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Review on Estimation of Eugenol in Herbal Plants

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Abstract
Eugenol is used as a flavor in the food industry, has a variety of biological activity, and can serve as a biomarker. Because eugenol is present in the leaves of Ocimum, which are used as a herbal medicines, a sensitive and reliable quantitative Ultra-violet spectroscopy and high-performance liquid chromatographic method has been established for quantification of the compound in the leaves of the plant. A methanol extract of the powder of dried leaves of Ocimum, was spotted on the Merck aluminium plate precoated silica gel F254 with 0.2 mm thickness. Meoh:Chloroform (95:5) and MeOH: H2O: ACN (50:25:75) used as mobile phase to isolate the eugenol and to prepare the sample for UV and HPLC analysis respectively. The UV and HPLC method proposed for the quantitative monitoring of eugenol in Ocimum leaf powder is rapid, simple, and precise. Hence from that UV and HPLC analysis it was conclude that the sanctum linn contains higher amount of Eugenol.

Keywords: Ocimum; Ultra-violet spectroscopy; high-performance liquid chromatography; Merck aluminium plate precoated silica gel.

Introduction
The spice known as clove is the dried flower bud of the clove tree, Eugenia Caryophylata. Eugenol is derived from the species name Eugenia Caryophyllata which contains high level of eugenol (45-90%). Clove has been used in ancient China as spice and fragrance. In Chinese traditional medicine, clove oil, has been used as carminative, antispasmodic, antibacterial and antiparasitic agent, while, the buds were used to treat dyspepsia, acute, chronic gastritis and diarrhea. Several scientific studies have been carried out on E.Caryophylata oil and its main volatile constituent eugenol, revealing pharmacological properties such as anesthetic, analgesic, antimicrobial, antioxidant, antiinflammatory, and anticonvulsant, anticarcinogenic, antimutagenic, repellant and antifumigant activities. Eugenol and its derivatives have been used in medicine as local antiseptic and anesthetic and in perfumeries and flavorings. Eugenol is also suggested to be a beneficial antioxidant. In dentistry, it is used in combination with zinc oxide for surgical dressing, temporary fillings, and caving liners. Eugenol is also used in food industry in restricted concentrations. FDA has approved clove oil for use in food as a flavoring agent. Eugenol has been classified as “generally recognized as safe (GRAS)’ by the U.S. Food and Drug Administration. However, in spite of extensive use and availability of clove oil, cytotoxicity and genotoxicity studies of eugenol is lacking. The aim of this study is to investigate the cytotoxic and genotoxic effects of eugenol. For toxicity V79 cells and Neutral Red Uptake Assay are used as an in vitro cytotoxicity test. Single cell Gel Electrophoresis (Comet) assay and Micronucleus assay are used as genotoxicity tests. Genotoxicity studies are carried in lymphocytes.

Uses of eugenol.
Clove has been used in medicine since ancient times. In traditional Chinese medicine clove oil has been used as an antimicrobial, antispasmodic and anti-parasitic agent. In the United States, clove oil has been marketed as a dental analgesic and antiseptic, a flavoring agent in food, mouthwashes, and pharmaceutical products, and also as an ingredient in aromatherapy. Eugenol is also used as fragrance and flavoring agent and as an insect repellent (1). Eugenol and its derivatives have been used in medicine as local antiseptic and anesthetic and in perfumeries and flavorings. They are used in the formulation of insect repellents and UV

