Pharmacogn. Rev.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
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REVIEW ARTICLE

A Comprehensive Review on Eugenol's Antimicrobial Properties and Industry Applications: A Transformation from Ethnomedicine to Industry

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ABSTRACT

Eugenol and eugenol-containing plants are used in ethno and modern medicine for various biological activities including antimicrobial activity. This review article provides an insightful transformation of eugenol from being an ethnomedicine to being a food protectant in the food industry. Scientific publications on the antimicrobial activity of eugenol and its respective advancements were collected from scientific databases such as Scopus, PubMed, and Google Scholar published between 1995 and June 2018. The eugenol has shown significant broad-spectrum antimicrobial activities against Gram-positive, Gram-negative, fungi, and virus. The eugenol has also shown synergistic effects with conventional antimicrobials. Formulations, such as micro- and nanoemulsions, nanocapsules, and nanoparticles, are prepared to improve the aqueous solubility and efficacy of eugenol. Eugenol is used as a food protectant in storing plants, grains, fruits, and livestock. This review covers eugenol's antimicrobial activities, formulations to improve aqueous solubility, and applications in the food industry. Extensive scientific investigations validated the ethnomedicinal uses of eugenol as an antimicrobial agent. Its activity on multidrug-resistant pathogens should further be explored to identify the molecular mechanisms and synergistic/antagonistic effects with conventional antimicrobials. There were no studies on investigating eugenol's potential in *in vivo* infectious animal models. This is the first review on eugenol that details the antimicrobial potential of eugenol and its possible applications as a protectant in the food industry.

Key words: Antimicrobial, antiviral, essential oil, eugenol, food protectant, food industry

INTRODUCTION

Despite the recognition of the ever-growing problem, global prevalence of microbial infections and associated health concerns continue to take a toll, resulting in increased cases of prolonged illness and death. This is worsened by increasing antimicrobial resistance that poses threats to the effective prevention and treatment of microbial infections. With this concern, various natural or synthetic compounds are actively explored for their efficacy in combating microbes. Eugenol, which is the major natural component found in clove oil, has been known for its versatility in pharmacological activities such as anti-inflammatory,[1,2] anticancer,[3-5] analgesic,[6,7] and anesthetic activities.[8-10] The chemical structure of eugenol is shown in Figure 1.

The eugenol is extracted from various plants such as cloves,[6,11-13] lemon grass,[14-16] tulsi,[17,18] and cinnamon[19,20] via numerous methods of extraction including steam distillation, microwave-assisted extraction, supercritical carbon dioxide extraction, and ultrasound-based extraction.[21] This prominent compound offers a number of therapeutic benefits via inhibition of generative reactive oxygen and nitrogen species, scavenging of free radicals, and disruption of biofilms of microbes.[21,22] The efficacy of eugenol and its mechanism of action against bacteria, fungi, and viruses are of interest. Due to the increasing incidence of microbial resistance to conventional antibiotics, the effects of eugenol that could work synergistically with the current antibiotics to improve their antimicrobial efficacy against different microbial strains were compiled and explicated. The applications of eugenol and its formulations in the food industry and antimicrobial activity of eugenol containing essential oils/plant extracts were also compiled as shown in Figure 2.

ANTIBACTERIAL ACTIVITY

One of the predominant causes of bacterial infections arises from biomaterial implant failures or bacterial adherence and biofilm formation on medical implants.[23] The prevalence of medical implants is increasing extensively in the past decades, and implant failure leads to relentless

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Access this article online

Quick Response Code:

Website: www.phcogrev.com

DOI: 10.4103/phrev.phrev_46_18

Cite this article as: Mak KK, Kamal MB, Ayuba SB, Sakirolla R, Kang YB, Mohandas K, *et al*. A comprehensive review on eugenol's antimicrobial properties and industry applications: A transformation from ethnomedicine to industry. Phcog Rev 2019;13:1-9.

