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COMPREHENSIVE REVIEW ON PHYSIOLOGICAL EFFECTS OF EUGENOL

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ABSTRACT

Medicinal plants are considered rich sources of traditional medicines, and many modern medicines are derived from them. For thousands of years medicinal plants have been used to treat different health disorders. Eugenol is a hydroxyphenyl propene, naturally occurring compound in the essential oils of various sort of plant belonging to limiaceae, lauraceae, myrtaceae and myristicaceae families. A large body of scientific evidence supports claims from traditional medicine that eugenol exert beneficial effect on human health and has several pharmacological activity. This review article is an attempt to update on the succinct study of pharmacological actions of eugenol. eugenol has anti-inflammatory, antibacterial, anti-fungal, antiviral, antiparasitic, and anticancer effect and also studies shows that it have cardio vascular and antidepressant effect but eugenol has been used for dental analgesic purposes

KEYWORDS: Eugenol, Essential oil, anti-inflammatory, anti-fungal, dental analgesic, anti-bacterial.

INTRODUCTION

Eugenol, a phenolic photochemical found in a number of essential oils, including clove oil, nutmeg, cinnamon, basil, and bay leaf, has antimicrobial and anticancer properties.[1] Since the dawn of time, medicinal plants, especially their active components, have been a reliable source of therapeutics for the treatment of a variety of ailments.[2] Eugenol was named after the first time it was extracted from the buds and leaves of Eugenia caryophyllata (clove).[3] Currently, eugenol can also be synthesized by allylation of guaiacol with allyl chloride having the similar kind of functional property. Eugenol is found in high concentrations in a

variety of medicinal herb extracts. So it has fascinated the attention of several researchers and opened up the gateway of research regarding its utilization as a medicine to cure various diseases.[4]

Eugenol is said to have anaesthetic activity, antioxidant potential, antimicrobial role, anti-inflammatory action, anti-carcinogenic effects, neuroprotective ability, hypolipidemic efficiency, and anti-diabetic effectiveness. eugenol belongs to the class of essential oils that is generally recognised as safe (GRAS) by the Food and Drug Administration.[5]

Eugenol remains a research priority due to its diverse biological and functional properties. As a result, it is critical to rationally combine research findings related to eugenol's therapeutic potential to elucidate its importance for human health and mechanisms involved in eugenol's functionality to avoid several lifestyle-related indispositions.

Physical and Chemical properties of eugenol

Eugenol is a chemical compound that belongs to the allyl-benzene class. It is guaiacol with an allyl chain substitution. Guaiacol is an organic compound that occurs naturally and has the formula C6H4(OH) (OCH3). It has the appearance of a clear to pale yellow oily liquid. Eugenol is highly soluble in organic solvents and only slightly soluble in water. Eugenol is a phenylpropanoids compound (C10H12O2). The compound's IUPAC name is 4-Allyl-2-methoxyphenol (Figure 1), and it has a molecular mass of 164.2g/mol and a pKa of 10.19 at 25°C. It has two isoforms: eugenol and iso eugenol. Caryophyllic acid is also known as allylguaiacol, 2-methoxy-4-(2-propenyl) phenol, and 4-allylcatechol2-methyl ether. Its antioxidant property is due to the phenolic group. It is partially soluble in water and becomes more soluble in organic solvents. The compound's colour ranges from clear to pale yellow [3]. The metabolism of eugenol produced conjugates with sulphate, glucuronic acid (major), and glutathione.[4]

