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Review Article

EUGENOL: A VERSATILE PHYTOMEDICINE

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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, Ocimmum 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 phytomedicine.

INTRODUCTION

Eugenol, a phenolic phytochemical extracted from certain essential oils especially from clove oil, nutmeg, cinnamon, basil and bay leaf. As it is extracted from the buds and leaves of *Eugenia caryophyllata (*clove) for the first time, 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, antihelmintic, 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. The food and Drug Administration (FDA) has 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 chemo-preventive 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 the research findings related to eugenol to highlight its importance in human health as well as to elucidate its mechanisms of action.

Physical and chemical properties of eugenol

Eugenol belongs to a class of phenyl propanoids ($C_{10}H_{12}O_2$). The IUPAC name of the compound is 4-Allyl-2-methoxyphenol (fig. 1), having molecular mass 164.2 g/mol with pKa=10.19 at 25 °C. Eugenol and isoeugenol are the two isoform of it. It is also known as caryophyllic acid, allylguaiacol, 2-methoxy-4-(2-propenyl) phenol, 4-allylcatechol-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 exhibited good ADME (absorption, distribution, metabolism and excretion) properties on oral and intraperitoneal administration [8, 9].

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 grattisimum, Ocimum tenuiflorum, Pimenta racemosa* etc. However, the principal source is clove oil which contains 45–90% eugenol of its constituent (table. 1) [1, 10-12].

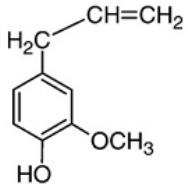

Fig. 1: Chemical Structure of Eugenol

Isolation of eugenol from plant

Eugenol was first isolated in 1929 and commercial production 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, 10, 13].

Therapeutic activities of eugenol

Eugenol exhibits versatile therapeutic activities (fig. 2).

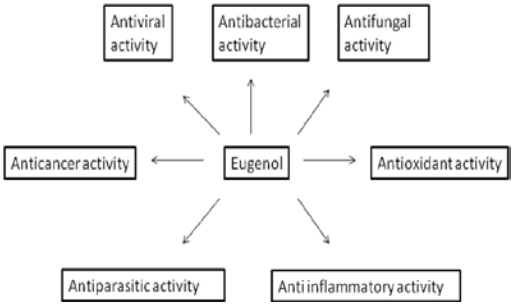

Fig. 2: Therapeutic activities of eugenol

Table 1: Presence of eugenol in different concentration in different type of plants [12]

Genus species	Common name of the Plant	Part	Concentration (ppm)
Syzygium aromaticum L.	Clove, 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
Alpinia galanga L.	Greater Galangal, Languas, Siamese Ginger	Rhizome	12 000
Syzygium aromaticum L.	Clove, Clovetree	Leaf; Stem	9000; 9000
Ocimum basilicum L.	Basil, Cuban Basil, Sweet Basil	Leaf	8575
Pimenta dioica L.	Allspice, Clover-Pepper, Jamaica-Pepper, Pimenta, Pimento	Leaf	8348
Daucus carota L.	Carrot	Seed	7000
Cinnamomum verum	Ceylon Cinnamon, Cinnamon	Bark	3520
Ocimum gratissimum L.	Agbo, Shrubby Basil, Ram Tulshi	Leaf and Plant	0-5340
Ocimum sanctum L.	Holy Basil, Tulsi	Leaf	4200-4970
Curcuma longa L.	Indian Saffron, Turmeric	Essential Oil	2100
Ocimum gratissimum L.	Agbo, Shrubby Basil	Shoot	0-4045
Ocimum kilimandscharicum	African Blue Basil, Kenyan Perennial Basil	Shoot	0-3000
Ocimum suave	Kenyan Tree Basil	Shoot	110-2860
Laurus nobilis L.	Bay, Bay Laurel, Bayleaf, Grecian Laurel, Laurel, Sweet Bay	Leaf	1335
Origanum majorana L.	Marjoram, Sweet Marjoram	Plant	1152
Cistus ladaniferus L.	Ambreine, Gum Cistus, Labdanum, Rockrose	Leaf	1050
Ocimum gratissimum L.	Agbo, Shrubby Basil	Seed	0-1670
Hyssopus officinalis L.	Hyssop	Flower; Leaf	624; 443
Ageratum conyzoides L.	Mexican ageratum	Shoot	0-800
Alpinia officinarum	Chinese Ginger, Lesser Galangal	Rhizome	400
Viola odorata L.	Common Violet, Sweet Violet	Flower	357
Mentha pulegium L.	European Pennyroyal	Plant	320
Myristica fragrans	Mace, Muskatnussbaum (Ger.), Nutmeg, nogal moscado (Sp.), nuez moscada (Sp.)	Seed	320
Cymbopogon winterianus	Java Citronella, Mahapengiri	Plant	233
Pycnanthemum setosum	Setose Mountain Mint	Shoot	93
Acorus calamus L.	Calamus, Flagroot, Myrtle Flag, Sweet Calamus, Sweetflag, Sweetroot	Rhizome	84
Origanum minutiflorum	Small-Flowered Oregano	Shoot	55-125
Umbellularia californica	California Bay	Plant	40
Ocimum kilimandscharicum	African Blue Basil, Kenyan Perennial Basil	Flower	0-35
Micromeria fruticosa subsp. barbata	Tea Hyssop, Zopha, Zuta	Shoot	0-26
Thymus capitatus L.	'Sicilian' Thyme, Spanish Origanum, Spanish Thyme	Shoot	0-21
Jasminum officinale L.	Jasmine, Poet's Jessamine	Flower	10
Lavandula latifolia	Aspic, Broad-Leaved Lavender, Spike Lavender	Plant	9
Micromeria congesta	Kaya Yarpuzu	Leaf	5-15
Ocimum basilicum L.	Basil, Cuban Basil, Sweet Basil	Plant	0-14
Hyacinthus orientalis L.	Hyacinth	Flower	4.6
Calamintha nepeta Glandulosa	Turkish Calamint	Shoot	0-8
Rosa gallica L.	French Rose	Flower	4
Glycyrrhiza glabra L.	Commom Licorice, Licorice, Licorice-Root, Smooth Licorice	Root	1
Elsholtzia blanda	Bantaluki, Bantulsi	Shoot	1>
Vaccinium corymbosum L.	Blueberry	Fruit	1>

