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Synthesis and Characterization of Eugenol Derivatives

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Dedication

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Introduction

Clove (Syzygium aromaticum L. Myrtaceae) is an aromatic plant widely cultivated in tropical and subtropical countries, rich in volatile compounds and antioxidants such as eugenol, β- caryophyllene, and α-humulene. Clove essential oil has received considerable interest due to its wide application in the perfume, cosmetic, health, medical, flavoring, and food industries. Clove essential oil has biological activity relevant to human health, including antimicrobial, antioxidant, and insecticidal activity. The impacts of the extraction method (hydrodistillation, steam distillation, ultrasound-assisted extraction, microwave-assisted extraction, cold pressing, and supercritical fluid extraction) on the concentration of the main volatile compounds in clove essential oil and organic clove extracts are shown. Eugenol is the major compound, accounting for at (76.8 %) . The remaining consists of (1.2 %)eugenyl acetate,(17.4 %)β-caryophyllene, and(2.1%)α- humulene. The main biological activities reported are summarized. Furthermore, the main applications in clove essential oil in the food industry are presented. This review presents new biological applications beneficial for human health, such as anti-inflammatory, analgesic, anesthetic, antinociceptive, and anticancer activity. This review aims to describe the effects of different methods of extracting clove essential oil on its chemical composition and food applications and the biological activities of interest to human health. Banerjee and coworkers observed the anti-inflammatory and wound healing ability of a clove oil emulsion in murine experiments. Eugenol-treated skin showed re-epithelialization 20 days after the wound. This result was similar to that of a diclofenac gel and a neomycin cream currently used to control inflammation and heal wounds Other research reported that eugenol did not modify interleukin 8 (IL-8) levels in human skin cells (HaCat) but instead targeted pro-inflammatory cytokines . The inhibition of voltage-gated Na+ channels modulate the analgesic effects of eugenol. Eugenol induces the activation of transient receptor potential cation channel V1 (TRPV1), an effect similar to local anesthetics such as lidocaine. Eugenol has shown potential anticancer activity against colon, gastric, breast, prostate, and skin cancer, as well as melanoma and leukemia. Eugenol inhibits tumor proliferation and formation, increases reactive oxygen species (ROS), generates apoptosis, and has a genotoxic effect in different They reported that eugenol reached plasma and blood in a half-life of 18–14 h. It also showed a cumulative effect in the treatment of neuropathic pain. Although the Food and Drug Administration (FDA) has confirmed the safety of CEO as a dietary supplement, much attention has recently been paid to its toxicity due to cytotoxic activity against human fibroblasts

and endothelial cells. They also reported that eugenol showed a spermicidal effect in vitro and allergic efficacy when used in dentistry.

CEO Composition

1. Eugenol

Eugenol is a phenylpropanoid compound found in S. aromaticum L., Cinnamomum spp., P. nigrum, Zingiber officinale, Origanum vulgare, and T. vulgaris . Eugenol is a volatile compound that varies from colorless to light yellow and has low water solubility (approximately 2460 mg/L at 25 °C), a strong odor, and an intense flavor. Among the reported biological activities of eugenol are insecticidal, antimicrobial, anti-inflammatory, wound healing, antiviral, antioxidant, and anticancer activity Banerjee and coworkers observed the anti-inflammatory and wound healing ability of a clove oil emulsion in murine experiments. Eugenol-treated skin showed re-epithelialization 20 days after the wound. This result was similar to that of a diclofenac gel and a neomycin cream currently used to control inflammation and heal wounds . Other research reported that eugenol did not modify interleukin 8 (IL-8) levels in human skin cells (HaCat) but instead targeted other pro-inflammatory cytokines . The inhibition of voltage-gated Na+ channels modulate the analgesic effects of eugenol. Eugenol induces the activation of transient receptor potential cation channel V1 (TRPV1), an effect similar to local anesthetics such as lidocaine.

Eugenol has shown potential anticancer activity against colon, gastric, breast, prostate, and skin cancer, as well as melanoma and leukemia . Eugenol inhibits tumor proliferation and formation, increases reactive oxygen species (ROS), generates apoptosis, and has a genotoxic effect in different They reported that eugenol reached plasma and blood in a half-life of 14–18 h. It also showed a cumulative effect in the treatment of neuropathic pain. Although the Food and Drug Administration (FDA) has confirmed the safety of CEO as a dietary supplement, much attention has recently been paid to its toxicity due to cytotoxic activity against human fibroblasts and endothelial cells. They also reported that eugenol showed a spermicidal effect in vitro and allergic efficacy when used in dentistry.