Biologically active synthetic derivatives of eugenol

Plant sources of eugenol

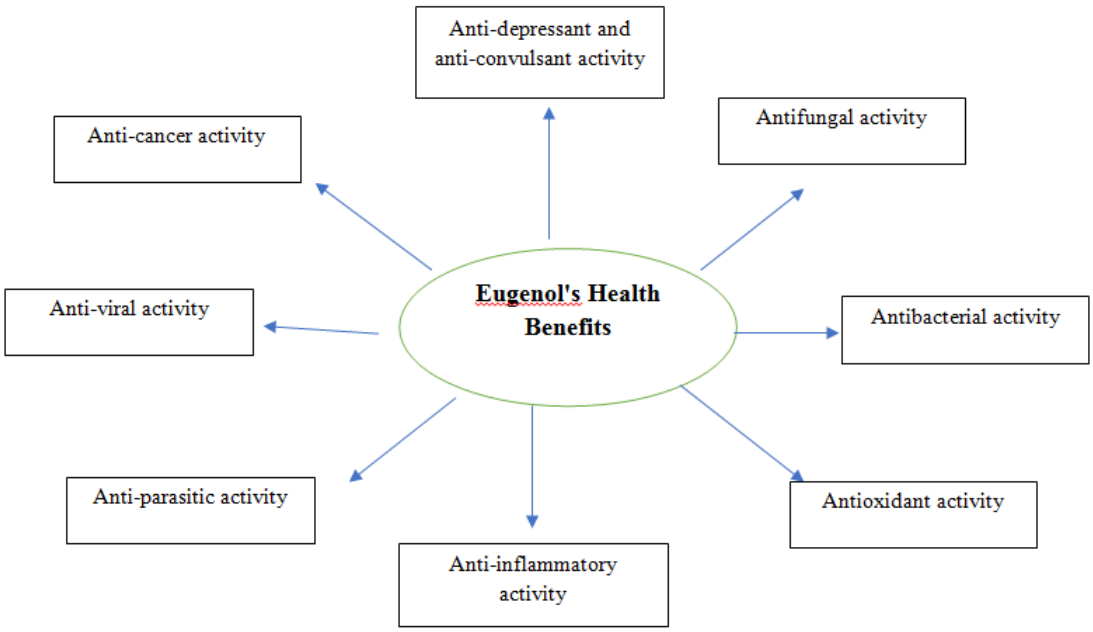
Eugenol is found in a variety of plants including clove buds, cinnamon bark and leaves, tulsi leaves, turmeric, pepper, ginger, oregano and thyme.[3] In addition, several other aromatic herbs including basil, bay, marjoram, mace and nutmeg are also claimed to have significant quantity of eugenol.[6] Concentration of eugenol in some plants is depicted in Table 1.

Pharmacokinetics properties of eugenol

Metabolism of eugenol in male and female healthy volunteers, the study found that after oral administration, eugenol is rapidly absorbed and metabolised. Within 24 hours, it is almost completely excreted in the urine. Only about 0.1 percent of the administered dose was excreted in urine unmetabolized. Eugenol was discovered in the urine as conjugates and metabolites.[6]

The amounts of the individual metabolites excreted were determined by g.1.c. 95 percent of the dose was recovered in the urine, the majority of which (> 99 percent) consisted of phenolic conjugates; 50 percent of the conjugated metabolites were recovered. Other metabolic routes observed were the epoxiddiol pathway, synthesis of a thiophenol and of a substituted propionic acid, allylic oxidation, and migration of the double bond. Eugenol which contains a free phenolic hydroxyl group, and thus could be expected to be readily conjugated and excreted as are other phenols, Studies in rats and guinea pigs showed that eugenol was effectively glucuronidated in these species and, as anticipated, the conjugation of eugenol with glucuronic acid and sulphate is also the major metabolic pathway of eugenol in man.7[21] pharmacokinetic parameters of eugenol using non-compartmental analysis after gavage administration in male Sprague–Dawley rats in a dose of 40 mg/kg. Plasma T1/2 of eugenol was found to be 14.0 hours and blood T1/2 was 18.3 hours.[8]

Pharmacological activities of eugenol



Anti-inflammatory action

It has been strongly suggested that eugenol has neuroprotective effects against various sorts of inflammatory events. Inducible cyclooxygenase (COX-2) is an important factor to drive inflammatory and carcinogenic processes in many organs including brain. Blockade of this enzyme can reduce inflammation. Each NSAID's anti-inflammatory effect is attributed to its inhibitory activity on COXs. Eugenol inhibits PGE2 production in lipopolysaccharide (LPS)-stimulated macrophages in vitro by suppressing gene expression of COX-2 but not of COX-1. Eugenol also inhibits enzymatic activity of COX-2. Thus, eugenol has a property of COX-2 specific anti-inflammatory reagent. Leukotrienes are bioactive 5-lipoxygenase products of arachidonic acid (AA) metabolism that have been linked to inflammation and allergic symptoms. Asthma and a number of other inflammatory pathologies such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, and glomerulonephritis have all been linked to LTs as mediators.

