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Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives

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Eugenol, a phytogenic bioactive component is frequently found in diversified herbal plants possessing well-defined functional attributes. Prominent sources of eugenol are clove, cinnamon, tulsi and pepper. Various extraction methods have been practiced globally for the extraction of eugenol and other nutraceutics from plants. The most extensively employed approaches in this regard include solvent extraction, hydro-distillation, microwave-assisted extraction, supercritical carbon dioxide extraction and ultrasound-based extraction. Eugenol has been approved to encompass numerous beneficial aspects against a capacious spectrum of life threatening indispositions including oxidative stress, inflammation, hyperglycemia, elevated cholesterol level, neural disorders and cancer. In addition, eugenol has also shown strong potential as an antimicrobial agent against wide ranges of pathogenic and spoilage causing microorganisms. Predominantly, the principle mechanistic approaches associated with the therapeutic potential of eugenol include its free radical scavenging activity, hindrance of reactive oxygen species' generation, preventing the production of reactive nitrogen species, enhancement of cyto-antioxidant potential and disruption of microbial DNA & proteins. Consequently, this article is an attempt to elucidate the general properties, sources, extraction methods, therapeutic role and associated mechanisms of eugenol.

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Eugenol: at a glance

Phytogenic components present in a variety of medicinal plants have been extensively used for the prevention and treatment of different lifestyle related risk factors. Traditionally, extracts of different parts of plants have been recommended to cure various complications including bronchitis, diarrhea, skin diseases, cancer, hyperlipidemia, liver ailments, hyperglycemia arthritis, cardiovascular diseases and inflammatory

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disturbances. The functionality of these plants is proposed due to the presence of a plethora of bioactive ingredients found in them. The most common plant based functional components include sterols, flavonoids, phenols, tocopherols and organic acids that possess tangible health benefits and have wide applications in the development of functional and nutraceutical foods.[1] This particular critique mainly emphases to the therapeutic potential of eugenol to curb commonly occurring disorders.

Eugenol is a phenolic component that can be obtained from a wide range of plant sources including clove oil, nutmeg oil, cinnamon extract and many other plants. It owns strong health promoting functions that make it a versatile natural ingredient. Eugenol was firstly extracted from the leaves and buds of *Eugenia caryophyllata* commonly named as clove. Currently, eugenol can also be synthesized by allylation of guaiacol with allyl chloride having the similar kind of functional property. Eugenol is present in significant amount in the extracts of numerous medicinal herbs 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. Eugenol is avowed to possess certain pharmacological properties including anaesthetic activity, antioxidant potential, antimicrobial role, anti-inflammatory action, anti-carcinogenic effects, neuroprotective ability, hypolipidemic efficiency and anti-diabetic effectiveness. Eugenol is declared as GRAS (generally recognized as safe) by World Health Organization (WHO) and is considered as non-mutagenic. The general physical and chemical properties of eugenol are discussed in Table 1. As a result of a wide spectrum of biological and functional properties, eugenol is still proclaimed as the priority of research. Therefore, it is of significant worth to rationally

Table 1 General physical and chemical properties of eugenol

Properties	Description
Class	Phenylpropanoids
IUPAC name	4-Allyl-2-methoxyphenol
Chemical formula	$C_{10}H_{12}O_2$
Molecular mass	164.2 g mol^{-1}
Solubility in water	Partially soluble
Solubility in organic solvents	Highly soluble
Color	Clear to pale yellow
Body metabolism	Absorption *via* small intestine
Excretion and elimination	Through urination and as expired CO_2

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confederate the research findings related to the therapeutic potential of eugenol to elucidate its importance for human health and mechanisms involved in the functionality of eugenol to obviate several lifestyle related indispositions.[2]

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. In addition, several other aromatic herbs including basil, bay, marjoram, mace and nutmeg are also claimed to have significant quantity of eugenol. Concentration of eugenol in some plants is depicted in Table 2. Among these plant sources, clove and cinnamon are considered as the prosperous provenances of eugenol containing 45–90% and 20–50% eugenol correspondingly, but the major problems linked with these sources are higher cultivation costs and commercial eugenol extraction. On the contrary, other cost effective and plentiful begetters include tulsi, ginger, bay, pepper and which can be used as an alternative to clove and cinnamon. Eugenol is mostly present in the aerial parts of plants such as leaves, bark and flowers because these parts contain a considerable amount of essential oils.[1,3] Tulsi leaves also contain good percentage of eugenol usually in the range of 40–71%. However, concentration of eugenol in different parts of plants varies with season. Studies reveal that maximum yield of eugenol can be obtained in the fall season as compared to the summer varieties.[4]

Extraction and isolation of eugenol

The most common method for the isolation of eugenol is steam distillation. In the extraction and isolation process of eugenol, firstly essential oil is extracted from the plants. Afterwards, the essential oils are mixed with 3% solution of sodium or potassium hydroxide for the extraction of eugenol. This reaction results in the formation of a phenolic alkali salt. The insoluble portion of the extract is then isolated by solvent extraction or steam distillation. The remaining alkaline solution is then acidified at refrigeration temperature followed by the liberation of eugenol by employing various techniques such as fractional distillation, high pressure liquid chromatography (HPLC) or thin layer chromatography (TLC). At the end, purity of the obtained eugenol is verified by employing modern spectroscopic techniques like Fourier transform infrared spectroscopy (FTIR), Fourier transform near infrared spectroscopy (FTNIR), mass spectroscopy (MS) and nuclear magnetic resonance (NMR).[5] Some important methods for the extraction of eugenol from various plant sources are described herein.