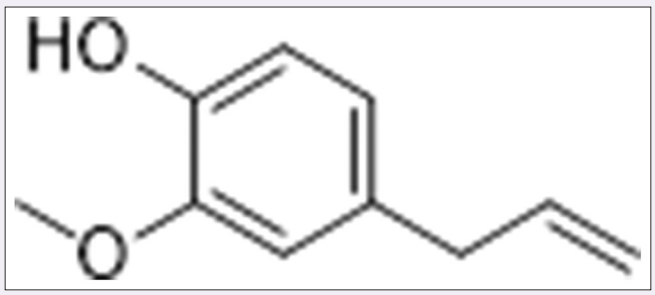

Figure 1: Chemical structure of eugenol ($C_{10}H_{12}O_2$). Eugenol's IUPAC name is 2-methoxy-4-(2-propenyl) phenol

challenges in the medical field as its application.[24] Accordingly, a hydrophilic copolymeric system using eugenol was tested and it showed success in inhibiting bacterial growth.[25] Eugenol has been scientifically proven to be pharmacologically active against a number of bacteria, both Gram-negative and Gram-positive, as well as fastidious and facultative anaerobic oral bacteria.[26] The current data that could substantiate these claims are concisely summarized in Table 1.

Eugenol is capable of significantly increasing the permeability profile of the membrane and has disruptive action on cytoplasmic membrane.[27] In addition, findings on the efficacy of eugenol suggest that inhibition of the production of virulence factors such as violacein, elastase, pyocyanin, and biofilm formation was successful.[28] Eugenol has also displayed high efficacy as an antistaphylococcal and antilisterial biofilm agent.[29]

In other industries – in broiler chicken for instance – eugenol-supplemented poultry feed reduces *Salmonella enterica* serovar Enteritidis colonization in chickens.[30] In the management of livestock waste on the other hand, eugenol has shown effectiveness in the inhibition of fermentation gas production, short-chain volatile fatty acids, and lactate and bacterial pollution in addition to its ability to stimulate lactate formation in cattle and swine waste.[31]

Resistant strains

Antibacterial effects of eugenol on multidrug-resistant bacteria also exhibited promising results. For instance, the *Salmonella* spp., *Salmonella typhimurium* SGI 1 (tet A), a Gram-negative bacterium that notably causes diseases of the intestines, is known to show resistance toward ampicillin, tetracycline, penicillin, bacitracin, erythromycin, and novobiocin (FIC <0.4) and nalidixic acid-resistant *Salmonella enteritidis*.[32] In contrast, the effects of eugenol on the aerotolerant Gram-positive *Streptococcus pyogenes* ermB to erythromycin (FIC <0.5) showed synergistic effects with ampicillin, tetracycline, penicillin, erythromycin, and novobiocin.[33]

Mechanisms investigated

To study the antibacterial studies of eugenol, a few discoveries regarding their mechanisms were made. The eradication of bloody diarrhea-causing agents such as *Pseudomonas aeruginosa* and enterohemorrhagic *Escherichia coli* was also possible as eugenol was found to successfully reduce the production of pyocyanin and PQS as well as inhibit the EHEC biofilm formation, respectively.[34] Eugenol was proven to possess the potential of a favorable target in the antibacterial area as it has the capabilities of downregulating YidC, a highly conserved bacterial protein which plays a vital role in membrane protein insertion.[35] In addition, eugenol showed competence in the inhibition and eradication of biofilms produced by methicillin-resistant and sensitive *Staphylococcus aureus*.[36] Another supporting study demonstrated membrane disruption in the bactericidal activity of *E. coli, Listeria monocytogenes,* and *Lactobacillus sakei*; this was achievable via the inhibition of their respective ATPase.[37] It is also believed that eugenol decreases the virulence factors that are produced by *E. coli* such as VT1 and VT2.[38] Another interesting finding involves the ability of eugenol to inhibit the FtsZ assembly that leads to the disruption of bacterial cell division.[39] It was also found that the effects of eugenol in downregulating the transcription of genes associated with *Acinetobacter baumannii* biofilm production contributed to the source of biofilm inhibition as well as disrupting the biofilm architecture.[40]

Table 1: Bacteria that are susceptible to eugenol in *in vitro* experimental models

Strain	Gram-stain	Experimental model
E. coli	Gram-negative	*In vitro* cultures - liquid and vapor phase[108]
P. fluorescens		
S. aureus	Gram-positive	
L. plantarum		
B. subtilis		
S. epidermis	Gram-positive	*In vitro* cultures - permeability studies[109]
S. pneumonia	Gram-positive	*In vitro* cultures - microdilution[110]
P. vulgaris	Gram-negative	*In vitro* cultures - antioxidant potential tests[111]
S. typhi		
B. bronchiseptica		
B. cereus	Gram-positive	
S. mutans		
S. typhi	Gram-negative	*In vitro* cultures - Disc diffusion method[27]
E. coli	Gram-negative	*In vitro* cultures - MIC detection[99]
Salmonella		
P. aeruginosa	Gram-negative	*In vitro* cultures[34]
S. aureus	Gram-positive	*In vitro* cultures - MIC detection[112]
E. coli	Gram-negative	
C. sakazakii	Gram-negative	*In vitro* cultures[113]
C. malonaticus		
P. mirabilis	Gram-negative	*In vitro* cultures - MIC detection[114]
H. pylori	Gram-negative	*In vitro* cultures - MIC detection[115]