absorbers, analgesics, biocides, and antiseptics (3).
Eugenol is also used in food industry such as ice cream, baked goods and candy in restricted concentrations. Although the first natural compound which used in the synthesis of vanillin was eugenol, nowadays vanillin is produced from lignin or phenol. Eugenol is also used as an industrial source in the production of isoeugenol and methyleugenol (6). Eugenol has also been shown to enhance skin penetration of various drugs. This agent is widely used in agricultural applications to protect foods from microorganisms such as *Listeria monocytogenes* and *Lactobacillus* during storage, as a pesticide and fumigant (7).Eugenol has been used to treat skin infections and digestive disorders. Ingested eugenol is also a beneficial antioxidant. In moderate amounts, some reports suggest that excessive doses of undiluted oil can cause symptoms. In fact, an excessive dose of eugenol was considered as a poison (2,7). The US Food and Drug Administration (FDA) approved clove oil for use in food as a flavoring agent, in dentistry as an analgesic and in dental cements, as a fragrance in personal care products and in aromatherapy oils (8). In the United States, eugenol and clove oil are generally recognized as safe (GRAS) food additive and have been approved for use in foods and dental products. Eugenol is also approved for use in the manufacture of textiles and textile fibers that contact food surfaces. Additionally, eugenol and clove oil are approved for use as fragrance (9, 10).

Agricultural applications

New potential safe strategies for control of postharvest decay in crops are needed due to the problems related to synthetic fungicides. Postharvest diseases cause heavy losses of fruits during storage. Species such as Phlyctema vagabunda, Penicillium expansum, Monilia fructigena and Botrytis cinerea are reported to damage apples in many regions of the world. The in vitro and in vivo activities of two eugenol formulations (eugenol-Tween®; eugenol-ethoxylate) against the four apple pathogens revealed growth inhibition of the pathogens incorporated in malt extract agar medium with a minimum inhibition concentration (MIC) value of 2 mg/ml. In addition, the mycelia growth of the four test pathogens was completely inhibited when treated with 150 µl/L of volatile eugenol (11) Combrinck and et al (12), investigated the effects of eugenol on various pathogens causing postharvest decay of fruits. The lowest concentration required achieving 100% inhibition for Lasodiplodia Theobromae, Alternaria citri, Penicillium Digitatum and B. Cinerea was 500 µM/L. Studies were conducted to determine the ability of eugenol to control spore germination of Alicyclobacillus acidoterrestris. The results indicated that spore germination could be inhibited through the use of 80 ppm of eugenol or alternatively through the combination of 40 ppm of eugenol with 20 ppm of cinnamaldehyde. The effect of eugenol alone and in combination with cinnamaldehyde against the wood decay fungi, white-rot fungus and brown-rot fungus was also evaluated using the MIC method which involved serial dilutions of the compound with sterilized potato dextrose agar. Eugenol exhibited good activity against white-rot fungus. Synergistic interactions were noted when eugenol and cinnamaldehyde were combined in a 1:1 ratio. This synergistic effect was attributed to the interference in fungal cell wall synthesis and cell wall destruction in addition to a radical scavenging effect. The combination of eugenol (0.5 mg/ml) and thymol (0.125 mg/ml) was found to induce a significant increase in the number of damaged cells in comparison to the corresponding single concentration of the two molecules after 4 hours incubation period (13). Inhibition of the wheat seed germination by clove oil was also investigated and eugenol was found to be responsible for its strong inhibitory activity (14). In recent years natural insecticides have been developed due to the global concern about air pollution because of the use of synthetic insecticides. Crude essential oils and some of their constituents have been identified as a source of natural pesticides. The repellent effects and fumigant potency of Ocimum gratissimum oil (64% of methyleugenol) and eugenol were evaluated against the rice weevil, one of the most severe stored-grain pests worldwide, named Rust Red Flour Beetle, and the Chinese Bean Weevil. The results showed that fumigant activity and repellency of the oil and eugenol were significantly influenced by concentration and time after treatment (15).

