Versatile and Synergistic Potential of Eugenol: A Review

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Abstract
Eugenol (1-allyl-4-hydroxy-3-methoxybenzene) is the phenolic component of essential oil and the main constituent of Eugenia caryophyllata, Ocimum gratissimum and several others medicinal plant. In view of its non-mutagenic and non-carcinogenic properties, eugenol is generally regarded as safe by the Food and Agricultural Organization of the United Nations. Eugenol has been recently shown to be effective for antimicrobials and treatment of different life threatening diseases including sepsis, leishmaniasis, and cancer. However overall, activity of eugenol is not discussed elsewhere. In this review, we discuss the current understanding of the mechanisms involved the antioxidant, antimicrobial, anticancer and anti-inflammatory potential of eugenol.

Keywords: Eugenol; Antioxidant; Antimicrobials; Anticancer; Anti-inflammatory potential

Introduction
Eugenol, a phenolic photochemical extracted from certain essential oils especially from clove oil, nutmeg, cinnamon, basil and bay leaf; possess a range of antimicrobials to anticancer activity. As it is extracted from the buds and leaves of Eugenia caryophyllata (clove) for the first time mainly, it’s named as eugenol. Now a day, eugenol can also be synthesized in laboratory scale and industrial scale by allylation of guaiacol with allyl chloride having the similar kind of functional property [1]. Being a major component in the extracts of various medicinal herbs it got much attention by the researchers and opened up a wide area of research in applying it as a medicine to cure various diseases. Eugenol is known to have several pharmacological properties i.e. anaesthetic, antioxidant, antimicrobial, antihelminthic, anti-inflammatory, anticarcinogenic, anti-fumigant, and anti-repellent properties. It has been in use as a traditional remedy for toothache and also for culinary purposes. This versatile molecule is a key ingredient in perfumes, cosmetics, flavorings and agents.

Both the Food and Agriculture Organization (FAO) and World Health Organization (WHO) have allowed an acceptable daily intake of eugenol of 2.5 mg/kg body weight for humans [2]. Moreover, the U.S. Food and Drug Administration (FDA) have proclaimed eugenol as safe and it is considered non-carcinogenic and non-mutagenic. In recent years, eugenol has fascinated the attention of researchers due to its anti-inflammatory and chemopreventive activity, as well as its superior anti-oxidant activity [3-6]. As a result of its broad range of pharmacological and biological activities, studies on eugenol and clove products still remains a research priority. It is therefore of significant value to rationally unite some of the most worth mentioning research findings related to eugenol to highlight its importance in human health as well as to elucidate its mechanisms of action where possible.

Physical and chemical properties of Eugenol
Eugenol belongs to a class of phenylpropanoids (C_{10}H_{12}O_2). The IUPAC name of the compound is 4-Allyl-2-methoxyphenol (Figure 1), having molecular mass 164.2g/mol with pK_a=10.19 at 25°C. Eugenol and isoeugenol are the two isomorph of it. It is also known as caryophylllic acid, allyglyuacil, 2-methoxy-4-(2-propenyl) phenol, 4-allylcathecol-2-methyl ether. The phenolic group confers the antioxidant property of it. It is partially soluble in water and its solubility increases with organic solvents. The colour of the compound ranges from clear to pale yellow [1,7]. Eugenol absorbed via small intestine when administered orally and rapidly distributed in all organ when administered intraperitoneally. According to Thompson et al. (1991), metabolism of eugenol resulted in the formation of conjugates with sulfate, glucuronic acid (major) and glutathione studied in vitro with 1mM concentration (lethal dose). Eugenol is eliminated and excreted as expired CO_2 and through urine studied in rabbit model (WHO, Food additive series 17 Eugenol, 1980).

Plant sources of Eugenol
Eugenol is extracted from several aromatic plants. Beside the Eugenia caryophyllata, it is also isolated from Myristica fragrans, Cinnamomum tamala, Zygium aromaticum, Ocimum basilicum, Ocimum gratissimum, Ocimum tenuiflorum, Pimenta racemosa etc. However, the principal source is clove oil which contains 45–90% eugenol of its constituent (Table 1) [1,8-10].

Isolation of Eugenol from plant
Eugenol was first isolated in 1929 and commercial production

Figure 1: Chemical Structure of Eugenol.
commenced in the United States in the 1940s [1]. However, eugenol is predominantly prepared from natural oil sources by mixing the essential oil with an excess of aqueous sodium (3%) or potassium hydroxide solution and shaking, leading to the formation of a phenolic alkali salt. The insoluble non-phenolic portion is then extracted with a solvent or via steam distillation. The undissolved portion is removed, the alkali solution acidified at low temperatures and the liberated eugenol purified by fractional distillation, thin layer chromatography, high pressure liquid chromatography. The presence and purity can be checked by FTIR, NMR and mass spectroscopy [3,8,11].