E. coli=Escherichia coli, P. fluorescens=Pseudomonas fluorescens, S. aureus=Staphylococcus aureus, L. plantarum=Lactobacillus plantarum, B. subtilis=Bacillus subtilis, S. epidermis=Staphylococcus epidermidis, S. pneumonia=Streptococcus pneumoniae, P. vulgaris=Proteus vulgaris, S. typhi=Salmonella typhi, B. bronchiseptica=Bordetella bronchiseptica, B. cereus=Bacillus cereus, S. mutans=Streptococcus mutans, P. aeruginosa=Pseudomonas aeruginosa, C. sakazakii=Cronobacter sakazakii, C. malonaticus=Cronobacter malonaticus, P. mirabilis=Proteus mirabilis, H. pylori=Helicobacter pylori

Synergistic effect

In addition to eugenol being used exclusively as a singular antibacterial agent, it has also successfully shown a synergistic effect with a few well-known antibacterial medications against various strains of bacteria as tabulated in Table 2.

ANTIFUNGAL ACTIVITY

Like antibacterial agents, new and potent antifungal agents are also invariably and actively discovered. Despite the discovery of many new antifungal agents which have greatly improved the treatment of invasive mycoses, these newer antifungal agents still face challenges as they present toxicity associated with long-term use;[41] therefore, the discovery of new antifungal agents with better safety profiles is highly driven by the side effects of current conventional antifungal medication and fungal resistance.[42]

Eugenol has exhibited significantly favorable antifungal activity on various preformed biofilms, adherent cells, subsequent biofilm formation, and cell morphogenesis of *Candida albicans*[43,44] without

Table 2: The synergistic effect of eugenol with current medications in combating bacteria

Strain	Gram stain	Current medication/ compound	Type of inhibition
S. pneumonia[110]	Gram-positive	Penicillin	Bactericidal
S. pyogenes ermB to erythromycin[33]	Gram-positive	Ampicillin Tetracycline Penicillin Erythromycin Novobiocin	Bactericidal
L. innocua[39]	Gram-positive	Carvacrol Thymol	Bactericidal
E. sakazakii[116]	Gram-negative	Nisin	Bacteriostatic
B. subtilis[117]	Gram-negative	Nisin	Bactericidal
L. innocua[117]	Gram-positive		
E. coli[118]	Gram-negative	Cinnamaldehyde Thymol Carvacrol	Bactericidal
S. typhimurium[119]	Gram-negative	Nalidixic acid	Biofilm eradication
E. coli[120]	Gram-negative	Potassium sorbate	Bacteriostatic
L. innocua[120]	Gram-positive		
S. typhimurium[120]	Gram-negative		
S. aureus[120]	Gram-positive		

S. pneumonia=Streptococcus pneumoniae, S. pyogenes=Streptococcus pyogenes, L. innocua=Listeria innocua, E. sakazakii=Enterobacter sakazakii, B. subtilis=Bacillus subtilis, E. coli=Escherichia coli, S. typhimurium=Salmonella typhimurium, S. aureus=Staphylococcus aureus

instigating hemolytic activity in human erythrocytes.[43] The battle to deal with fungal infections in its numerous guises has been on-going for the past few decades. The assortment of strains of fungus that are currently proven to be susceptible to eugenol is summarized in Table 3.

Resistant strains

Remarkably, eugenol displayed tremendous fungicidal activity against isolates of pathogenic yeasts that have shown resistance toward azoles, and this was due to the inhibition of H^+-ATPase activity.[45] Antigonorrheal activity was also shown in a number of multi-resistant strains of *Neisseria gonorrhoeae*.[46] Interestingly, eugenol was found to be more active against fluconazole-resistant *Candida dubliniensis* than synthetic antiseptic chlorhexidine gluconate, cetylpyridinium chloride, and triclosan.[47]