5-Lipoxygenase (5-LOX) is an enzyme that is essential in the biosynthesis of leukotrienes. Eugenol inhibited the 5-LOX enzyme in human polymorphonuclear leukocytes with an IC50 value of 26.0 M. Eugenol also inhibited leukotriene C4 (LTC4) formation in a concentration-dependent manner, with an IC50 value of 30.0 M. The study also concluded that eugenol's inhibitory effect on the 5-LOX enzyme was non-competitive.[9]

Antibacterial activity

The combination of eugenol with a conventional antibiotic has been evaluated to detect the synergistic effect against Gram negative bacteria. In the eugenol treated cells, 50% loss of membrane integrity was demonstrated which enhanced the activity of studied antibiotics. The combination of eugenol and two antibiotics, vancomycin and a -lactam, increased membrane damage in bacteria, indicating a synergistic effect. It has also been demonstrated that the penetration of vancomycin and -lactam in combination with eugenol increased, resulting in a greater antimicrobial effect.[10]

Eugenol induces cell lysis through the leakage of protein and lipid in the cell membrane. Furthermore, it reveals that the time of exposure of cells to eugenol is important. Because both the cell wall and membrane of the treated Gram-negative and Gram-positive bacteria were considerably damaged after about 120 min of exposure.[11] In vitro, eugenol had a synergistic effect with colistin against a panel of clinical E. coli strains.[9] The interaction of eugenol primarily is with the cytoplasmic membrane of the bacteria. Due to this action it is

able to enhance the activity of antibiotics such as ampicillin, penicillin, oxacillin, erythromycin, norfloxacin, tetracycline, chloramphenicol, vancomycin, rifampin and polymyxin B.[2]

Bacteria within a biofilm are frequently more antibiotic resistant than planktonic (free-living) bacteria, and biofilm infections are resistant to antibiotic treatment. The MIC eugenol concentration had a significant inhibitory effect on the biofilm formation of carbapenem-resistant Klebsiella pneumoniae. Bacterial biofilms on medical implants and biomaterials are another common source of bacterial infection. A hydrophilic copolymer system based on eugenol was found to effectively inhibit the growth of such bacteria in studies. Eugenol's general mechanism of action on bacterial biofilm includes biofilm inhibition and decreased viability of biofilm-forming cells. Other effects included cell dispersion in the biofilm matrix, biofilm bacterial cell inactivation, and inhibition of biofilm-associated gene expression.[12]

Antifungal activity of eugenol

The essential oil of clove (Eugenia caryophyllata) containing eugenol as a major constituent was evaluated against human pathogenic yeasts using a disc paper diffusion method and it showed antifungal effect against the tested strains. New Mannich base-type eugenol derivatives were synthesized and evaluated for their anticandidal activity using a broth microdilution assay. Among different synthesized eugenol derivatives, 4-allyl-2-methoxy-6-(morpholin-4-ylmethyl) phenyl benzoate and 4-{5-allyl-2-[(4-chlorobenzoyl)oxy]-3-methoxybenzyl}morpholin-4-ium chloride were found to be the most effective antifungal compounds even comparable with fluconazole.[3] Eugenol MIC values for eugenol were 400–800 µg/mL for Candida tropicalis and 200–400 µg/mL for Candida krusei. Synergistic effects of eugenol and voriconazole were observed for Candida tropicalis (83.3%) and Candida krusei (77.7%), and no antagonistic activity occurred. Consequently, eugenol is a potential antifungal agent designed to fight the genital Candida yeast. Moreover, the combination therapy of eugenol and voriconazole may prove effective in antimicrobial resistance in mares with genital candidiasis.[13]

Antiviral activity

Eugenol has the ability to inhibit viral replication and reduce viral infection specifically against herpes simplex-1 (HSV-1) and herpes simplex -2 (HSV-2) with interesting IC50 values ranging 16.2–25.6 µg/ml determined by plaque reduction assay. Eugenol is also effective against clinical isolates of HSV-1.synergistic interactions with a combination of

eugenol and acyclovir, a known antiviral drug. In a mouse model, eugenol application was shown to delay the development of herpes virus-induced keratitis.[14]