**Therapeutic activities of Eugenol**

Eugenol exhibits versatile therapeutic properties (Figure 2).

**Antioxidant activity of Eugenol**

Eugenol and Clove oil have the ability to scavenge the free radicals.
Eugenol exhibited potent antibacterial activity against various strains of Gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Enterococcus faecalis* and *Listeria monocyctogenes*) and Gram-negative (*Escherichia coli*, *Salmonella typhi*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Yersinia enterocolitica*, *Proteus vulgaris*) bacteria [22-29]. Eugenol induced cell lysis of Gram-negative and Gram-positive bacteria by damaging the cell wall and membrane caused leakage of protein and lipid contents (Figure 4) [29]. In *vitro* and *in vivo* studies on biofilms revealed that eugenol has strong inhibitory and eradicative effect. It exhibited inhibition against the formation of biofilms by MRSA and MSSA strains. At a concentration of 0.5×MIC it showed 50% inhibition against MRSA and MSSA strains. At sub-MIC, eugenol significantly decreased 88% *S. aureus* colonization in rat middle ear. MBEC (minimum biofilm eliminating concentration) of eugenol and carvacrol combination decreased the already formed biofilms by 99% [30]. Eugenol at 0.5 MIC was able to induce an inhibition of ≥ 90% of *P. aeruginosa* biofilms [31]. Combinational therapy helps to reduce the risk of resistant microbes. Eugenol exhibited synergistic interaction with vancomycin, gentamicin and β-lactam antibiotics lead to greater antimicrobial effect [28,32]. Eugenol also exhibited synergic interactions with cinnamate, cinnaamaldehyde, thymol and carvacrol, resulting greater antibacterial activity [33-34]. Sub-inhibitory concentrations of eugenol (16-128 µg/mL) dose-dependently decreased the necrosis factor-inducing and haemolytic activities of culture supernatants and significantly reduced the production of staphylococcal enterotoxin A [35]. The drawbacks of eugenol i.e, low solubility, liability to sublimation and strong odor, could be overcome by glycosylation to eugenol α-D-glucopyranoside (α-EG), which is more effective than that of pure eugenol as tested with *Staphylococcus aureus* and *E. coli* [36].

**Antifungal activity of Eugenol**

The essential oil of clove (*Eugenia caryophyllata*) containing eugenol as a major constituent was evaluated against 53 human pathogenic yeasts using a disc paper diffusion method and it showed antifungal effect (Figure 4) against the tested strains [37]. New Mannich base-type eugenol derivatives were synthesized and evaluated for their antifungal 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. The most significant IC50 values were ranging 0.063-1.23 µM against *C. krusei*, *C. glabrata*, and *C. albicans* [38]. Fractional inhibitory concentration indices (FICI) for carvacrol-fluconazole and eugenol-fluconazole combinations for *C. albicans* biofilm formation were 0.31 and 0.25, respectively [39]. Eugenol treatment significantly reduced the adherence and metabolic activity of biofilms of *C. albicans* isolated from the oral cavity of HIV infected patients [40]. Exposure of Candida cells to eugenol resulted in reduction of ergosterol biosynthesis followed by apoptosis [41]. Eugenol has the ability to alter the morphogenesis of *C. albicans*. Certain combinations of eugenol and thymol led to a synergistic effect, which is interesting in the view of potentiating their inhibition of *C. albicans* colonization and infectivity [42].

**Antiviral activity**

Eugenol has the ability to inhibit viral replication and reduce viral infectivity 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 [43-44]. Eugenol is also effective against clinical isolates of HSV-1 [45]. Unfortunately, it has been found that cytotoxicity of eugenol as a single compound is negligible against HSV-1, but in combination with acyclovir exhibits

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**Figure 2:** Therapeutic properties of eugenol.

**Figure 3:** The overall mechanism of antioxidant potential of eugenol.
leishmanial activity than the native form against promastigotes and amastigotes of *Leishmania infantum chagasi* [54]. Clove essential oil having eugenol showed strong trypanocidal activity (inhibition of epimastigotes and trypomastigotes) comparable with basil and yarrow [55]. Eugenol also extended its arm in antimalarial research. Eugenol exhibited antimalarial activity with an IC50 value of 753 μM against the chloroquine-resistant strain *Plasmodium falciparum* (FCR-3) [25].