Mechanisms involved

It is believed that cell wall alteration contributes to the attainment in antifungal activity of eugenol against *Saccharomyces cerevisiae*.[48] The fungicidal causative effects of eugenol in *C. albicans* are mainly due to the disruption of membrane integrity[44] as well as significantly weakening the defense system through free radical cascade-mediated LPO, which subsequently leads to membrane lesions.[49] Interestingly, the interference with amino acid permeases contributes to the inhibitory effect of eugenol. A study has shown inhibitory effects of eugenol on two permeases (Tat1p and Gap1p) that are responsible for the transport of amino acids through the yeast cytoplasmic membrane.[50] In addition, the synergistic antifungal effect of eugenol with cinnamaldehyde is a result of the interference of fungal cell wall synthesis as well as cell wall destruction in addition to a radical scavenging effect.[51]

Synergistic effect

The synergistic effects of eugenol with currently available antifungal drugs and other compounds are listed in Table 4.

Table 3: Fungus strains that are susceptible to eugenol

Fungus strain	Experimental model
C. albicans	*In vivo* immunosuppressed rats' vaginas[121] *In vivo* immunosuppressed oral candidiasis[121,122]
Aspergilli	*In vitro* cultures[123]
A. niger	
A. terreus	
E. nidulans	
Penicillium	
P. expansum	
P. glabrum	
P. italicum	
Fusaria	
F. oxysporum	
F. avenaceum	
F. verticillioides	*In vitro* and in artificially infected kernels[124]
Fluconazole-sensitive *Candida* isolates	*In vitro* antifungal activity[125]
Fluconazole-resistant *Candida* isolates	
S. cerevisiae	*In vitro* cultures[48]
T. mentagrophytes	Irreversible cellular disruption in *in vitro* cultures[126]
L. betulina	*In vitro* cultures[127]
L. sulphureus	
T. rubrum	*In vitro* cultures[128]
Onychomycosis strains	*In vitro* cultures[129]
Candida spp.	*In vitro* cultures[130]
M. canis	
A. fumigatus	*In vitro* cultures[131]
P. citrinum	*In vitro* culture media and different Spanish cheeses[132]
A. fumigatus	*In vitro* zone inhibition cultures[133]
A. niger	
A. flavus	
A. parasiticus	*In vitro* cultures[134]
C. albicans	*In vitro* culture[135]

A. niger=Aspergillus niger, A. terreus=Aspergillus terreus, E. nidulans=Emericella nidulans, P. expansum=Penicillium expansum, P. glabrum=Penicillium glabrum, P. italicum=Penicillium italicum, F. oxysporum=Fusarium oxysporum, F. avenaceum=Fusarium avenaceum, F. verticillioides=Fusarium verticillioides, S. cerevisiae=Saccharomyces cerevisiae, T. mentagrophytes=Trichophyton mentagrophytes, L. betulina=Lenzites betulina, L. sulphureus=Laetiporus sulphurous, T. rubrum=Trichophyton rubrum, M. canis=Microsporum canis, A. fumigatus=Aspergillus fumigatus, P. citrinum=Penicillium citrinum, A. flavus=Aspergillus flavus, C. albicans=Candida albicans, A. parasiticus=Aspergillus parasiticus

ANTIVIRAL ACTIVITY

As a potent natural product, eugenol is heavily investigated to understand its biological activity and therapeutic potential as an antimicrobial agent. There is also the synergistic effect between eugenol and acyclovir in the inhibition of herpes virus *in vitro*; on its own, topical application of eugenol was found to delay the growth of herpes virus-induced keratitis in mouse models.[38] Eugenol displayed antiprotozoal activity against *Leishmania*, which is an assembly of diseases responsible for a wide spectrum of clinical manifestations,[52] ovicidal activity against *Haemonchus contortus*, a parasite that resides in the gastrointestinal tract[53] and virucidal activity against HSV-1 and HSV-2 viruses.[54,55]

ESSENTIAL OILS

Eugenol essential oil can be extracted from plant sources, such as cloves, cinnamon, tulsi, and pepper[56-58] as well as the leaves of *Lippia multiflora*, *Mentha piperita,* and *Ocimum basilicum* from Burkina Faso.[59] Eugenol is commonly the main constituent in these plants, and this essential oil has

Table 4: The synergistic effect of eugenol with current medication in combating fungi

Strain	Current medication/ compound	Type of inhibition
C. albicans	Thymol	Inhibits colonisation and infectiousness[135]
C. albicans	Amphotericin B	Antifungal and suppress the effects of AMPH treatment[44,136]
L. sulphureus	Cinnamaldehyde	Against wood decay fungi[51]
C. albicans	Fluconazole	Antifungal[44,125,137]