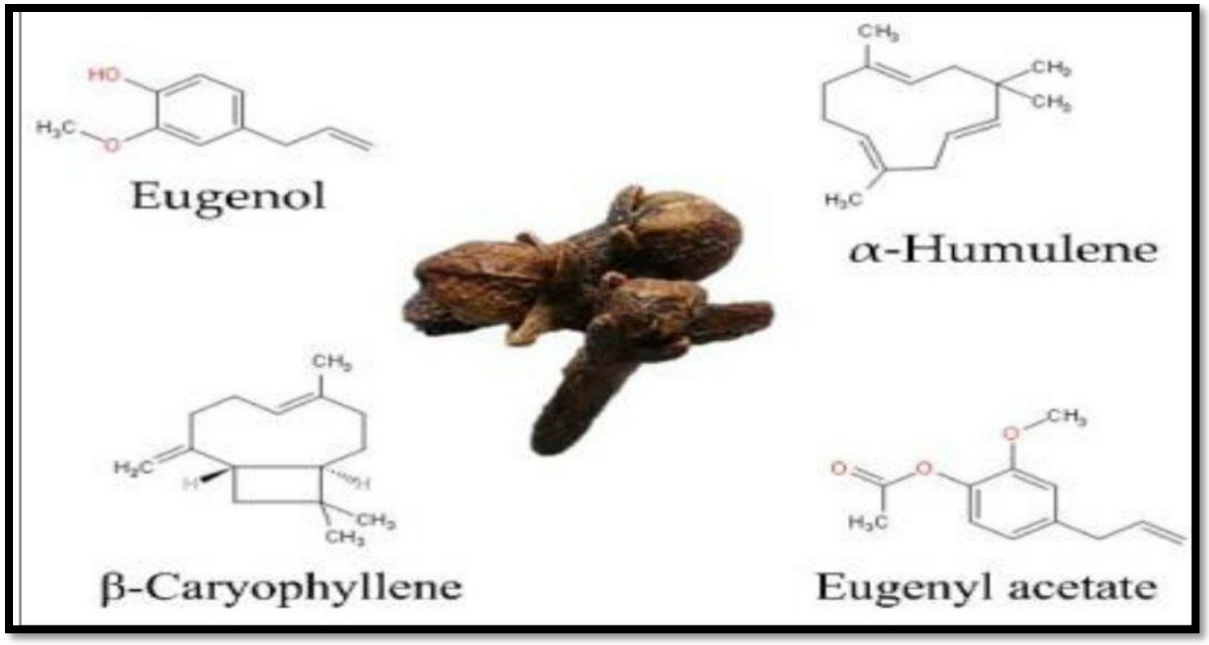

Figure (1): Clove essential oil components.

2. Eugenyl Acetate

Eugenyl acetate is a phenylpropanoid derivative of eugenol that exhibits antibacterial, anticancer, antimutagenic, antioxidant, and anti-virulence activity. It showed inhibition of 94.5, 92.1, and 100% at 200 µg/mL against Fusarium moniliforme, Harpophora oryzae, and Rhizoctonia solani, respectively . Eugenyl acetate has been described as a potent antioxidant agent; it showed 90.30% DPPH free radical scavenging at 35 µg/mL, and 89.30% NO free radical scavenging at 60 µg/mL. It also exhibited potential antifungal activity against Candida spp. and inhibited biofilm formation capacity. Pasay et al. (2010) reported high toxicity against human scabies mites . Eugenyl acetate also showed 100% toxicity against Artemia salina at 0.3 µg/mL. The low lethal concentrations obtained for eugenyl acetate could also indicate toxicity to other organisms, such as disease vector insect larvae . Eugenyl acetate had an LC50 of 0.1 mg/mL against Aedes aegypti, showing potential utility as a larvicide . The larvicidal action is mainly due to interference with the octopaminergic system . The antioxidant, antimicrobial, antitumor, and larvicidal properties have increased its demand in the food and cosmetic industries .

3. β-Caryophyllene

β-Caryophyllene is a sesquiterpene found in clove (S. aromaticum L.), hemp (Cannabis sativa L.), black pepper (P. nigrum L.), Eugenia cuspidifolia, Eugenia tapacumensis, and guava leaves (Psidium cattleianum Sabine) . β-Caryophyllene is insoluble in water but is soluble in ethanol. It has demonstrated antimicrobial, anticarcinogenic, anti-inflammatory, antioxidant, anxiolytic-like, and local anesthetic effects and anticancer properties, including against prostate, breast, pancreatic, skin, leukemia, lymphatic, and cervical cancer . These studies suggest that β-caryophyllene decreases cell growth and proliferation in colon cancer, interfering with the stages of tumor development and reducing the activity of extracellular matrix metalloproteinases. β-Caryophyllene can act as a chemosensitizer, improving the effectiveness of drugs against tumor cells . It is also effective against Anopheles subpictus (LC50 = 41.66 μg/mL), Aedes albopictus (LC50 = 44.77 μg/mL), and Culex tritaeniorhynchus (LC50 = 48.17 μg/mL). Dahham et al. reported that the radical scavenging ability of β-caryophyllene was approximately 1.25 and 3.23 μM by the DPPH and FRAP scavenging methods, respectively. These results indicate that β-caryophyllene has high antioxidant activity.