Antiparasitic activity

In vitro studies on eugenol show that at higher concentrations, it has anti-giarddial, anti-leishmanial, trypanocidal, and anti-malarial properties. In the case of leishmaniasis, eugenol concentrations ranging from 100 to 1000 g/ml inhibited the growth of Leishmania amazonensis. Ultrastructural changes such as swelling, inner membrane collapse and increase in number of cristae were observed when the promastigotes were treated with eugenol (IC50: 80 µg/ml) About 30 percent of eugenol treated promastigotes and amastigotes were found to contain two or more flagella or nuclei indicating the arrest of cell division.Eugenol has also branched out into antimalarial research.Eugenol had antimalarial activity against the chloroquine-resistant strain Plasmodium falciparum, with an IC50 value of 753 M. (FCR-3).[8]

Antioxidant activity

The prevention of free radical development, the removal of radicals until they cause damage, the repair of oxidative damage, the elimination of damaged molecules, and the prevention of mutations are all essential mechanisms in cancer prevention.[1] Eugenol protects neurons from neurotoxicity caused by N-methyl-D-aspartate by inhibiting excitotoxic or oxidative injury. The effect of eugenol on lipid peroxidation and low-density lipoprotein oxidation was investigated. It also protected in vivo inhibition of lipid peroxidation propagations.[15] In vitro, eugenol appeared to protect murine peritoneal macrophages from nicotine-induced superoxide-mediated oxidative damage. Eugenol is a well-known antioxidant and monoamine oxidase (MAO) inhibitor with neuroprotective properties. Various internal and external sources tend to produce reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and singlet oxygen, as well as other free radicals such as nitrogen free radicals, in the human body. ROS and other free radicals are byproducts of normal cellular metabolism in aerobic life, where molecular oxygen is ubiquitous. ROS are generated during irradiation by ultraviolet light, x-rays, and gamma rays, eugenol had the most powerful antioxidant activity and radical-scavenging activity. eugenol had marked Fe3+ -Fe2+ – and Cu2+ - Cu+reducing ability and electrondonor properties for neutralising free radicals by forming stable product.[16]

Antidepresent and Anticonvulsions

Since eugenol and dopamine have structural similarities, it was recently discovered that eugenol inhibits monoamine oxidases (MAOs).Interestingly, many compounds structurally related to eugenol inhibited MAOs, but only those that inhibited MAO-A exhibited antidepressant activity. It is thus concluded that the antidepressant-like effect of this class of compounds is dependent on its MAO-A inhibitory activity.[5] Eugenol reduces seizure stage and duration, as well as mortality, in a lithium-pilocarpine model of epilepsy.

Eugenol therapy maintained neuronal numbers in epileptic animals, while eugenol alone decreased neuronal numbers in all hippocampal sub-regions. Eugenol can prevent behavioural convulsions and have neuroprotective effects by enhancing neuronal survival, most likely by lowering MFS and raising glutathione peroxidase, an antioxidant marker.[17]

Reactive oxygen species have been related to seizure-induced neurodegeneration and are thought to play a role in the production of seizures in pathological situations. Eugenol has a number of antioxidative properties, including a strong protective effect against hydroxyl radicals (OH) produced by the Fe2+-mediated fenton reaction. Eugenol acts as a direct hydroxyl radical scavenger without chelating iron.[18]

Anticancer activity

Eugenol has anticancer activity against a variety of cancers and has been shown to have an excellent inhibitory effect on a number of cancer cell lines.[19]

Nuclear factor-kappa B (NF-B) has been shown in recent studies to play a critical role in the regulation of over 200 genes involved in inflammation, stress response, immune functions, apoptosis, cell proliferation, cell survival, metastasis, and angiogenesis. Eugenol induces apoptosis via a Reactive Oxygen Species (ROS) and mitochondria-dependent mechanism, and it has the potential to be developed as a chemotherapeutic or chemopreventive agent.[20]