Antioxidant activity of eugenol

Eugenol and Clove oil have the ability to scavenge the free radicals [6, 14-16]. At lower concentrations, it acts as an antioxidant by inhibiting DPPH and Hydroxyl free radicals and at higher concentrations it acts as pro-oxidant by forming free radicals [17]. This property of eugenol and its isomer isoeugenol was tested by the iron-mediated lipid peroxidation and auto oxidation of Fe^{2+} [18]. 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 the 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 (fig. 3) [3,19-23]. Wie *et al.* reported that eugenol reversed neuronal excitotoxic or oxidative injury and had protective effect against N-methyl-D-aspartate–induced neurotoxicity [24].

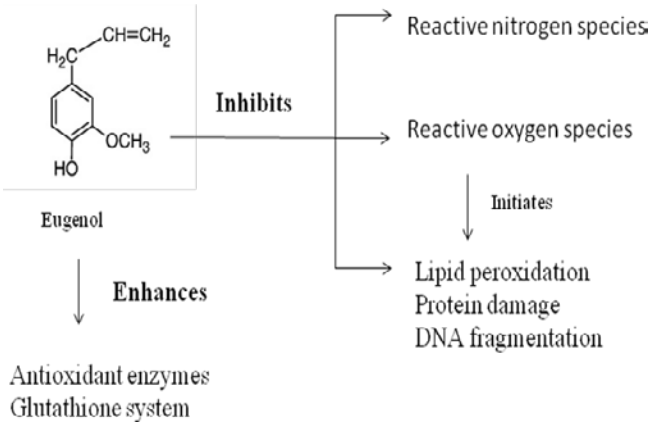

Fig. 3: The overall mechanism of antioxidant activity of eugenol

Antibacterial activity of eugenol

Eugenol exhibited potent antibacterial activity against 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 [25-32]. 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 (fig. 4) [32]. *In vitro* and *in vivo* studies on bacterial 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% [33]. Eugenol at 0.5 MIC was able to induce an inhibition of ≥90% of *P. aeruginosa* biofilms [34]. Combinational therapy helps to reduce the risk of resistant microbes. Eugenol showed synergistic interaction with vancomycin, gentamicin and β-lactam antibiotics lead to greater antimicrobial effect [31, 35]. Eugenol also exhibited synergic interactions with cinnamate, cinnamaldehyde, thymol and carvacrol, resulting greater antibacterial activity [36, 37]. 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 [38]. 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* [39].