4. α-Humulene

α-Humulene is a sesquiterpene found in S. aromaticum L., Senecio brasiliensis, Humulus lupulus L., and Salvia officinalis L. This compound has shown anti-inflammatory and antitumor activity in lung, colon, prostate, and breast cancer. Some studies reported that α-humulene demonstrated antiproliferative activity and alteration of the mitochondrial cell membrane in colon cancer cells . It can also improve the antiproliferative effect of cytostatic drugs and other anticancer bioactivities . Nguyen et al. [reported that α-humulene inhibits the activity of the CYP3A enzyme, a drug-metabolizing enzyme in humans' and rats' liver microsomes . Fernandes et al. reported that oral treatment with α-humulene and β-caryophyllene (50 mg/kg) produced comparable anti-inflammatory effects with dexamethasone treatment in model mice and rats. α-Humulene prevents the generation of TNFα, while β-caryophyllene only decreases its release. In addition,

they reduce the production of prostaglandin E2, the inducible expression of nitric oxide synthase, and cyclooxygenase. α-Humulene exhibited larvicidal activity against three vector mosquitoes, An. Subpictus (LC50 = 10.26 µg/mL), Ae. albopictus (LC50 = 11.15 µg/mL), and Cx. tritaeniorhynchus (LC50 = 12.05 µg/mL) but was shown to be safe for Gambusia affinis (LC50 = 1024.95 µg/mL). It showed larvicidal LC50 of 20.86 µg/mL and EC50 of 77.10 µg/mL on Helicoverpa armigera eggs. α-Humulene has also been evaluated against beetle species that attack stored products . The toxicity of α-humulene against Sitophilus granarius was LC50 = 4.61 µL/mL, and it reduced the respiration rate of S. granarius at 1 and 3 h after exposure.

Biological Activities of CEO

CEO has been shown to have different health benefits, mainly due to the eugenol content. However, the other compounds have various health benefits too.

Principal biological activities of CEO.

1. Antimicrobial

CEO has shown broad-spectrum inhibitory activity against pathogens. The antibacterial mechanism has been related to the -OH groups located at the meta and ortho positions, respectively, in the main chemical composition. These functional groups can interact with the cytoplasmic membrane of microbial cells . CEO can permeate through the cell membrane due to its lipophilic properties. The interaction of CEO with polysaccharides, fatty acids, and phospholipids causes loss of cellular membrane integrity, leakage of cellular contents, and interference with proton pump activity, leading to cell death . CEO can inhibit Gram-negative bacteria (E. coli, Salmonella, Klebsiella pneumoniae, Erwinia carotovora, Agrobacterium,

and Pseudomonas aeruginosa) and Gram-positive bacteria (S. aureus, Streptococcus, and L. monocytogenes), Aspergillus (A. flavus, A. parasiticus, and A. ochraceus), Penicillium, C. albicans, and yeast . CEO inhibits Gram-positive bacteria to a greater extent than Gram-negative bacteria. This is attributed to a diffusible mucopeptide layer in Gram-positive bacteria that makes them susceptible to antimicrobial agents. In contrast, the complex layer of lipopolysaccharide in the outer cell membrane of Gram-negative bacteria can significantly reduce the diffusion rate of lipophilic antibacterial compounds through the cell membrane . Likewise, food-related pathogens have shown greater sensitivity to CEO than probiotics and fungi.

2. Antioxidant

CEO has the antioxidant compounds eugenol, eugenyl acetate, β-caryophyllene, and α-humulene, which protect cells from free radical oxidation. Diseases such as cancer, arteriosclerosis, Alzheimer's disease, and Parkinson's disease are related to the presence of ROS compounds . CEO has shown scavenging activity on radicals and inhibition of lipid peroxidation . The hydroxyl group available in eugenol on the aromatic ring is responsible for the antioxidant activity [99]. The phenolic compounds transfer electrons or hydrogen atoms and neutralize them to free radicals, resulting in a blocked oxidative process .