Table 2 Eugenol occurrence and concentration in various plants[a]

Source	Specified parts	Concentration (mg g^{-1})
Clove	Flowers and buds	180
Clover pepper	Fruit	36
Betel pepper	Leaves	17.85
Cinnamon	Bark	3.52
Tulsi	Leaves	4.2–4.97
Bay	Leaves	1.34
Turmeric	Leaves and essential oil	2.1
Nutmeg	Seeds	0.32
Thyme	Shoots	0.021

[a] Source: (Raja *et al.*, 2015).

Solvent extraction

Solvent extraction is one of the most common and extensively employed methods for the extraction of essential oils from plants. Accordingly, eugenol has also been extracted using various solvents like methanol, ethanol, petroleum ether and *N*-hexane. The major hindrances of solvent extraction are inclusion of other soluble residues undesirable flavor changes in the food.[6] However, still this method has wide applications for the extraction of eugenol and other essential oils from various aromatic herbs. In a typical solvent extraction process of eugenol from clove, the clove buds are ground and wrapped in filter paper followed by subjecting the filter paper to the extraction thimble and inserting into the reflux flask having 500 mL capacity. Afterwards, extraction is carried out by using a suitable organic solvent in Soxhlet apparatus.[7] The process ends by concentrating the obtained extracts at 50 °C using rotary vacuum evaporator.

Several modifications have been made in the conventional solvent extraction process, which show higher efficiency as compared to the traditional method. As an instance, batch extraction process is an attractive alternative to the Soxhlet extraction. This method employs the use of reactor equipped with agitator having four blades and motor having 1200 rpm speed. Recently, this method was studied by Garkal *et al.*[8] who extracted eugenol from leaves of tulsi plant using methanol as solvent and reported satisfactory extraction efficiency. They further reported that extraction efficiency of eugenol was not affected by agitation speed.

Hydro distillation

Hydro-distillation is also one of the mostly used methods for the extraction of essential oils.[9] During hydro distillation method, powdered sample (100 g dried and ground clove buds) is soaked into water. To carry out hydro-distillation, dried clove sample is taken into 500 mL volumetric flask and subjected to hydro-distillation for 4–6 hours. Subsequently, the volatile distillate is collected and saturated with sodium chloride following the addition of petroleum ether or other suitable organic solvent. Later, hydro and ether layers are separated and dehydrated by using anhydrous sodium sulphate. Eventually, the sample is heated in water bath at 60 °C for the recovery of ether and concentration of extract. The average yield of oil using hydro-distillation is about 11.5% whereas reported eugenol concentration is 50.5–53.5%.[6] However, extraction yield can be increased by reducing the particle size of ground clove buds.[10]

Microwave-assisted extraction of eugenol

Traditional methods practiced for the extraction of eugenol from various plant sources are associated with several

drawbacks like hydrolysis, thermal degradation and leaching of some fragrance components.[11] To combat these problems, several modern extraction methods have been introduced which provide high extraction yield along with reduced processing time and energy demands. Among these approaches microwave assisted extraction (MWAE) is regarded as a green extraction approach having ability to produce eugenol and other essential oils with same sensorial attributes and quality as those attained by conventional methods. This technique also provides rapid extraction rate at lower cost as compared to the traditional methods.[12]

Several configurations have been developed using microwave extraction process. These techniques include microwave-assisted hydro-distillation (MWHD),[13] coaxial microwave-assisted hydro-distillation (coaxial MWHD), microwave assisted hydro-diffusion and gravity (MWHG)[14] and microwave steam distillation (MWSD).[15] Amongst these green extraction approaches, coaxial MWHD extraction is reported to be advantageous with high savings in heating time (400%) and energy demands (30%). Besides, this method is safe, cost effective and has easy scale-up configuration as compared to the other microwave extraction techniques.

More recently, Gonzalez-Rivera *et al.*[16] explored the potential of coaxial MWHD for the extraction of essential oils from various herbs (clove, lavender, sage, rosemary and fennel). In parallel, traditional hydro-distillation method was also used for comparative studies. Results of the study revealed that eugenol and other essential oil extracted through coaxial MWHD showed higher thermal stability as compared to those extracted through conventional hydro-distillation. Additionally, extraction time was quite less as compared to the conventional extraction process. Conclusively, it can be stated that microwave assisted extraction methods are quite mature to be used at industrial scale as a replacement of conventional techniques for the extraction of eugenol.