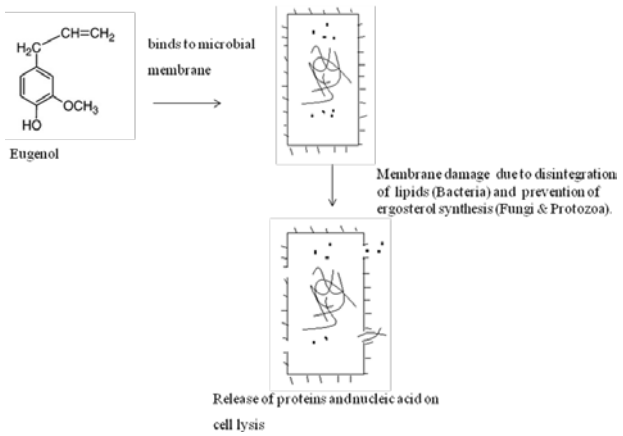

Fig. 4: Schematic diagram of antimicrobial activity of eugenol

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 potential against the tested strains [40]. New Mannich base-type eugenol derivatives were synthesized and evaluated for their anticandidal activity. 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 IC_{50} values were ranging 0.063–1.23 µM against *C. krusei, C. glabrata*, and *C. albicans* [41]. Fractional inhibitory concentration indices (FICI) for carvacrol-fluconazole and eugenol-fluconazole combinations for *C. albicans* biofilm formation were 0.311 and 0.25, respectively [42]. Eugenol treatment significantly reduced the adherence and metabolic activity of biofilms of *C. albicans* isolated from the oral cavity of HIV infected patients [43]. Exposure of *Candida* cells to eugenol resulted in reduction of ergosterol biosynthesis followed by apoptosis (fig. 4) [44]. 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 [45].

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 IC_{50} values ranging 16.2–25.6 µg/ml determined by plaque reduction assay [46,47]. Eugenol is also effective against clinical isolates of HSV-1 [48]. Unfortunately, it has been found that cytotoxicity of eugenol as a single compound is negligible against HSV-1, but in combination with acyclovir exhibits a promising antiviral property [49]. This compound also acts against human cytomegalovirus (CMV), murine CMV (MCMV) and hepatitis C virus (≥90% inhibition at 100 µg/ml) [50, 51]. Eugenol inhibited autophagy and influenza-A virus replication, via hindering the activation of ERK, p38MAPK and IKK/NF-κB signal pathways and antagonizing the effects of the activators of these pathways. Eugenol also ameliorated the oxidative stress and inhibited the expressions of autophagic genes. The mechanisms underlying assume to be, eugenol inhibited the oxidative stress and the activation of ERK1/2, p38MAPK and IKK/NF-κB pathways, subsequently repressed the dissociation of Beclin1-Bcl2 heterodimer and autophagy, and finally impaired IAV replication These suggested that eugenol is a promising inhibitor for autophagy and IAV infection [52].

Anti-parasitic activity

In vitro studies on eugenol suggested 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 [53]. 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 (IC_{50}: 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 [54]. It showed anti-leishmanial activity against *L. major* promastigote with IC_{50} value of 47.2 µg/ml [55]. Methanolic extract of *Piper betle* containing eugenol exhibited anti-leishmanial potential against *Leishmania donovani* [56]. Benzylated and acetylated derivatives of eugenol exhibited better anti-leishmanial activity than the native form against promastigotes and amastigotes of *Leishmania infantum chagasi* [57]. Clove essential oil having eugenol showed strong trypanocidal activity (inhibition of epimastigotes and trypomastigotes) comparable with basil and yarrow [58]. Eugenol also extended its arm in antimalarial research. It exhibited antimalarial activity with an IC50 value of 753 µM against the chloroquine-resistant strain *Plasmodium falciparum* (FCR-3) [28].

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 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 neuro ectodermal tumour cells by partially triggering apoptosis [59]. The epoxide form of eugenol is a potential drug candidate for inducing apoptosis in human breast cancer cells [60]. ROS plays a critical role in eugenol and eugenol loaded nano emulsion induced apoptosis in HB8065 and HTB37 cells [61]. Volatile extracts obtained by hydro distillation 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 [62]. Eugenol at the low dose (2 μM) has specific toxicity against different breast cancer cells. This killing effect was mediated mainly through inducing the intrinsic apoptotic pathway and strong down-regulation of E2F1 followed by its downstream anti-apoptotic target surviv in, 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 [63]. 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 extracts and ericifolin eugenol fraction against prostate cancer [64]. 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 [65]. Eugenol inhibited matrix metalloproteinase-9 activities in PMA-stimulated HT1080 cells via inactivation of ERK. Therefore, these results suggest that eugenol could be available as an excellent agent for prevention of metastasis related to oxidative stress [66]. 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 [67]. Eugenol and its chemically synthesized derivatives proved its activity against melanoma, skin tumors, prostate cancer, gastric cancer and leukemia via oncogene regulation and caspase dependent pathway which extensively reviewed by [68].

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 revealed 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 [69]. 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 the lungs [70]. 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.

CONFLICT OF INTERESTS

Declare None

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