CEO has a protective effect on biochemical changes and histopathological injuries in the kidney, liver, and brain induced by ROS. The main ROS changes inhibited were increased lipid parameters (HDL-C, TC, LDL-C, and VLDL), blood electrolyte (Na^+, K^+, and Cl^-) and creatinine levels in the liver, hepatic enzymes, blood urea, increased liver and kidney weight, increased serum creatinine, and decreased total protein and albumin . Marmouzi et al. reported that CEO antioxidant activity in three test methods was 150 mg TE/g EO for DPPH, 110 mg TE/g EO for $ABTS^+$, and 34 mg AAE/g EO for FRAP.

3.Insecticidal

Insect-borne diseases are an ongoing challenge to public health. Some species are invasive urban pests, transmitting numerous pathogenic microorganisms and causing allergic reactions and asthma in young and older people. Commonly used insecticides cause significant health problems and have long-lasting adverse effects on the environment. Moreover, an increase in resistance against insecticides has been reported. Due to this, investigations have focused on developing natural insecticides based on EOs to control agricultural and urban pest. However, their high volatility decreases the time during which EOs remain in the human body, so sometimes several applications are required in a day.

CEO has shown high levels of repellency and fumigant toxicity on flea, aphids, nymphal instars, mites, imported red fire ants, C. pipiens, and American and German cockroaches . The oviposition-deterrent activity of CEO can be found in other mosquito species (Anopheles stephensi, An. subpictus, Ae. aegypti, C. pipiens, Ae. albopictus, Culex quinquefasciatus, and Cx. tritaeniorhynchus) . It targets the egg stage as an oviposition deterrent and the larval stage as a larvicide against Ae. japonicus, Ae. aegypti, and Cx. quinquefasciatus. CEO has shown repellent action in the laboratory and field settings against adult Ae. aegypti, Ae. cinereus, and Ae. Communis. The primary targets of CEO and other EOs are octopamine and gamma-aminobutyric acid (GABA) receptors and transient receptor potential (TRP) channels . The dose–response ratio of CEO showed an increased mortality rate with increasing concentration . CEO increased permeability activity on the cell membrane, disrupted the cytoplasmic membrane, and interacted with proteins, ATPase, histidine decarboxylase, amylase, and protease enzymes, which were also inhibited. Lambert et al. evaluated the activity of CEO against adult C. felis felis and the development of their eggs. The LC50 was 5.70 μg/cm2 against adult fleas and 0.30 g/cm2 against flea eggs; however, the insecticidal activity of eugenol was three times higher . Toledo et al. reported that it had activity against aphids, but not against ladybugs. They reported an LC95 of 0.17 μL/cm2 for aphids, while the same dose only had a lethality of less than 18% for Corymbia maculata. The ladybugs that were exposed to CEO did not exhibit impaired locomotion ability. Therefore, it was concluded that the application of CEO represents an alternative to control aphid infestations . Elzayyat et al. evaluated the insecticidal activity against adults and larvae of Culex pipiens, and reported an LC50 of 0.374 and 0.036%, respectively . Neupane et al. observed that CEO, eugenol, and eugenyl acetate applied at 4.0 mL/cm2 provided 95, 85, and 87% mortality of German cockroaches, respectively.

They also reported repellency for 30 min by applying 80% CEO. Reuss et al. observed that CEO functions as an oviposition repellent and a larvicide, with an LC50 of 17 mg/L. CEO and its main constituents are products that have low toxicity to mammals and zero residual concentration. Its application is limited to plague insect control, which is essential to prevent infestations in the environment.

4.Antiviral

CEO has shown antiviral activity against Ebola , influenza A virus , and herpes simplex virus types 1 and 2 . Recent studies by de Oliveira et al. showed that eugenol derivatives could inhibit the activity of the West Nile Virus, providing a promising compound against flaviviruses such as dengue, Zika, and yellow fever . Eugenol has also been studied as a possible inhibitor of the initial stage of HIV-1 infection because it can reduce virus replication. Likewise, eugenol can increase lymphocyte production; therefore, the lymphocyte proliferation capacity of eugenol may be responsible for its anti-HIV-1 activity .

CEO has demonstrated antiviral activity against feline calicivirus, which is used as a substitute for human norovirus. For this reason, the application of CEO in the process of washing fruits and vegetables eliminates any viral load that may exist. In addition, the application of CEO in cleaning wipes allows the decontamination of surfaces . Furthermore, CEO has been shown to increase the resistance of tomato plants to tomato yellow leaf curl virus more than moroxydine hydrochloride.