Supercritical carbon dioxide extraction

Supercritical carbon dioxide (SC-CO_2) extraction is another efficient method for the extraction of eugenol from clove buds.[17] The efficiency of SC-CO_2 extraction has been reported through several investigations. For example, Gopalakrishnan *et al.*[18] compared the efficiency of liquid and SC-CO_2 extraction and found higher extraction rate of clove oil using SC-CO_2 extraction. Likewise, Reverchon and Marrone[19] concluded that extraction yield of eugenol can be increased by increasing the flow rate of CO_2 in supercritical extraction. Additionally, Wenqiang *et al.*[6] and Hong-Peng *et al.*[20] conducted experiments to study the composition of clove bud oil extracted by SC-CO_2 and other conventional techniques (solvent extraction, steam distillation, molecular distillation) and reported very similar composition as obtained by traditional methods. Although several studies have shown the potential of SC-CO_2 extraction as an efficient tool for the extraction of clove oil however studies on optimization of the yield of the SC-CO_2 extracted eugenol are scanty. Hence, further studies are needed to highlight the potential of SC-CO_2 extraction method for the recovery of eugenol from the clove bud oil.

Ultra-sound assisted extraction of eugenol

Even though several non-conventional techniques successfully control the drawbacks associated with the use of traditional methods however they may differ in extraction effectiveness. Recently, another green extraction method called ultrasound (US) extraction has been introduced that has significantly accelerated the extraction process and reduced the energy demand. Other advantages linked with the use of US extraction are easy handling of extract, rapid execution, no residues, high yield, eco-friendly, enhanced quality and prevention of extract degradation.[21]

Alexandru *et al.*[22] assessed the efficiency of ultrasound-assisted extraction and traditional maceration for the extraction of clove oil. Purposely, they selected three different flow modes (450, 900, 1350 mL min^{-1}) for US extraction and reported higher extraction rate at 1350 mL min^{-1}. Similarly, Tekin *et al.*[23] employed US extraction for the extraction of clove essential oils using central composite design (CCD). The independent variables of the study were temperature (32–52 °C), extraction time (30–60 min) and plant concentration (3–7%) while the dependent variable was clove extract. The frequency of the ultrasound bath was fixed at 53 kHz. The findings of the study exposed that extract yield was highly influenced by temperature. The obtained extract was composed of eugenol, α-caryophyllene and 2-methoxy-4-(2-propenyl)phenol acetate. These investigations concluded that ultrasound-assisted extraction is an effective tool that can pave the road for the setup of commercial applications of this method.

Validated pharmacological properties of eugenol

Numerous investigations have documented the therapeutic potential of eugenol that highlights its importance as one of the principal bioactive components having several health promoting functions. The pharmaceutical functions of essential oils extracted from various plants have also thought to be due to the presence of eugenol.[24] A number of studies have been carried out in order to explain the mechanism involved behind the therapeutic activity of eugenol. On a general node, mechanistic approach of functional activeness of eugenol proposed through several trials is its free radical scavenging activity, prevention of reactive oxygen species (ROS) & reactive nitrogen species (RNS) generation, DNA and protein damage, and elevation of cellular antioxidant potency.[25] Eugenol is vindicated to be effective against a number of lifestyle related threats including nervous disorders, digestive complications, reproductive derangements, blood cholesterol irregularity, hyper-tension, elevated blood glucose level, microbial infections, inflammatory actions and carcinogenesis.[26] This section will summarize the ameliorative vivacity of eugenol to encounter several metabolic ailments and mechanisms associated with the functional and health promoting attributes of eugenol (Table 3).

Antioxidant activity of eugenol

Many human related disorders like cancer, diabetes, arthritis, Parkinson's disease, AIDS and Alzheimer's complications are

Table 3 Molecular mechanisms of eugenol in various nutraceutical properties

Bioactivities	Mechanism(s)	References
Antioxidant activity	Inhibitory effect on lipid peroxidation Inhibits ROS & RNS formations	28, 29 and 30
Antimicrobial activity	Induces cell lysis in Gram positive & negative bacteria Inhibition of IKK/NF-κB, ERK and p38MAPK signaling pathways	2 and 35
Anti-inflammatory potential	Prevents inflammatory cytokine expression Inhibitory effect on prostaglandin synthesis Suppresses COX-2 activity Inactivates TNFα factors Inhibits NF-kappaB pathways	41–45
Anti-cancer activity	Trigger cell apoptosis Target E2E1/survivin pathways Apoptosis in MCF-7 human breast cancer cells Suppresses COX-2 gene in HT-29 human colon cells Inhibits prostaglandin E_2 production Reduces DNA oxidation Inhibits matrix metalloproteinase activity (MMP-9) Inactivates ERK proteins/pathways	42, 49, 55, 56, 59, 73 and 74
Neuro-protective & anti-stress related perspectives	Inhibits lipid peroxidation Prevents reduction in dopamine contents Inhibitory action on 5-lipoxygenase activity Reduces NO, MDA and ROS concentrations Augments glutathione contents in cortex and cerebellum regions Attenuate acetylcholinesterase activity and cytosolic calcium content in brain regions Regulated serotonergic system in amygdala region Diminishes RS-induced rise in plasma corticosterone levels Augmented changes in serotonin (5-HT) levels Decreases level of norepinephrine in brain regions Reduces ulcer index Modulates HPA & BMS	77–80
Antidiabetic potential	Inhibits α-glucosidases activity Inhibits formation of AGE Prevents attachment of glucose to serum albumin Upregulates the concentration of antioxidative enzymes	85–88
Hypocholesterolemic perspectives	Scavenges free radicals Maintains antioxidant status of body Reduces cellular oxidative damage Increases the concentration of SOD enzymes Decreases the levels of serum MDA Inhibitory effect on HMG-CoA reductase	99–101

prompted and exaggerated due to redundant group of the free radicals. Reports have indicated that fruits and vegetables containing cache of phytonutrients like polyphenols, flavonoids and anthocyanin are observed to be efficacious in scavenging the free radicals.[27] Eugenol, a potent phenolic component in clove oil is chiefly responsible for its antioxidant and free radical scavenging activity. Antioxidant power of eugenol can be elucidated by forming complexes with reduced metals. Potent inhibitory effect on lipid peroxidation by isoeugenol and eugenol is administrated to be due to eradication of free radical and formation of iron–oxygen chelate complex, by keeping iron and copper at a reduced state respectively.[28]