The composition, structure, sources, and applications of eugenol

What do cloves, cinnamon, nutmeg, and basil have in common? Maybe you recognize each of them as common culinary herbs, which they are, but there is actually a characteristic molecule that is found to some extent in each, referred to as eugenol. Eugenol is an organic molecule consisting of a six carbon ring of alternating double-bonds; two oxygen atoms are singlybonded to carbons in the ring, one of the oxygen atoms is single-bonded to another carbon with three hydrogen atoms and the other is bonded only to hydrogen; another carbon atom is singly-bonded to a remaining carbon in the ring, which is bonded to two other carbons each of which are also bonded to hydrogen atoms. Whew, that was a lot of words. The best way to understand how the atoms fit together is to see its skeletal formula and a ball and stick model, each of which are in the public domain and can be found on Wikipedia [1], but are shown in the margin for convenience. It is considered a phenylpropene molecule [1] , the "phenyl" part of the word meaning that it contains a modified benzene ring (6 carbon atoms with hydrogen normally attached to each) with an allyl group attached (a side group H2C=CH-CH2R, where R is the rest of the molecule [2]). The terminology given and how all this fits together will be interesting to learn about in organic chemistry. Its molecular formula is C10H12O2. Cloves, cinnamon, nutmeg, and basil are some of the well-known natural (plant) sources of eugenol. It varies in concentration in each type of plant from which it can be extracted as an essential oil, which is said to have the "essence" of the plant that gives it its taste and smell. For instance, the essence of cloves can be generally extracted using an organic solvent such as olive oil which will dissolve some of the eugenol in the plant matter [3]. This would work given the principle of like molecules dissolving like molecules. The smaller the plant can be broken up the better, which increases the surface area of the particles being dissolved. The olive oil will have the essence of cloves, but this is not pure eugenol.

Fig. 1: Pictured are the spices cloves, cinnamon, and nutmeg with a basil plant.

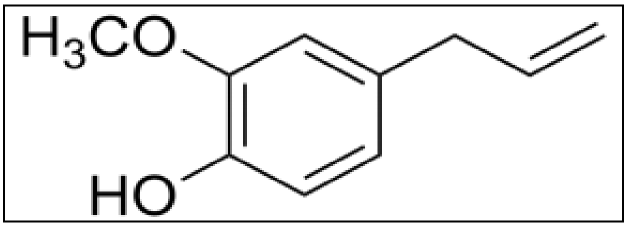

Fig. 2: Skeletal formula of eugenol.

To further refine the plant towards pure eugenol, distillation and selective precipitation processes would need to be employed. If purification was needed, distillation would take the place of the olive oil. Eugenol has a boiling point of 254°C and can be steam distilled from freshly ground clove, which sounds like a fun organic chemistry experiment (there looks to be several example experiments available online) [4]. Cloves' distinctive taste is said to carry the flavor of eugenol [5] , which makes sense given the consistently high concentration of eugenol in cloves, which is on the order of 72-89% in the extract [6][7]. The cloves that we eat come from the flower part of an evergreen tree, Syzygium aromaticum, native to Southeast Asia [8]. The flower is dried (see figure 5) and then often ground to a powder to be paired with compatible foods, like coupled with apple cider and cinnamon! The distinctive and familiar flavor of cinnamon is not due to eugenol, which has a very different taste than cloves. This may be due to the part of the plant we eat, which is from the inner bark of a tree (see figure 6), as eugenol is said to be found primarily in the leaves of a cinnamon tree [9]. There is also a lot of variation in the genus Cinnamomum that seems to have a large affect on the concentration of eugenol found in its leaves, from trace levels to about 80% in its essential oil [10]. Given the general association that eugenol tastes like cloves, when compared to cinnamon or to even nutmeg and basil which generally contain smaller amounts of eugenol, the vastly different flavors signify that the differences in taste are due to other chemicals present within the parts of the plants we eat. In light of the information we have such as the relatively consistently high concentration of eugenol in cloves, and using taste as a guide, it seems that the most efficient source of eugenol is cloves.

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. In conclusion, the results of this study suggest that eugenol might have cytotoxic effects in a dose dependent manner. However, eugenol in the concentrations used below the IC_{50} values showed no significant genotoxic effects. Our results of MN assay also showed that eugenol might protect against H_2O_2-induced genotoxicity. As our study is composed only an *in vitro* experiments, further *in vivo* animal studies are required to understand the genotoxic and antigenotoxic properties of eugenol in detail.

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