It was discovered that eugenol can inhibit the proliferation of melanoma cells. This effect resulted in a significant delay in tumour growth and a 40% reduction in tumour size. An in vitro study conducted on human embryonic lung fibroblast MRC-5 and lung cancer adenocarcinoma cells A549 found that even a low dose of eugenol interfered with the migration and invasion of carcinogenic cells, inhibited lung cancer cell viability, and prevented metastasis by blocking the PI3K/Akt pathway (an intracellular signalling pathway

involved in cell cycle regulation) and inhibiting MMP (matrix metalloproteinase) activity. At higher doses (1000 M), the compound was also found to have cytotoxic effects on normal cells and lung cancer cells.[21] The combination of cisplatin and eugenol has chemotherapeutic effects on TNBC cells that are synergistic. In humanised breast cancer tumour xenografts, cisplatin alone had moderate effects, but cisplatin plus eugenol synergistically inhibited tumour growth, induced apoptosis, and suppressed angiogenesis and the pro-metastatic EMT process. Through strong down-regulation of the proinvasive proteins MMP-2 and MMP-9, eugenol enhanced the inhibitory effect of cisplatin on the invasive capacity of TNBC cells. In vitro and in vivo, eugenol can enhance cisplatin's anti-cancer properties. In cervical cancer cells, eugenol improves growth inhibition and apoptosis induced by 5-fluorouracil and gemcitabine.[22]in agarose gel electrophoresis, eugenol treated human promyelocytic leukaemia cell(HL-60) exhibited apoptotic features such as DNA fragmentation and the formation of DNA ladders.[23]

Role of eugenol in dentistry

Zinc oxide eugenol cement (ZOE) is currently widely used in dentistry for indirect pulp capping, as well as a temporary filling and root canal sealer. Eugenol is well-known for its antioxidant and anti-inflammatory properties. However, high concentrations of eugenol have been reported to have some cytotoxic properties.[24]

Eugenol has been used for dental and oral care since ancient times: it has antimicrobial activity against bacteria associated with dental caries and periodontal disease, as well as disinfectant properties, and has been shown to relieve local pain, such as pulpitis and dentinal hypersensitivity, as a topical analgesic.[25] It is combined with zinc oxide in dentistry to form an amorphous chelate compound that is used to cover the pulp indirectly, dress endodontic treatment, and temporarily fill cavities. It is also used to fill root canals in liquid form in special pastes such as mummification pastes (e.g., Caryosan and Endomethazone).[26]

Toxicity of eugenol

Eugenol is considered safe as a food additive, but due to the wide variety of applications, as well as the widespread use and availability of clove oil, there has been considerable concern about its toxicity in recent years. The oral administration of eugenol in various doses over a 15-day period may cause some changes in blood chemistry. Furthermore, it raises aspartate aminotransferase, alanine aminotransferase, and total bilirubin levels, but these effects do not appear to be dose dependent.[27]

Cytotoxicity of eugenol

In a human submandibular cell line, the cytotoxic effects of eugenol, induction of reactive oxygen species (ROS), and decreased levels of GSH were investigated. It is suggested that formation of benzyl radicals is the main cause of low GSH of eugenol is found to be related to ROS-independent mechanisms. Eugenol has been shown to have less cytotoxic effects than isoeugenol, and these effects are dose dependent.[27]

Immunotoxicity of eugenol

The effectiveness of eugenol and clove oil in inducing allergy and hypersensitivity is debatable. Several negative effects have been reported following the use of eugenol-containing dental products. Different studies have reported localised skin irritation, ulcers, allergic dermatitis, tissue necrosis, and, on rare occasions, anaphylactic-like shock. eugenol plays both protective and curative roles in reducing Chlorpyrifos-induced immunotoxicity.[28]

Genotoxicity of eugenol

In MCF-7 cells, the chemopreventive effect of eugenol on DNA damage caused by 7,12 dimethylbenzanthracene(DMBA) was investigated. The findings suggested that eugenol was effective at protecting DNA from genotoxic damage caused by DMBA. Eugenol has the ability to inhibit DMBA activation and may be used as a chemopreventive agent.[29] The wing spot test of Drosophila melanogaster was used to assess the genotoxicity of various phenolic compounds such as eugenol, isoeugenol, and safrole (common fruit fly). The results of this experiment demonstrated that isoeugenol was not genotoxic at the same concentrations. Observations also revealed that eugenol and safrole can elicit a positive recombinogenic response, which is associated with a high CYP P450 activation capacity. Eugenol's genotoxicity is linked to reactive metabolites and recombinogenic compounds.[7]

The Ames Salmonella/microsome test was used to assess eugenol's effect on tobacco-induced genotoxicity. The results showed that eugenol has an inhibitory effect on tobacco-induced mutagenicity at concentrations ranging from 0.5 to 1 mg/plate. However, ten non-smoking healthy male adults were given 150 mg/day of eugenol, and the results indicated that eugenol has no antigenotoxic activity in human.[27]

Table 1: Plant sources of eugenol.