5.Antinociceptive

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs to treat inflammatory nociceptive pain. Their principal mechanism is cyclooxygenase (COX) inhibition, decreasing the prostaglandins that cause nociceptive pain. The antinociceptive and anti-inflammatory activities of eugenol are related to COX-2 inhibition and vanilloid transient receptor potential (TRPV) by high-voltage $Ca2+$ current inhibition in primary afferent neurons [. This antinociceptive response is related to opioid, cholinergic, and α2-adrenergic receptors, but not serotoninergic receptors. The antinociceptive effect of eugenol is probably related to gamma-aminobutyric acid (GABA) receptor modulation, because eugenol administration inhibits GABA receptor currents in trigeminal ganglion neurons and inhibits GABA $\alpha 1\beta 2\gamma 2$ expressed in these neurons .

6.Anti-Inflammatory and Wound Healing

Oxidative stress and inflammation are near-related processes in many pathophysiological conditions such as diabetes, hypertension, and cardiovascular and neurodegenerative diseases . The anti-inflammatory properties of CEO and eugenol are comparable to diclofenac gel, reducing inflammation by 60 to 20% after 3 h. Likewise, induced wounds in rats treated with CEO showed a significant contraction of more than 95% in the first 15 days. These results demonstrate that animals treated with CEO underwent similar healing to those treated with neomycin, which is currently used to control inflammation and heal wounds. Therefore, the chronic and acute side effects of synthetic antibiotics can be avoided, especially if they are given frequently . CEO inhibited important antiproliferative biomarkers whose activity depends on their concentration. It decreased the levels of inflammatory biomarkers such as VCAM-1, IP-10, I-TAC, and MIG, in addition to inhibiting the tissue remodeling protein molecules collagen I, collagen III, M-CSF, and TIMP-1 [26,87,109]. The application of CEO can reduce epidermal thickness and the number of inflammatory cells expressing COX-2 without affecting COX-1. The mechanism of eugenol, as an anti-inflammatory, inhibits the expression of COX-2 and reduces the production mediators of inflammation . Eugenol has also been reported to not alter IL-8 levels in human skin keratinocytes but to target other pro-inflammatory cytokines in pre-inflamed human dermal cells . These results suggest that CEO possesses anti-inflammatory activity and favors wound healing.

7.Analgesic

Headaches, joint pain, toothaches, and oral hygiene issues have traditionally been treated with aromatherapy and CEO. The CEO and eugenol are safe, effective, and inexpensive analgesics, and the analgesic effect of eugenol in different pain models has been well documented . Khalilzadeh et al. reported that the analgesic effect of CEO is mediated by the opioidergic and cholinergic systems. The analgesia produced by CEO in acute corneal pain appears to depend on the cholinergic activity. The analgesic and local anesthetic effects of eugenol can be modulated by its inhibitory effect on voltage-gated channels (Na^+ and Ca^{2+}) and activation of TRPV1. The analgesic effects of CEO and eugenol are very similar to those of

lidocaine. Correia et al. demonstrated the analgesic efficacy of CEO in fish. When it was used in concentrations between 40 and 80 µL/L in procedures that were invasive or could cause pain, an analgesic effect in animals was reported, minimizing the effects of harmful stimuli. CEO has potential for use in painful procedures, to minimize the effects of harmful stimuli for ethical reasons, and to ensure the welfare of the animal, avoiding stress and its negative consequences .

8.Anesthetic

CEO is recognized as an anesthetic at low concentrations (50–500 µL/L) in vertebrates and invertebrates without side effects. It induces anesthesia faster, has brief reflex recovery, and shows a low mortality rate without affecting external stimulus response . Recent studies showed that topical application of CEO and eugenol reduces corneal sensitivity in rats similar to lidocaine . The maximum level and duration of anesthesia depending on the concentration and time of exposure, which differs between chemicals. CEO efficiently induces anesthesia in Nile tilapia, cardinal tetra, ringed cichlid, and angelfish, affecting swimming ability and balance, and decreasing the response to external stimuli until complete immobilization. Depending on the concentration of the dose, the time to achieve full anesthesia is decreased. Furthermore, there are no side effects of CEO based on the concentration and time of exposure when recovering from anesthesia . CEO is an effective anesthetic for red claw crayfish and other crustaceans, including Nephrops norvegicus and grass shrimp. Induction and recovery times increase with increased crayfish size, as these are related to oxygen demand. Absorption and elimination of CEO are measured by the oxygen consumption rate, the relationship between the body and the gill surface, and the gill infusion rate. Size is inversely related to anesthetic efficacy . For invasive and painful procedures, the use of CEO is recommended due to its better anesthetic effect .