C. albicans=Candida albicans, L. sulphureus=Laetiporus sulphureus

prospects as a nutraceutical. Among many beneficial effects induced by eugenol such as anti-inflammation, anti-hyperglycemia and anticancer, it is also a highly potent antimicrobial agent.[21,60]

Antibacterial

Eugenol essential oil showed potent anthelmintic activity in a *Caenorhabditis elegans* model.[61] A few essential oils extracted from flowering plants showed antibacterial activity. For instance, eugenol essential oil is extracted from *Syringa oblata* flower buds against *Ralstonia solanacearum*[62] and *Syzygium aromaticum* flower buds against *Leishmania donovani*.[63]

The essential oil of eugenol and its interaction with ten antibiotics of hydrophobic and hydrophilic characteristics were studied against Gram-negative bacteria.[64]

Antifungal

As previously discussed, eugenol essential oil conferred antifungal activity against *Fusarium oxysporum* f. sp. *Lycopersici* 1322, which suggests that eugenol can be used in preventive and therapeutic applications.[65] Eugenol extracted from the essential oil of *Piper divaricatum* showed activity against cladosporioides and *Cladosporium sphaerospermum*[66] while essential oil from *Nephrolepis exaltata* and *Nephrolepis cordifolia* showed antibacterial and antifungal activities.[67] The eugenol-containing essential oil extracted from *Cinnamomum verum*, *S. aromaticum, Cymbopogon* citratus and *Cymbopogon martini* were found to transform the hyphal ultrasound and virulence factors of *Aspergillus fumigatus* and *Trichophyton rubrum*.[68] Eugenol from *Ocimum sanctum* showed activity against *Aspergillus flavus* NKDHV8 that causes biodeterioration of food stuff.[69] It was also reported that volatile oil of *Cinnamomum zeylanicum Blume*'s leaves and barks showed fungal inhibitory activity against *A. flavus, Aspergillus ochraceus, Aspergillus niger, Aspergillus terreus, Penicillium citrinum*, and *Penicillium viridicatum*. The antifungal activity of eugenol-containing essential oils is summarized in Table 5.

FORMULATIONS

Beyond its role as a simple antimicrobial agent, eugenol was investigated in numerous formulations. Eugenol/cyclodextrin inclusion complexes encapsulated in electrospun polyvinyl alcohol nanofibers were shown to have enhanced thermal stability and subsequently result in the slow release of eugenol.[70] Eugenol-containing microemulsion can be prepared by simply solubilizing the eugenol (0.75–1.5 wt.%) essential oil in surfactant micelles (Surfynol 465; 5–10 wt. %).[71] In addition, findings from another study reported that eugenol-incorporated micelles had higher efficacy than that of pure application of eugenol.[72]

A variety of formulated products that incorporated eugenol were found to be potent. The consecutive application of eugenol and lauric arginate was shown to inhibit the growth of *Staphylococcus carnosus, Listeria innocua, L. monocytogenes, E. coli* (K12 and O157:H7), *Pseudomonas fluorescens*, and *S. enteritidis*.[73,74] Synergistic antimicrobial activity was exhibited in the multi-agent formulation comprising eugenol/beta-pinene/salicylic acid and eugenol/beta-pinene/2-phenoxyethanol/potassium sorbate where the mechanism is facilitated via cellular permeabilization in addition to inhibition of efflux pump activity.[75] Another formulation that yielded higher antimicrobial activity than that of eugenol itself is the inclusion complex between water-soluble β-cyclodextrin-grafted chitosan derivatives (βCD-g-CS) and eugenol to produce a mucoadhesive drug carrier. This formulation has potent antimicrobial activity against *C. albicans, S. oralis*, and *S. mutans*[76] and antifungal activity against *Peronophythora litchi*.[77]