Genus species	Common name of the Plant	Part	Concentration (ppm)
Syzygium aromaticum L.	love, Clovetree	Flower	180,000
Pimenta dioica L.	Allspice, Clover-Pepper, Jamaica-Pepper, Pimenta, Pimento	Fruit	36,000
Pimenta racemosa	Bayrum Tree, West Indian Bay	Leaf	19,100
Piper betel L	Betel Pepper	Leaf	17,850
Daucus carota L.	Carrot	Seed	8,575
Pimenta dioica L.	Allspice, Clover-Pepper, Jamaica-Pepper, Pimenta, Pimento		8,348
Ocimum basilicum L. B	Basil,Cuban Basil,Sweet Basil	Leaf	8,575
Syzygium aromaticum L.	Clove, Clovetree	Leaf; Stem Leaf	9,000; 9,000
Alpinia galanga L.	Greater Galangal, Languas, Siamese Ginger	Rhizome	2,000

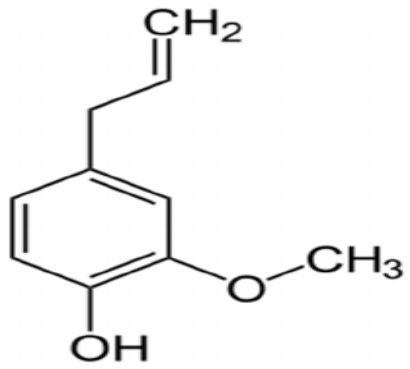


Figure 1: Chemical structure of eugenol.

CONCLUSIONS

Several spices and medicinal herbs contains eugenol. This Review article discusses the use of eugenol as a treatment method that can be added to a variety of foods and herbal remedies to treat serious metabolic disorders. Although eugenol has been found effective in patients with neurological diseases/disorders like AD, depression, PD and cancer, Inflammatory reactions. However, rigorous toxicity tests should be carried out to ensure that eugenol is safe for the general population.

In conclusion, various properties and activities of eugenol remain largely unknown and should be investigated further through additional in vitro and in vivo long-term human research.

REFERENCES

1. Irie Y. Effects of eugenol on the central nervous system: its possible application to treatment of alzheimer's disease, depression, and parkinson's disease. Current Bioactive Compounds, 2006; 1, 2(1): 57-66.
2. Hemaiswarya S, Doble M. Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. Phytomedicine, 2009; 1, 16(11): 997-1005.
3. Raja MC, Srinivasan V, Selvaraj S, Mahapatra SK. Versatile and synergistic potential of eugenol: a review. Pharm. Anal. Acta, 2015; 6(5): 367.
4. Thompson DC, Constantin-Teodosiu D, Moldéus P. Metabolism and cytotoxicity of eugenol in isolated rat hepatocytes. Chemico-biological interactions, 1991; 1, 77(2): 137-47.
5. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygium aromaticum L.(Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules, 2020; 10(2).
6. Pramod K, Ansari SH, Ali J. Eugenol: a natural compound with versatile pharmacological actions. Natural product communications, 2010; 5(12): 1934578X1000501236.
7. Munerato MC, Sinigaglia M, Reguly ML, de Andrade HH. Genotoxic effects of eugenol, isoeugenol and safrole in the wing spot test of Drosophila melanogaster. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 2005; 4, 582(1-2): 87-94.
8. Guénette SA, Ross A, Marier JF, Beaudry F, Vachon P. Pharmacokinetics of eugenol and its effects on thermal hypersensitivity in rats. European journal of pharmacology, 2007; 7, 562(1-2): 60-7.