9.Anticancer

The eugenol, α-humulene, and β-caryophyllene components of CEO, which have cytotoxic and antitumor activity, have been used as alternatives in the prevention and co-treatment of cancer. Some reports suggest that EOs reduce the side effects of chemotherapy, which include nausea, vomiting, loss of appetite, and weight loss . The anticancer activity is mainly attributed to the antioxidant and anti-inflammatory activity, since the production of ROS specifically activates signaling pathways and contributes to the development of tumors by regulating cell proliferation, angiogenesis, and metastasis . CEO has been tested against different cancer types, such as colon , lung , breast , pancreatic , leukemia , cervical , and prostate .

The anticancer properties are due to the following mechanisms: the activation of detoxifying enzymes, the destruction of DNA by oxidative stress, antimetastatic and cytotoxic activity, decreased viability, cell cycle arrest or apoptosis, the reduction of phosphate-Akt expression levels, and MMP-2 and protein leakage . CEO has shown a low cytotoxic effect on normal cells, improving their antiproliferative activity .

The Aims of studies

The overall objective of this research was to develop new compounds with potential biological activity from readily accessed natural products, in particular eugenol. Eugenol has been reported to posses antioxidant and anticancer properties. In an attempt to enhance intrinsic activity of this natural compound.

Materials

1. Eugenol
2. L-pro-OH
3. tritylglycin-OH
4. tritylsulfanyl propanoic acid
5. DMAP
6. DCC
7. Chloroform
8. methanol.

Methods

1.In round flask was mixed eugenol (1gm,6.0901 mmol) ,L_pro _OH (2.055gm,6.0901mmol),4_dimethylaminopyridine (0.186gm,1.523mmol) and dicyclohexylcarbodiimide (1.257gm,6.0901mmol) in chloroform under stirring for 10 hours. The reaction was followed by thin layer chromatography (TLC). Then the mixture was filter and the crude was recrystalized from the chloroform and washed with methanol.

2.In round flask was mixed eugenol (1gm,6.0901 mmol) , (TritylN_ Glyc(1.933gm,6.0901mmol),4_dimethylaminopyridine (0.186gm,1.523mmol) and dicyclohexylcarbodiimide (1.257gm,6.0901mmol) in chloroform under stirring for 10 hours. The reaction was followed by thin layer chromatography (TLC). Then the mixture was filter and the crude was recrystalized from the chloroform and washed with methanol.

3.In round flask was mixed eugenol (1gm,6.0901 mmol) and Ttritylsulfanyl propanoic acid (2.122gm,6.0901mmol) ,4_dimethylaminopyridine (0.186gm,1.523mmol) and dicyclohexylcarbodiimide (1.257gm,6.0901mmol) in chloroform under stirring for 10 hours. The reaction was followed by thin layer chromatography (TLC). Then the mixture was filter and the crude was recrystalized from the chloroform and washed with methanol.

Results :

