in vitro and in vivo investigations. In addition, in vivo benefits of clove, clove oil, extracts of clove, and individual constituents toward established biomarkers of chronic disease need to be conducted using culinary-relevant doses. Estimates of individual human consumption of clove and clove oil are available[112] that can be used to design studies with greater nutritional relevance. These studies also can provide useful information about bioavailability of constituents and possible mechanisms of action when this spice is used as a dietary ingredient.

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FDA TAKES ANOTHER STEP ON INFANT FORMULA PROTECTIONS

The FDA finalized manufacturing guidelines for infant formula makers that aim to ensure products sold for babies meet certain quality controls to keep them safe. Under the final rule, standards include the following:

- current good manufacturing practices specifically designed for infant formula, including required testing for the harmful pathogens (disease-causing bacteria) *Salmonella* and *Cronobacter*
- a requirement that manufacturers demonstrate that the formulas they produce support normal physical growth
- a requirement that infant formulas be tested for nutrient content in the final product stage, before entering the market, and at the end of the products' shelf life

The final rule applies only to infant formulas intended for use by healthy infants without unusual medical or dietary problems.

The FDA notes that many companies now manufacturing infant formula for the US market have been producing safe products and have voluntarily applied many of the current good manufacturing practices and quality control procedures included in the final rule. But this rule will set in place federally enforceable requirements for the safety and quality of infant formula. The FDA does not approve infant formulas before they can be marketed. However, all formulas marketed in the United States must meet federal nutrient requirements, which are not changed by the new rule. Infant formula manufacturers are required to register with the FDA and provide the agency with a notification prior to marketing a new formula. The FDA conducts yearly inspections of all facilities that manufacture infant formula and collects and analyzes product samples. It also inspects new facilities. If the FDA determines that an infant formula presents a risk to human health, the manufacturer of the formula must conduct a recall.

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