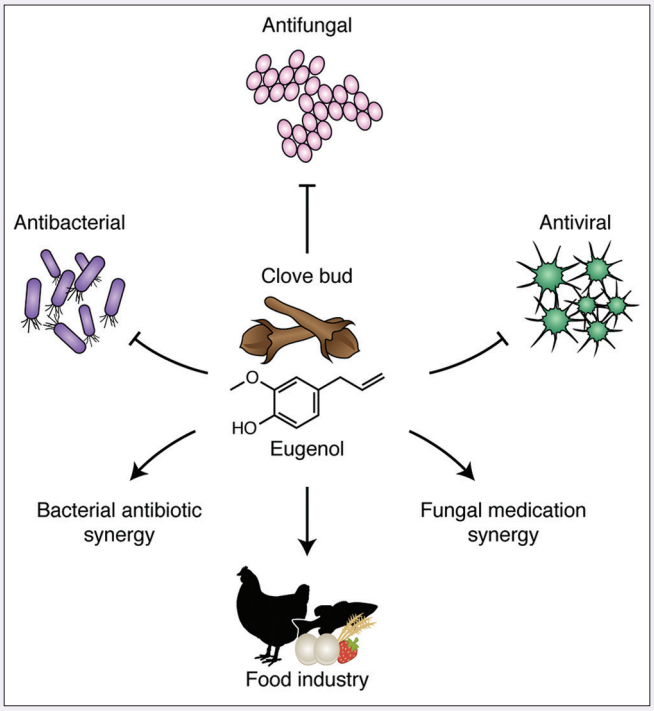

Figure 2: The multifaceted applications of eugenol

Aqueous solubility

Eugenol is sometimes encapsulated as it can improve the water solubility profile; such preparation showed growth inhibition of *E. coli* O157:H7 (H1730, F4546, 932, and E0019) and *L. monocytogenes* (Scott A, 101, 108, and 310).[78] Another way of improving the water solubility profile is by formulating eugenol as nanoparticles in water-based microemulsion systems.[60]

Emulsions

Microemulsions of eugenol inhibited the growth of foodborne pathogens such as *L. monocytogenes, E. coli* (O157:H7 and C 600), and *L. innocua* completely.[112,113] Formulated carbopol hydrogels incorporated with eugenol-loaded solid lipid nanoparticles (EG-SLN) were beneficial for the epidermal treatment in the attempt to treat fungal infection in the skin.[81] Various nanoemulsions with incorporated eugenol were prepared and tested against different pathogens. For example, sesame oil-blended eugenol-loaded nanoemulsion displayed antibacterial activity against *S. aureus,*[82] eugenol-chitosan particles exhibited significant antibacterial activity against *E. coli* and *S. aureus*,[83] and eugenol-beta-cyclodextrin nanoparticles showed antivirulence activity against *E. coli* and *S. aureus*.[84] Dispersion of eugenol in nanocapsules

Table 5: Source of eugenol essential oil extracts and their respective susceptible strains

Source of extracted eugenol essential oil	Common name	Against strains	Biological activity
O. sanctum	Tulsi	*C. elegans*	Anthelmintic activity[61]
S. oblate	Lilac	*R. solanacearum*	Antibacterial activity[62]
S. aromaticum	Clove	*L. donovani*	Antileishmania activity[62]
Clove oil		*F. oxysporum f.* sp. *Lycopersici* 1322	Bio fungicide[65]
Piper divaricatum		*C. sphaerospermum*	Antifungal activity[66]
C. verum	Cinnamon	*A. fumigatus*	Alters hyphal ultrastructure and virulence factors[68]
S. aromaticum	Clove	*T. rubrum*	
C. citratus	Lemon grass		
C. martini	Lemon grass		
O. sanctum	Tulsi	*A. flavus* NKDHV8	Antifungal activity[69]
Cinnamomum zeylanicum Blume	Cinnamon tree leaf and bark	*P. citrinum* *A. flavus* *A. ochraceus* *A. niger* *A. terreus* *P. citrinum* *P. viridicatum*	Antifungal activity[138,139]
N. exaltata *N. cordifolia*	Sword fern Tuber ladder fern	*S. typhimurium* *K. pneumoniae* *S. flexneri* *E. coli* *P. vulgaris* *K. pneumoniae* *S. flexneri*	Antibacterial and antifungal activity[67]

Ocimum sanctum=O. sanctum, S. aromaticum=Syzygium aromaticum, S. oblate=Syringa oblate, C. verum=Cinnamomum verum, S. aromaticum=Syzygium aromaticum, C. citratus=Cymbopogon citratus, C. martini=Cymbopogon martini, O. sanctum=Ocimum sanctum, N. exaltata=Nephrolepis exaltata, N. cordifolia=Nephrolepis cordifolia, C. elegans=Caenorhabditis elegans, C. elegans=Caenorhabditis elegans, R. solanacearum=Ralstonia solanacearum, L. donovani=Leishmania donovani, F. oxysporum=Fusarium oxysporum, C. sphaerospermum=Cladosporium sphaerospermum, A. fumigatus=Aspergillus fumigatus, T. rubrum=Trichophyton rubrum, A. flavus=Aspergillus flavus, A. ochraceus=Aspergillus ochraceus, A. niger=Aspergillus niger, A. terreus=Aspergillus terreus, P. citrinum=Penicillium citrinum, P. viridicatum=Penicillium viridicatum, S. typhimurium=Salmonella typhimurium, S. flexneri=Shigella flexneri, E. Coli=Escherichia coli, P. vulgaris=Proteus vulgaris, K. pneumoniae=Klebsiella pneumoniae