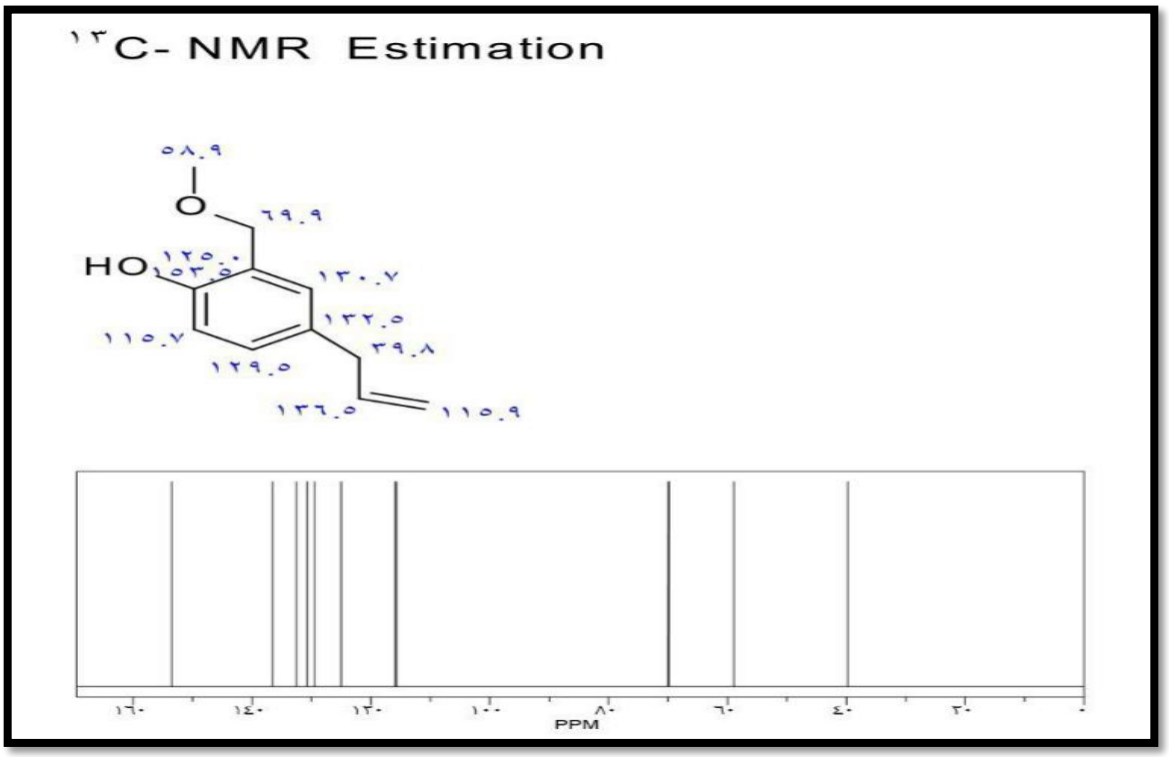

Figure (2): Estimated 13C -NMR for eugenol.

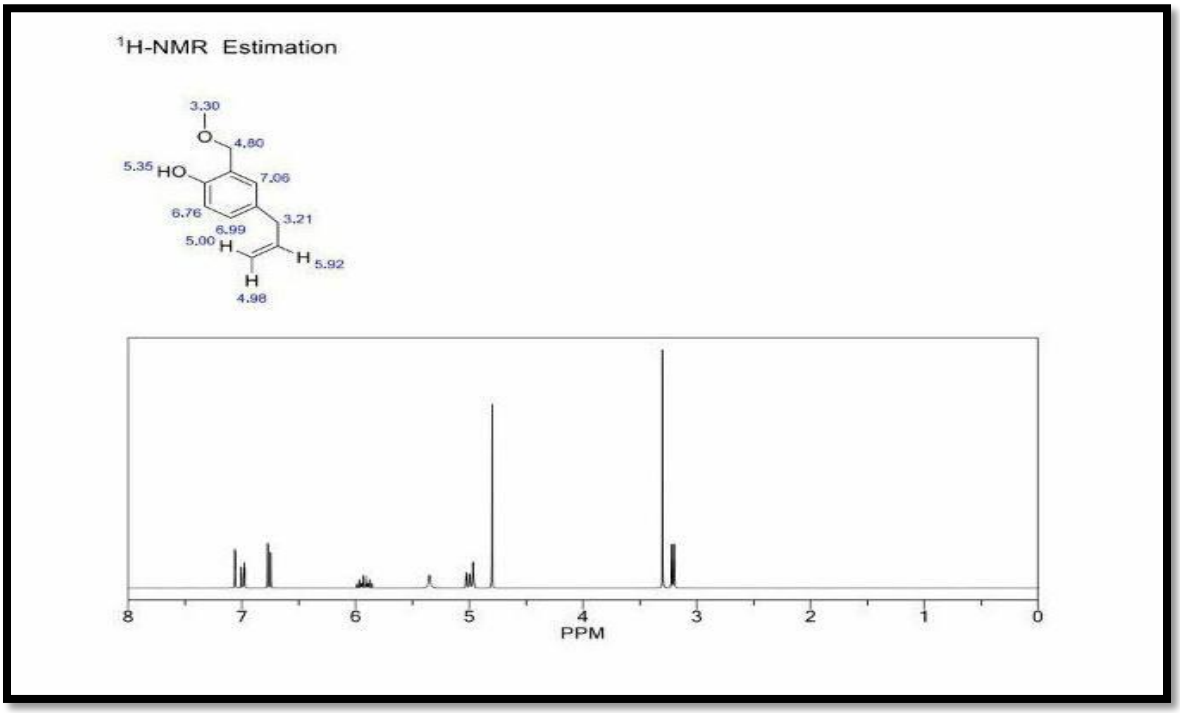

Figure (3): Estimated 1H-NMR for eugenol.