In an experimental trial, Gulcin[29] revealed the antioxidant potential of eugenol and reported 96.7% (r^2 = 0.9319) inhibitory effect on lipid peroxidation of linoleic acid emulsion at a concentration of 15 μg mL^{-1} comparable to butylated hydroxytoulene (BHT). Same study suggested DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity having lowest IC_{50} value of 16.06 μg mL^{-1} (r^2 = 0.9823). This scavenging effect of eugenol is due to presence of allyl group in its structure. Another study by Gulcin *et al.*[30] elucidated 93.3% and 97.9% inhibition on lipid peroxidation of linoleic acid emulsion at 20 and 60 μg mL^{-1} correspondingly.

Additionally, Lee and Shibamoto[31] showed that aromatic extract containing eugenol as major bioactive ingredient from clove inhibited the hexanal oxidation at a level of 50 μg mL^{-1} for 30 days. By gas chromatography/mass spectrometry (GC-MS) analytical technique twenty two different compounds were quantified in aromatic extracts, implying 24.37 mg g^{-1} and 2.35 mg g^{-1} of eugenol and eugenyl acetate to be the key ones. Eugenyl acetate and eugenol repressed 79% and 88% malonaldehyde formation from cod liver oil@160 μg mL^{-1}.

Antimicrobial activity of eugenol

Microbial food-borne illness is currently one of the foremost concerns for food safety authorities, food processing industry and ultimately the consumers. Simultaneously, consumers are concerned regarding the safety aspects of synthetic and artificial food preservatives. Dissemination of methicillin resistant *Staphylococcus aureus* (MRSA) one of the antibiotic resistant pathogen has provoked researchers to revive the quest for antimicrobial complexes from natural plant sources. Since ancient times, herbs and spices are supplemented in food system, not only to enhance flavoring profile, but also as food preservative and folk medicine. Due to their diversified biological and biochemical functions, plant phytogenic compounds have established a great deal of attention, over past few decades.[32]

Clove oil polyphenol eugenol illustrates potent antibacterial potential against numerous strains of Gram-positive (*Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis*) and Gram-negative (*E. coli*, *Proteus vulgaris*, *Salmonella choleraesuis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Helicobacter pylori* and *Yersinia enterocolitica*) bacteria. Eugenol damages cell membrane and cell wall, inducing cell-lysis in Gram negative and Gram positive bacteria resulting in leakage of intracellular fluid along with lipid and protein contents.[33,34] Strong eradicative and inhibitory effect of eugenol has been documented according to *in vitro* and *in vivo* studies on biofilms. At concentration of 0.5× MIC (minimum inhibitory concentration) 50% inhibition was recorded in case of biofilms formed by methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin susceptible *Staphylococcus aureus* (MSSA) strains. Minimum biofilm eliminating concentration (MBEC) of already formed biofilms was reduced to 99% by collective application of both carvacrol and eugenol.[2]

Eugenol has potentiality to constrain viral infection and replication categorically against herpes simplex viruses *i.e.* HSV-1 and HSV-2 exhibiting IC_{50} values between 16.2 μg mL^{-1} and 25.6 μg mL^{-1}, explored by plaque reduction assay (PRA). Eugenol has been validated to be adequate against herpes simplex virus-1 (HSV-1) clinical isolates.[35] Eugenol inhibits influenza-A virus's replication and autophagy; by inhibiting the initiation of IKK/NF-κB and ERK, p38MAPK signal pathways.[2]

Anti-inflammatory potential of eugenol

Inflammation is known as adaptive immunity response of body that is stimulated by noxious stimuli and other various conditions, for instance tissue injury and infection.[36] It could either be classified as acute or chronic inflammation. Acute inflammation also known to be physiological inflammation is a valuable and constructive host response against tissue damage, but if in time remediation is delayed, it leads to metabolic-associated syndromes as cancer, inflammatory bowel disease (IBD) and rheumatoid arthritis. It is an initial response that results in movement of macrophages and neutrophils from blood stream into infected tissues.[37] Alternatively, chronic inflammation is related to tissue malfunctioning mainly due to persistent exposure to noxious stimuli that results in changes allied with the development of cancer through attraction of bioactive lipids such as eicosanoids, soluble pro-inflammatory mediators TNF-α and transcription activation factors NF-κB.[38,39] In the last decade, nutraceutical prospects of eugenol and its derivatives have been exploited by scientists to formulate novel drugs from plant origin having low drug toxicity, mainly those implicating anti-inflammatory potential, to be used in treatment of various maladies.[40]