**Anti-parasitic activity**

The treatment of cancer lies in prohibiting the cell proliferation and destruction of the malignant cells. Eugenol and its derivatives were investigated for their anti-cancer property. *In vitro* studies showed that eugenol and its monomeric forms did not inhibit the cell proliferation. The biphenyl forms of eugenol however, had some effect. Eugenol related biphenyl (S)-6,6′-dibromo-dehydrodieugenol elicits specific antiproliferative activity on neuroectodermal tumour cells by partially triggering apoptosis [56]. The epoxide form of eugenol is a potential drug candidate for inducing apoptosis in human breast cancer cells [57]. ROS plays a critical role in eugenol and eugenol loaded nano emulsion induced apoptosis in HB8065 and HTB37 cells [58]. Volatile extracts obtained by hydrodistillation of bark and roots of *Uvariodendron angustifolium* contains 68.3% and 85.3% of methyl eugenol respectively and exhibits interesting cytotoxic properties on human breast cancer cells MCF-7 [59]. Eugenol at low dose (2 μM) has specific toxicity against different breast cancer cells. This killing effect was mediated mainly through inducing the internal apoptotic pathway and strong down-regulation of E2F1 and its downstream anti-apoptosis target survivin, independently of the status of p53 and ERα. Eugenol also inhibited several other breast cancer related oncogenes, such as NF-κB and cyclin D1. Moreover, eugenol up-regulated the versatile cyclin-dependent kinase inhibitor p21WAF1 protein, and inhibited the proliferation of breast cancer cells in a p53-independent manner. Importantly, these anti-proliferative and pro-apoptotic effects were also observed *in vivo* in xenografted human breast tumors. Hence, eugenol exhibits anti-breast cancer properties concentration both *in vitro* and *in vivo*, indicating that it could be used to consolidate the adjuvant treatment of breast cancer through targeting the E2F1/survivin pathway, especially for the less responsive triple-negative subtype of the disease [60]. Eugenol 5-O-β-(6′-galloylglucopyranoside) or ericifolin, showed antiproliferative, pro-apoptosis and anti-androgen receptor transcription activities, which suggested the potential use of aqueous allspice extract and ericifolin eugenol fraction against prostate cancer [61]. Cytotoxic concentrations of eugenol induced the reduction of ATP of oxidative stress and an increase in the polyamines and glycolytic metabolites, in normal oral cells and oral squamous cell carcinoma, suggests the induction of non-apoptotic cell death by eugenol [62]. Eugenol inhibited matrix metalloproteinase-9 activities in PMA-stimulated HT1080 cells via triggering apoptosis [56]. The epoxide form of eugenol is a potential drug candidate for inducing apoptosis in human breast cancer cells [63]. Combination therapy is the most effective treatment strategy in cancer to overcome drug toxicity and drug induced resistance. Eugenol in combination with 5-fluorouracil exhibited more cytotoxicity against the cervical cancer cells (HeLa). Flow cytometry results indicated that the combination of eugenol and 5-fluorouracil increased the number of cells in the S and G2/M phases when compared to treatment with the individual compounds alone. This indicated that eugenol possessed different cell cycle targets and induced apoptosis in the cancer cells [64]. Eugenol and its chemically synthesized derivatives proved its activity against melanoma, skin tumors, prostate cancer, gastric cancer and leukemia via oncogene activation.
regulation and caspase dependent pathway which extensively reviewed by [65].

**Anti-inflammatory potential of Eugenol**

The anti-inflammatory action of eugenol arises from inhibition of prostaglandin synthesis and neutrophil/macrophage chemotaxis. In *in vitro* studies also reveal that this bioactive compound inhibited nuclear factor-κB (NF-κB) activation induced by tumor necrosis factor (TNFα) and blocked cyclooxygenase activity (COX-2) in LPS stimulated macrophages. COX-2 expression is triggered by growth factors, cytokines and LPS [66]. Eugenol showed reduced inflammation by decreasing TNF-α and infiltration of neutrophils during pulmonary infection in animals. The compound when administered at a dosage of 160 mg/kg body weight showed reduction in alveolar collapse and PMN infiltration in lungs [67]. Eugenol also protected chemical-induced cellular dysfunction of macrophages and balanced the pro/ anti-inflammatory mediators in mouse peritoneal macrophages [5].

**Conclusion**

Eugenol, a natural bioactive compound has high potential as a therapeutic agent which can be incorporated in the treatment of cancers, leishmaniases and several other disorders. It serves as a broad spectrum drug against bacterial, viral, fungal and parasitic infections. The combinational therapy of eugenol with standard drugs has great spectrum drug against bacterial, viral, fungal and parasitic infections. Studies also reveal that this bioactive compound inhibited nuclear factor-κB (NF-κB) activation induced by tumor necrosis factor (TNFα) and blocked cyclooxygenase activity (COX-2) in LPS stimulated macrophages. COX-2 expression is triggered by growth factors, cytokines and LPS [66]. Eugenol showed reduced inflammation by decreasing TNF-α and infiltration of neutrophils during pulmonary infection in animals. The compound when administered at a dosage of 160 mg/kg body weight showed reduction in alveolar collapse and PMN infiltration in lungs [67]. Eugenol also protected chemical-induced cellular dysfunction of macrophages and balanced the pro/anti-inflammatory mediators in mouse peritoneal macrophages [5].

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