9. Raghavenra H, Diwakr BT, Lokesh BR, Naidu KA. Eugenol—the active principle from cloves inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells. Prostaglandins, Leukotrienes and Essential Fatty Acids, 2006; 1, 74(1): 23-7.
10. Wang YM, Kong LC, Liu J, Ma HX. Synergistic effect of eugenol with Colistin against clinical isolated Colistin-resistant Escherichia coli strains. Antimicrobial Resistance & Infection Control, 2018; 7(1): 1-9.
11. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 2013; 6(12): 1451-74.
12. Qian W, Sun Z, Wang T, Yang M, Liu M, Zhang J, Li Y. Antimicrobial activity of eugenol against carbapenem-resistant Klebsiella pneumoniae and its effect on biofilms. Microbial pathogenesis, 2020; 1, 139: 103924.
13. Sharifzadeh A, Shokri H. In vitro synergy of eugenol on the antifungal effects of voriconazole against Candida tropicalis and Candida krusei strains isolated from the genital tract of mares. Equine veterinary journal, 2021; 53(1): 94-101.
14. Tragoolpua Y, Jatisatienr A. Anti-herpes simplex virus activities of Eugenia caryophyllus (Spreng.) Bullock & SG Harrison and essential oil, eugenol. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 2007; 21(12): 1153-8.
15. Mahapatra SK, Chakraborty SP, Majumdar S, Bag BG, Roy S. Eugenol protects nicotine-induced superoxide mediated oxidative damage in murine peritoneal macrophages in vitro. European Journal of Pharmacology, 2009; 25, 623(1-3): 132-40.
16. Gülçin İ. Antioxidant activity of eugenol: A structure–activity relationship study. Journal of medicinal food, 2011; 1, 14(9): 975-85.
17. Joushi S, Salmani ME. Effect of eugenol on lithium-pilocarpine model of epilepsy: Behavioral, histological, and molecular changes. Iranian journal of basic medical sciences, 2017; 20(7): 745.
18. Wie MB, Cheon BH, Lee SY, Son KH, Song DK, Shin TK, Kim HC. Eugenol inhibits excitotoxins-induced delayed neurotoxicity, oxidative injury and convulsion. Toxicological Research, 2006; 22(3): 275-82.
19. Liu H, Schmitz JC, Wei J, Cao S, Beumer JH, Strychor S, Cheng L, Liu M, Wang C, Wu N, Zhao X. Clove extract inhibits tumor growth and promotes cell cycle arrest and apoptosis. Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics, 2014; 30, 21(5): 247-59.

20. Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. Cancer immunology research, 2014; 1, 2(9): 823-30.
21. Fangjun L, Zhijia Y. Tumor suppressive roles of eugenol in human lung cancer cells. Thoracic cancer, 2018; 9(1): 25-9.
22. Islam SS, Al-Sharif I, Sultan A, Al-Mazrou A, Remmal A, Aboussekhra A. Eugenol potentiates cisplatin anti-cancer activity through inhibition of ALDH-positive breast cancer stem cells and the NF-κB signaling pathway. Molecular carcinogenesis, 2018; 57(3): 333-46.
23. Yoo CB, Han KT, Cho KS, Ha J, Park HJ, Nam JH, Kil UH, Lee KT. Eugenol isolated from the essential oil of Eugenia caryophyllata induces a reactive oxygen species-mediated apoptosis in HL-60 human promyelocytic leukemia cells. Cancer letters, 2005; 8, 225(1): 41-52.
24. Koch T, Peutzfeldt A, Malinovskii V, Flury S, Häner R, Lussi A. Temporary zinc oxide–eugenol cement: eugenol quantity in dentin and bond strength of resin composite. European journal of oral sciences, 2013; 121(4): 363-9.
25. Ulanowska M, Olas B. Biological Properties and Prospects for the Application of Eugenol—A Review. International Journal of Molecular Sciences, 2021; 22(7): 3671.
26. Nielsen JP. Basic Metallurgy of Dental Casting Alloys. InDental Materials Research: Proceedings of the 50th Anniversary Symposium, US National Bureau of Standards, 1972; 13: 67.
27. Nejad SM, Özgüneş H, Başaran N. Pharmacological and toxicological properties of eugenol. Turkish Journal of Pharmaceutical Sciences, 2017; 14(2): 201.
28. Sarrami N, Pemberton MN, Thornhill MH, Theaker ED. Adverse reactions associated with the use of eugenol in dentistry. British dental journal, 2002; 193(5): 257-9.
29. Han EH, Hwang YP, Jeong TC, Lee SS, Shin JG, Jeong HG. Eugenol inhibit 7, 12-dimethylbenz [a] anthracene-induced genotoxicity in MCF-7 cells: Bifunctional effects on CYP1 and NAD (P) H: quinone oxidoreductase. FEBS letters, 2007; 20, 581(4): 749-56.