The principal methoxyphenol of clove oil; eugenol has documented anti-inflammatory potential. Eugenol suppresses cyclooxygenase (COX)-2 expression and tumor necrosis factor (TNF) signaling, whereas eugenol oligomers avert inflammatory cytokine expression in macrophages and NF-kappaB (nuclear factor-kappaB) activation.[41] The anti-inflammatory mode of action of eugenol is mainly due to its inhibitory effect on prostaglandin synthesis and neutrophils/macrophages chemotaxis.[42]

According to findings of Koh *et al.*,[43] eugenol at concentration 5–500 μM significantly accelerated IL-8 (interleukin-8) production in HGF (hepatocyte growth factor) cells, resulting insubstantial stimulation at 5 μM and significant inhibition at 500 μM. Another study concluded beneficial effect of eugenol administrated at 5 and 10 mg kg^{-1} per B.W. against lipopolysaccharide (LPS) induced acute lung injured (ALI) mice, for this purpose 0.5 mg kg^{-1} LPS was intratracheally infused. Examination of lung tissues and bronchoalveolar lavage fluid (BALF) suggested anti-inflammatory effect due to reduced production of pro-inflammatory cytokines.[44]

Additionally, *in vitro* studies revealed that clove oil polyphenol inhibits nuclear factor-κB (NF-κB) activation in lipopolysaccharides initiated macrophages induced by inactivated cyclooxygenase activity (COX-2) and tumor necrosis factor (TNFα). Cyclooxygenase activity is prompted by LPS, cytokines and growth factors.[42] During pulmonary inflammation in mouse, elevated TNF-α and neutrophils were significantly reduced by eugenol at a dose of 160 mg kg^{-1} per body weight. It also protected against chemically induced dysfunction of macrophages and balanced the pro-inflammatory mediators.[45]

Further clinical studies are required to authenticate the use of eugenol and its other active derivatives as anti-inflammatory agents on dendritic cells, along with their modulating effects on cytokines and autoimmune inflammatory diseases. Eventually, results from these clinical trials would significantly improve the immune-protective application of eugenol.

Anti-cancer activity of eugenol

Cancer is broadly known as rampant cell division that eventually causes cell aggregation leading to tumors formation. Cancer is documented as second leading single reason of mortality claiming annual death toll of six million lives globally.[46] Numerous factors are involved in onset of cancers, for instance smoking, ingestion of heavy metals & pesticides, genetic mutations and indeed lack of safe & healthy diet.[47] Inflammation results in cellular aggregation due to improper functioning of signaling pathways which is related to

pathogenesis of cancers.[48] Treatment of cancer lies in inhibition of cell propagation and annihilation of malicious cells.[49]

Recent clinical studies of medicinal plants used in numerous traditional medicines have triggered the invention of various chemotherapeutic drugs, like camptothecin, vinblastine, taxol and vincristine.[50] Several scientific studies have pointed out chemo-protective potential of eugenol against different form of cancers.[51] *In vitro* studies have proven the potential of eugenol and their chemically synthesized derivatives against gastric cancer, colon cancer, leukemia, breast cancer, prostate cancer, melanoma and skin tumors *etc.*[49]

Breast cancer is the most prevalent type of malignant cancer among women and is ranked fourth common reason of cancer related deaths in the world.[52] Mammary homeostasis depends upon balance among cycles of proliferating mammary epithelial cells and their death through the process of apoptosis.[53] Risk associated to instinctive breast cancer is linked to elevated escalation of mammary epithelial cells that is eventually responsible for the genetic alteration. Eugenol associated biphenyl (*S*)-6,6′-dibromo-dehydrodieugenol elicits particular anti-proliferative activity on neuro-ectodermal tumor cells by partially triggering apoptosis.[54] Vidhya and Devaraj[55] provided some evidence that eugenol has anticancer potential in human breast cancer cells (MCF-7) as epoxide form of eugenol is considered as anticancer drug regarding induction of apoptosis in MCF-7 cells. Eugenol repressed growth of human breast cancer cells by initiation of cell death both in time and dose dependent manner.[56]

Essential oils being extracted by hydro distillation of roots and barks of *Uvariodendron angustifolium* containing 85.3% and 68.3% of methyl eugenol respectively, unveiled cytotoxic potential in MCF-7 cancer cells.[57] Cytotoxic properties of eugenol and its related dimers were may be due to interaction of lipophilic radical with the cell membrane and this cytotoxic characteristic was observed highest in dehydrodiisoeugenol followed by di-isoeugenol, isoeugenol, eugenol and least by bis-eugenol.[58] Both *in vitro* and *in vivo* studies on anti-breast cancer activity of eugenol have revealed that it could augment the treatment of human breast cancer by targeting the E2F1/survivin pathway.[59]

Inducible cyclooxygenase (COX-2) inhibitors have been considered as cancer chemo-preventive and anti-inflammatory agents. According to findings of Kim *et al.*,[42] eugenol at concentration 10 μg mL^{-1} was found to significantly inhibit (98.3%) prostaglandin E_2 production in LPS-activated (lipopolysaccharide) mouse macrophage cells (RAW264.7). Isolated eugenol not only suppressed COX-2 genes in HT-29 human colon cancer cell line, but also exhibited inhibition of PGE-2 considerably, having IC_{50} value as 0.37 μM. They also revealed that eugenol inhibited the propagation of HT-29 cells and the mRNA expression of COX-2 gene. Remedies for colon cancer through administration of eugenol resulted in decreased concentration intracellular non-protein thiols. Additionally, DNA fragmentation caused by amplification of reactive oxygen species (ROS) is considered as a core reason for apoptosis in eugenol-subjected colon cancer cells. Eugenol not only transmitted apoptotic signal through reduction of non-protein thiols resulting in decreased potential of mitochondrial membranes, eventually leading to increased production of ROS.[60,61] Similarly, an earlier investigation by Jaganathan *et al.*[62] suggested that intraperitoneal administration of eugenol at a concentration of 100 mg kg^{-1} inhibited the growth of solid carcinoma and Ehrlich ascites by 24.35 and 28.88%, respectively.

