Versatile and Synergistic Potential of Eugenol: A Review

Mamilla R Charan Raja, Varsha Srinivasan, Sharmila Selvaraj and Santanu Kar Mahapatra*

Medicinal Chemistry and Immunology Lab, AS-K-II, School of Chemical and Biotechnology, SASTRA University, Thanjavur 613401, Tamil Nadu, India

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, antifumigant, and antirepellent 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 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 pKa=10.19 at 25°C. Eugenol and isoeugenol are the two isomerof of it. It is also known as caryophyllic acid, allyguaiaicol, 2-methoxy-4-(2-propenyl) phenol, 4-allyl-catechol-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 CO2 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 grattissimum, 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

Received April 15, 2015; Accepted April 29, 2015; Published May 06, 2015


Copyright: © 2015 Raja MRC, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 showed inhibition of DPPH radical and hydroxyl radical at concentrations dependent manner. As the concentration of eugenol increases, it is shown to possess the activity of pro-oxidants by forming free radicals also [15]. This property of eugenol and its isomer iso-eugenol was tested by the iron-mediated lipid peroxidation and auto-oxidation of Fe²⁺ [16]. These functional properties of eugenol strongly suggested the dual role that possessed the versatility of eugenol. Besides the free radical scavenging activity, eugenol also has nitric-oxide scavenging activity, and strong reducing power while determined by Griess reagent and FTC method respectively [6]. Not only the direct free radical scavenging activity in chemical system, eugenol also protected in vitro and in vivo ROS generation and ROS-induced lipid-protein and DNA damage as well as increased the cellular anti-oxidant, specifically, glutathione system (Figure 3) [3,17-21]. Wie et al. (1997) reported that eugenol reversed neuronal excitotoxicity and oxidative injury and had protective effect against N-methyl-D-aspartate– induced neurotoxicity.

**Antioxidant activity of Eugenol**

Eugenol exhibited potent antioxidant activity against various strains of Gram-positive (*Bacillus cereus*; *Bacillus subtilis*; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Streptococcus pneumonia*; *Streptococcus pyogenes*; *Enterococcus faecalis* and *Listeria monocytogenes*) 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 cinnamates, cinnamaldehyde, 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].

**Anticancer 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 enhanced antiviral activity. 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].
Leishmania donovani exhibited anti-leishmanial potential against [53]. Methanolic extract of *Piper betle* containing 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-cancer 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 it 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′-S)-dibromo-dehydrodieugenol elicits specific cytotoxic effects. The biphenyl forms of eugenol however, had some effect. Eugenol and it 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].

**Anti-parasitic activity**

*In vitro* studies on eugenol suggest its anti-giardial, anti-leishmanial, trypanocidal, and anti-malarial potential at higher concentrations. It inhibited *G. lamblia* trophozoites adherence since the third hour but did not induce cell death. The main morphological alterations were modifications on the cell shape, presence of precipitates in the cytoplasm, autophagic vesicles, internalization of flagella and ventral disc, membrane blebs and intracellular/nuclear clearing [50]. In case of leishmaniasis, 100 to 1000 μg/ml of eugenol concentration restricted the growth of the *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 % of eugenol treated promastigotes and amastigotes were found to contain two or more flagella or nuclei indicating the arrest of cell division [51]. It showed anti-leishmanial activity against *L. major* promastigote with IC50 value of 47.2 μg/ml [52]. Methanolic extract of *Piper betle* containing eugenol exhibited anti-leishmanial potential against *Leishmania donovani* [53]. Benzylated and acetylated derivatives of eugenol exhibited better anti-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].

---

**Figure 4:** Schematic diagram of antimicrobial activity of eugenol.
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 vitro studies also reveal that this bioactive compound inhibited nuclear factor-xB (NF-xB) 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, leishmaniasis 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 potential to clear the drug resistant strains. Being a component of naturally obtained essential oil, it has far less drawbacks than other synthetically prepared compounds. However, in most of the cases, the activity is concentration dependant. The derivatives of this compound have opened up a new era in the field of pharmacology, kindling the research interests on this compound.

References

13. Slamenov B, Horvatdovic E, Vojislova L, Sramkovic M, Navarovic J (2009) Potential to clear the drug resistant strains. Being a component of spectrum drug against bacterial, viral, fungal and parasitic infections. It serves as a broad therapeutic agent which can be incorporated in the treatment of cancers, leishmaniasis 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 potential to clear the drug resistant strains. Being a component of naturally obtained essential oil, it has far less drawbacks than other synthetically prepared compounds. However, in most of the cases, the activity is concentration dependant. The derivatives of this compound have opened up a new era in the field of pharmacology, kindling the research interests on this compound.