formulated with conjugates of whey protein isolate and maltodextrin also showed potency against *E. coli* O157:H7 strains ATCC 43889 and 43894 and *L. monocytogenes* strains Scott A and 101.[85] Encapsulation of eugenol in poly (DL-lactide-co-glycolide) (PGLA) nanoparticles enhanced its antimicrobial delivery against the growth of *Salmonella* spp and *Listeria* spp.[86] Solid lipid nanoparticles (SLN) loaded with eugenol showed *in vivo* antifungal activity in immunosuppressed rats of oral candidiasis.[87] A gum Arabic and lecithin-eugenol incorporated nanoemulsion prepared as a food-grade natural emulsifier successfully displayed antimicrobial activity against *L. monocytogenes* and *S. enteritidis*.[88]

The significant leishmanicidal activity of eugenol against promastigotes and intracellular amastigotes of *L. donovani* carried out *in vitro* and *in vivo* was shown when it was formulated in an oil-in-water emulsion.[89] In addition, eugenol emulsions showed antibacterial activity against *Xanthomonas campestris* pv. *Phaseoli* var. *fuscans*.[90] The eugenol-incorporated formulations reported in the literature and their respective antimicrobial activities are summarized in Table 6.

APPLICATIONS IN THE FOOD INDUSTRY

Productive applications in science stem from the ability to connect unanticipated observations to develop new areas of explorations – the usage of eugenol in the food industry for instance. From the identification of eugenol as a potent antimicrobial agent, it has been gradually introduced to the food industry [Table 7]. Some of the most prevalent foodborne pathogenic bacteria affected by the antibacterial activity of eugenol include *S. aureus, E. coli, S. enterica* serovar *Typhimurium*, and *L. monocytogenes*[91-93] and fungi comprising *Cladosporium* spp. (MIC: 100 mug/mL), *Aspergillus* spp. (MIC: 100 mug/mL), *Cladosporium* spp. (MIC: 350 mug/mL), and *Aspergillus* spp. and *Cladosporium spp*. as shown in Table 2.[98]

Plants and grains

The potency of eugenol as a prospective biocontrol agent in grains was suggested as it contributes to the inhibition of ochratoxin A production caused by *A. ochraceus* – a frequent contaminant found in the storage of grains.[94] Another possibility is to diminish the population of *Salmonella* in soil to reduce probable contamination of fresh organic produce.[95]

Livestock

The broad spectrum of eugenol's protective effects can be observed in various foodborne pathogens. Eugenol worked against the common nalidixic acid-resistant *S. enteritidis* found in contaminated eggs[32,96,97] and it was found to inhibit the growth and colonization in the chicken reproductive tract.[98] In a more general application, eugenol was capable of enhancing food safety and stability without instigating any bacterial adaptation of *S. enterica* serovar *Typhimurium*.[99] Treatment and prevention of bacterial diseases in fish is also possible as eugenol is active against a fish pathogen called *A. hydrophila* without posing toxicity effects to the fish.[100]

Fruits

In the fruit sector, eugenol has been commonly used as fungicides. Eugenol was found to inhibit the growth of fungi that causes decay of strawberries.[101] Eugenol oil inhibited the growth in both *in vitro* and *in vivo* tests of numerous apple fungus, namely *P. vagabunda, P. expansum, B. cinerea*, and *Monilinia fructigena*.[102] A few fungi such

Table 6: Summary of current eugenol-incorporated formulations in the enhancement of antimicrobial activity