Novel strategies in cancer prevention have initiated the use of eugenol as a potential chemotherapeutic agent. Synergistic effect of eugenol with chemo-preventive drugs has been reported to reduce the drug toxicity on normal cells and augment the cytotoxicity of administrated synthetic agents.[63] Purposely, to mitigated the effect of drug induced resistance various phenyl-propanoids including eugenol was examined in combination with 5-fluorouracil; an anticancer drug against human cervical cancer cell line (HeLa cells). Among all investigated phenyl-propanoids, eugenol explicated maximum cytotoxicity and reduced hemolytic activity validating its use as a chemo-protective agent without any adversative toxicity.[64,65] Moreover, synergistic interactions of eugenol and gemcitabine impelled apoptosis and growth inhibition at lower concentrations as compared to individual agents. Eugenol (150×10^{-6} M) and gemcitabine (15×10^{-6} M) reduced cell viability from 84% (eugenol) and 51% (gemcitabine) to 47% in combination.[66,67]

Currently, chemo-protective agents being practiced to cure malignant melanoma; typically the most antagonistic skin cancer occurring in melanocytes are unsatisfactory.[68,69] Pisano *et al.*[54] conducted *in vitro* trials to assess anti-proliferative potential of eugenol and its six derivatives against melanoma cells lines. They suggested inhibitory mode of action of eugenol as it causes cell cycle arrest and induces apoptosis. Likewise, anti-proliferative mode of action of eugenol and iso-eugenol against melanoma cancer cells has also been examined in B16 xenograft model, resulting decrease (40%) in tumor size due to production of ROS having inhibitory effect on DNA synthesis.[70,71]

In an experimental trial Koh *et al.*[72] suggested the induction of non-apoptotic cell death by clove oil bioactive ingredient, eugenol. Cytotoxic concentrations of eugenol resulted in reduction of ATP utilization of oxidative stress and an increase in the polyamines and glycolytic metabolites, in normal oral cells and oral squamous cell carcinoma. Another group of researchers have highlighted the inhibitory potential of eugenol against expression of matrix metalloproteinase (MMP-9) activity in PMA-stimulated HT1080 cells *via* inactivation of ERK proteins. Consequently, they evaluated that eugenol from cloves, explicitly revealed highest inhibitory effect on hydrogen peroxide (H_2O_2) than other ROS (reactive oxygen species), and was able to halt lipid peroxidation and DNA oxidation, induced by hydroxyl radical. Therefore, eugenol could be accessible as an outstanding agent for prevention of metastasis associated to oxidative stress.[73,74] Eugenol and its allied derivatives require further investigation in order to be considered as chemotherapeutic.

Neuro-protective & anti-stress related perspectives of eugenol

Eugenol, an indispensable component predominantly found in spices such as nutmeg, clove, cinnamon, basil *etc.* has been

stated to possess potent neuro-protective and anti-stress related properties. Evidently, eugenol acts as neuro-protective agent against amyloid-β peptide, ischemia & excito-toxicity by improving neuronal complications and inhibiting the transmission in sciatic nerves.[75] Ancient pharmacopoeias exposed that majority of herbal medications designated for Alzheimer disease (AD) comprised of a herb Acori Graminei Rhizoma which was rich in eugenol.[76] *In vitro* cell model studies have revealed inhibitory activity of eugenol on 5-lipoxygenase and ameliorating effect against oxidative & excito-toxic injured neuronal cells. Further, eugenol significantly inhibited lipid-peroxidation and prevented reduction in dopamine contents in experimental rats.[77]

Several studies have been performed to study the neuro-protective and anti-stress related potential of eugenol. For instance, Prasad and Muralidhara[78] investigated the neuro-protective effectiveness of eugenol and iso-eugenol against acrylamide (ACR) induced neuropathy model in male albino rats. They intraperitoneally administrated ACR (50 mg kg^{-1} per B.W.) thrice a week for 5 consecutive weeks for the induction of neuropathy. Supplementation of functional ingredient eugenol and iso-eugenol to ACR-induced rats (10 mg kg^{-1} per B.W.) for five weeks resulted in noticeable enhancement in behavioral index gait score. Momentously, both active ingredients reduced oxidative stress markers *i.e.* nitric oxide (NO), malondialdehyde (MDA) & reactive oxygen species (ROS) and augmented the glutathione concentrations in brain [cortex (Ct), cerebellum (Cb)] as well as in sciatic nerve (SN) regions. Furthermore, they effectually diminished the acetylcholinesterase activity, levels of cytosolic calcium and dopamine in brain regions. Conclusively, they were of a view that eugenol and iso-eugenol have tendency to curtail acrylamide induced neuropathy in rats and therefore possibly could be used as a diet based regime to attenuate various forms of neuropathy in human beings.

Additional studies illuminate the protecting potential of eugenol by modulation of hypothalamic pituitary adrenal cortex (HPA) axis and brain mono-aminergic pathways against restraint stress irritable bowel syndrome (RS-IBS) in rats. Oral administration of eugenol (50 mg kg^{-1}) reduced (80%) restraint stress induced upsurge in fecal pellets comparable with the medication of ondansetron. Eugenol regulated serotonergic system in amygdala & PFC and extenuated (80%) RS-induced rise in levels of plasma corticosterone. Alongside, eugenol also deflated RS-induced alterations in levels of norepinephrine and elevated antioxidant immune system in all regions of brain.[79]

Stress is foremost psychopathological reason for numerous psychological and mental impairments. Hypothalamic pituitary adrenal (HPA), sympatho-adrenal and brain monoaminergic systems (SA & BMS) mediates various psychological and physiological responses in stress conditions. Eugenol is stated to significantly moderate brain functions by regulating the release of neurotransmitters. Garabadu *et al.*[80] assessed the anti-stress potential of eugenol on rats that were subjected to 4 h restraint stress model. Exposure to stress not only elevated the ulcer index but also increased the plasma norepinephrine and corticosterone levels. However, eugenol administration for 7 days showed significant effect on hypothalamic pituitary adrenal (HPA) axis by reducing the stress-induced upsurge in ulcer index and plasma corticosterone levels. Moreover, eugenol also augmented the stress-induced changes in serotonin (5-HT) levels in all regions of brain, while decrease in norepinephrine levels was also observed in all portions of brain except for hippocampus. Outcomes of this study suggested anti-stress activity of eugenol is mainly due to modulation of hypothalamic pituitary adrenal (HPA) and brain monoaminergic systems (BMS). Several research mediations suggested the importance of eugenol as neuro-protective agent which could further be used in stress related pathological conditions.

Anti-diabetic potential of eugenol

Abnormalities in glucose metabolism result in degenerative disease broadly known as diabetes mellitus (DM). It is mostly associated with reduced production of pancreatic insulin and or passivity of body organs to insulin resulting in hyperglycemia, a pre-condition stage for onset of diabetes.[81] Abnormalities in machinery of glucose metabolism and persistent efforts of physiological system to revert metabolic discrepancies pose an extra exertion on hormones secreted by endocrine system eventually causing disturbance in endocrine control. Disruption of endocrine control infuriates the metabolic deterioration by varying glucose metabolic enzymes that induces hyperglycemia.[82] Prolonged hyperglycemia precedes assistance in disintegration of β-secreting insulin cells, commonly known as glucose toxicity.[83] Prevalence of hyperglycemia in diabetic subjects not only causes impairment in nucleic acids, cellular proteins and membrane lipids, but also escalates the rate of outset of respective ailment.

Nowadays, researchers have shifted their preference towards diet based régime to cure various metabolic ailments owing to presence of nutraceutical agents mainly responsible for augmenting potentials against diseases. Amongst various strategies to combat diabetes, diet plays a vital part in maintaining blood sugar level, hyper-glycosylation of biologically active molecules linked with various metabolic pathways and inhibition of pathologies. For this purpose, innumerable polyphenolic molecules present in various food commodities irrespective of plant or animal origin are incessantly being exploited to assess their therapeutic potential in precluding and/or controlling diabetes.[84] Phenolic acids known to be secondary metabolites are predominantly found in several fruits, vegetables and spices. Various epidemiological investigations have illustrated the positive relationship among the utilization of phenolic rich diet and prevention of diabetes.[85]

Various experiments have shown that eugenol is the key bioactive molecule present in spices with anti-diabetic potential. In this context, Srinivasan *et al.*[86] assessed the hypoglycemic properties of eugenol by determining the activities of main enzymes associated to glucose metabolism in streptozotocin induced (40 mg kg^{-1} per B.W.) male diabetic rats. The normal control group (I) was vehicle treated (olive oil), group II (normal rats) subjected to eugenol (10 mg kg^{-1} per B.W.), group III was (diabetic control) while group IV, V and VI comprised of

diabetic rats that were administrated to 2.5, 5.0 and 10 mg kg^{-1} per body weight (B.W.) of eugenol. Serum biochemistry after 30 days showed that all doses of eugenol significantly ($p < 0.05$) declined plasma glucose and increased insulin level. However, dose of 10 mg kg^{-1} per B.W. eugenol momentously reduced the concentration of blood glucose (70%) and glycosylated hemoglobin (25.70%) whereas an increase in plasma insulin level (46.15%) was observed as compared to diabetic control at the termination of experimental trial. Furthermore, activities of various enzymes involved in carbohydrate metabolism increased for instance hexokinase (62.25%), pyruvate kinase (68.57%), glucose-6-phosphate dehydrogenase (31.05%) while reduction in levels of glucose-6-phosphatase (24.45%), fructose-1,6-bisphosphatase (32.55%), AST (27.12%), ALT (39%), ALP (41.70%), creatine kinase (38.57%) and blood urea nitrogen (34.01%) of experimental diabetic rats was observed due to administration of eugenol. Additionally, orally administrated eugenol elevated the levels of hepatic glycogen demonstrating the anti-hyperglycemic capabilities in diabetic rats.