Strain	Gram stain/ identity	Formulation	Experimental model
L. monocytogenes	Gram-positive	Microemulsion	*In vitro* cultures[79,80]
E. coli (O157:H7 and C 600)	Gram-negative		
L. innocua	Gram-positive		
S. aureus	Gram-positive	Sesame oil blended eugenol-loaded nanoemulsion	*In vitro* cultures[82]
E. coli	Gram-negative	Eugenol-chitosan particles	*In vitro* cultures - DPPH[83]
S. aureus	Gram-positive		
E. coli	Gram-negative	Eugenol-beta-cyclodextrin nanoparticles	*In vitro* cultures - MIC and agar diffusion[84]
S. aureus	Gram-positive		
E. coli O157:H7 strains ATCC 43889 and 43894	Gram-negative	Eugenol in nanocapsules formulated with conjugates of whey protein isolate and maltodextrin	*In vitro* cultures - MIC and MBC[85]
L. monocytogenes strains Scott A and 101	Gram-positive		
Salmonella spp.	Gram-negative	Encapsulation of eugenol in poly (DL-lactide-co-glycolide) (PGLA) nanoparticles	*In vitro* cultures[86]
Listeria spp.	Gram-positive		
Candida spp.	Fungus	SLN loaded with eugenol	*In vivo* immunosuppressed rats of oral candidiasis[87]
L. monocytogenes	Gram-positive	Gum Arabic and lechitin-eugenol incorporated nanoemulsion	*In vitro* cultures[88]
S. enteritidis	Gram-negative		
L. donovani	Parasite	Eugenol in an oil-in-water emulsion	*In vitro* cultures
			In vivo - Intraperitoneal administration to 8 weeks-infected BALB/c mice[89]

L. donovani=Leishmania donovani, E. Coli=Escherichia coli, L. monocytogenes=Listeria monocytogenes, L. innocua=Listeria innocua, S. aureus=Staphylococcus aureus, S. enteritidis=Salmonella enteritidis, SLN=Solid lipid nanoparticles, MIC=Minimum inhibitory concentration, MBC=Minimum bactericidal concentration, DPPH= 2,2-diphenyl-1-picrylhydrazyl assay

Table 7: List of pathogens that are susceptible to the antimicrobial activity of eugenol

Strains	Application	Biological activity
Bacteria		
L. plantarum	Fruit juice	Antibacterial activity[104]
L. brevis		
B. coagulans		
Nalidixic acid resistant *S. enteritidis*	Contaminated eggs Chicken reproductive tract	Antibacterial activity[32,96] Inhibits the growth and colonization[98]
A. hydrophila	Fish	Treats and prevents bacterial diseases in fish[100]
Fungus		
S. bayanus	Fruit juice	Partial inhibition[104]
R. bacarum		
P. vagabunda	Apples	Fungicidal[102]
P. expansum		
B. cinerea		
M. fructigena		
A. ochraceus	Grains	Inhibits Ochratoxin A production[94]

A. ochraceus=Aspergillus ochraceus, S. enteritidis=Salmonella enteritidis, L. plantarum=Lactobacillus plantarum, L. brevis=Lactobacillus brevis, B. coagulans=Bacillus coagulans, A. hydrophila=Aeromonas hydrophila, S. bayanus=Saccharomyces bayanus, R. bacarum=Rhodotorula bacarum, P. vagabunda=Phlyctena vagabunda, P. expansum=Penicillium expansum, B. cinerea=Botrytis cinerea, M. fructigena=Monilinia fructigena, A. ochraceus=Aspergillus ochraceus

as *Sclerotinia sclerotiorum* (Lib.), *R. stolonifera* (Ehrenb. ex Fr.) *Vuill*, and *Mucor spp.* (Fisher) cause deterioration and decay in peaches especially during marketing, shipping, and storage; eugenol works synergistically with linalool as a potent fungicide.[103]

In a study looking at the type of microflora that are responsible in fruit juice spoilage, eugenol was also biologically active against foodborne bacteria such as *L. plantarum, L. brevis*, and *B. coagulans* and foodborne yeasts such as *Saccharomyces bayanus, Pichia membranifaciens*, and *Rhodotorula bacarum*.[104]

Shelf life

Eugenol is incorporated into polyhydroxybutyrate-based antimicrobial films as an approach to enhance the shelf life of food.[105] As a preservative, positive results were also observed against *S. cerevisiae* and *Zygosaccharomyces bailii*.[106]

Certain results suggest a mechanism that involves the inhibition of tumor necrosis factor-inducing and hemolytic activities of *S. aureus* supernatants by reducing the production of staphylococcus enterotoxin A and B and toxic shock syndrome toxin 1 as well as the expression of alpha-hemolysin.[107]

Acknowledgement

The authors would like to thank the International Medical University for the facilities and the Ministry of Higher Education for their financial support.

Financial support and sponsorship

The study was funded by the ERGS grant from the Ministry of Higher Education of Malaysia (ERGS/1/2012/STG01/IMU/02/1).

Conflicts of interest

There are no conflicts of interest.

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