Later, Tahir *et al.*[87] examined anti-diabetic potential of essential oils from clove and cumin and their respective emulsions in dose dependent manner (1–100 μg mL^{-1}) by application of α-amylase inhibitory assay. Highest anti-diabetic activity was noticed at maximum dose *i.e.* 100 μg mL^{-1} for both experimented clove and cumin essential oils. In this experiment, five emulsions were formulated using different concentrations (5–25%) of each clove and cumin essential oils. Results of α-amylase inhibition assay unveiled maximum anti-diabetic potential (83.09% & 95.30%) in emulsions containing 25% of cumin and clove essential oils, respectively. Besides this, bioactive components responsible for potent anti-diabetic activity of clove (eugenol; 18.70%) and cumin (α-pinene 18.80%) essential oils were quantified using GC-FID. Furthermore, Singh *et al.*[88] proposed dual anti-diabetic mechanism of eugenol; primarily by inhibiting alpha-glucosidases followed by preventing formation of advanced glycation end products (AGE). Moreover, there is a dire need for various community based trials and clinical studies to comprehend the antidiabetic and hypoglycemic potential of eugenol and its derivatives.

Eugenol: a hypo-lipidemic agent

Hyperlipidemia owing to its positive connection between lipids related abnormalities and cardiovascular diseases (CVD) is known to be the most vital socio-economic issue in common people along with health professionals.[89] Sedentary lifestyle, along with intake of fat rich diet and less physical activity, expressively contributes in onset of hyperlipidemia and CVD.[90] Elevated levels of low density lipoprotein cholesterol (LDL-c) causing atherogenesis and toxicity to vascular tissues, amass in extracellular sub-endothelial regions of arteries resulting in hypertension, diabetes, atherosclerosis, obesity and inappropriate functioning of vital body organs like heart, liver and kidneys.[91] Scientific trials have publicized that mortality and morbidity linked with CVDs could be alleviated by reducing the dietary intake of lipids.[92] Reduction in LDL-c levels has also been found to reduce the propagation of cardiovascular complications and ameliorate atherosclerotic conditions.[93,94]

Reactive oxygen species (ROS) results in progression of oxidative stress conditions responsible for proliferation of various chronic diseases such as coronary heart disease (CHD) and atherosclerosis.[95] Increased production of free radicals in body usually escorts disturbance in antioxidant status, leading to cellular oxidative damage.[96] Dyslipidemia is stated to be allied with oxidative stress conditions originated with the increased formation of ROS, resulting in improper functioning of the antioxidative defense system.[97] Diet modulation is the foremost choice to address atherosclerosis and dyslipidemia. The propagation of atherosclerotic conditions is significantly assuaged through if the diet comprising of antioxidants and cholesterol lowering phytochemicals. In the last two decades, rice bran has gained special attention by the researchers for its anti-atherogenic, cholesterol lowering and free radical scavenging potential.[98]

Many studies have been conducted to assess the anti-hypercholesterolemic anti-atherogenic potential of eugenol. In this context, Venkadeswaran *et al.*[99] evaluated anti-hyperlipidemic potential of eugenol in intraperitoneally injected Triton WR-1339 (300 mg kg^{-1} per B.W.) induced hyperlipidemic Wister male rats. Eugenol administration reduced LDL-cholesterol (79.48%), total cholesterol (55.88%) and triglycerides (64.30%) levels. The hyperlipidemia ameliorating potential of eugenol was comparatively effective as that of lovastatin; a lipid lowering drug. Additionally, hypercholesterolemic rats subjected to eugenol showed significant reduction in activities of ALP, LDH and ALT enzymes as compared to positive controls.

In hypercholesterolemia, concentration of superoxide dismutase (SOD) decreases and that of malondialdehyde (MDA) increases rapidly in serum profile. Munisa *et al.*[100] examined the effectiveness of cloves leaf extract mainly containing eugenol as bioactive phytochemical against SOD and MDA profiles in hypercholesterolemic rats. They were of a view that cloves leaf extract significantly reverted the levels of MDA and SOD in normal ranges. Similarly, Jin and Cho[101] also illustrated hypocholesterolemic potential of clove extract (eugenol) in hyperlipidemic zebrafish model. They concluded that clove essential oil reduced serum cholesterol and triglycerides up to 68 and 80%, respectively. Besides, without outcomes of clinical trials, it is not recommended to suggest eugenol and its derivatives to patients suffering from dyslipidemia.

Conclusion

This review article explicates the effectiveness of eugenol as a therapeutic tool that can be incorporated to various foods and herbal medicines for contending considerable metabolic disorders. It also contains considerable antimicrobial properties and can be employed to inhibit the growth of microbial populations in many foods. Additionally, derivatives of eugenol have unlocked a new era in the domain of pharmacology, kindling the research interests on this compound. Nevertheless, more studies are required to specify the dosage level of eugenol

for various functional applications and to explore several other hidden potentials of eugenol for the betterment of mankind.

Conflicts of interest

Authors declare no conflicts of interest.

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