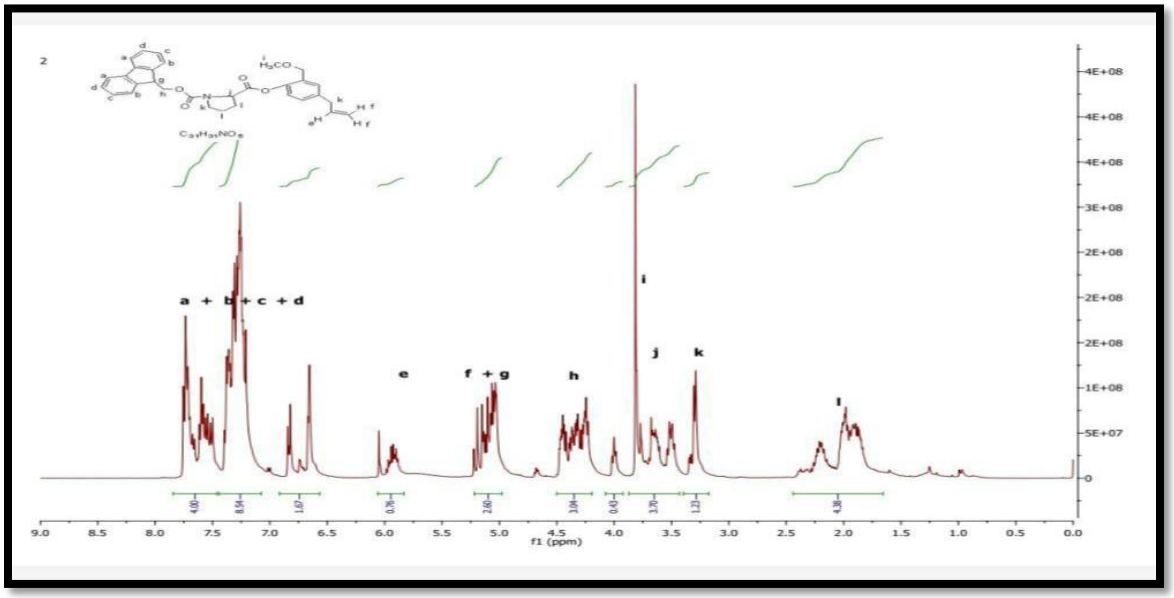

Figure (4): Experimental 1H-NMR for compound 1.

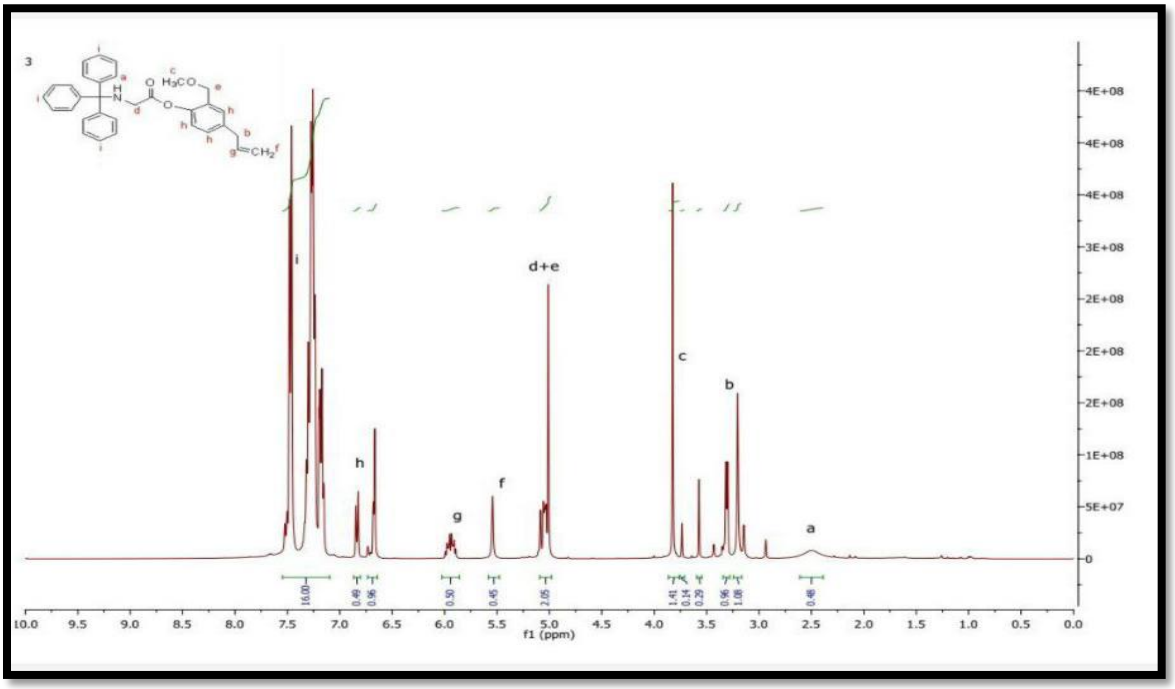

Figure (5): Experimental 1H-NMR for compound 2.

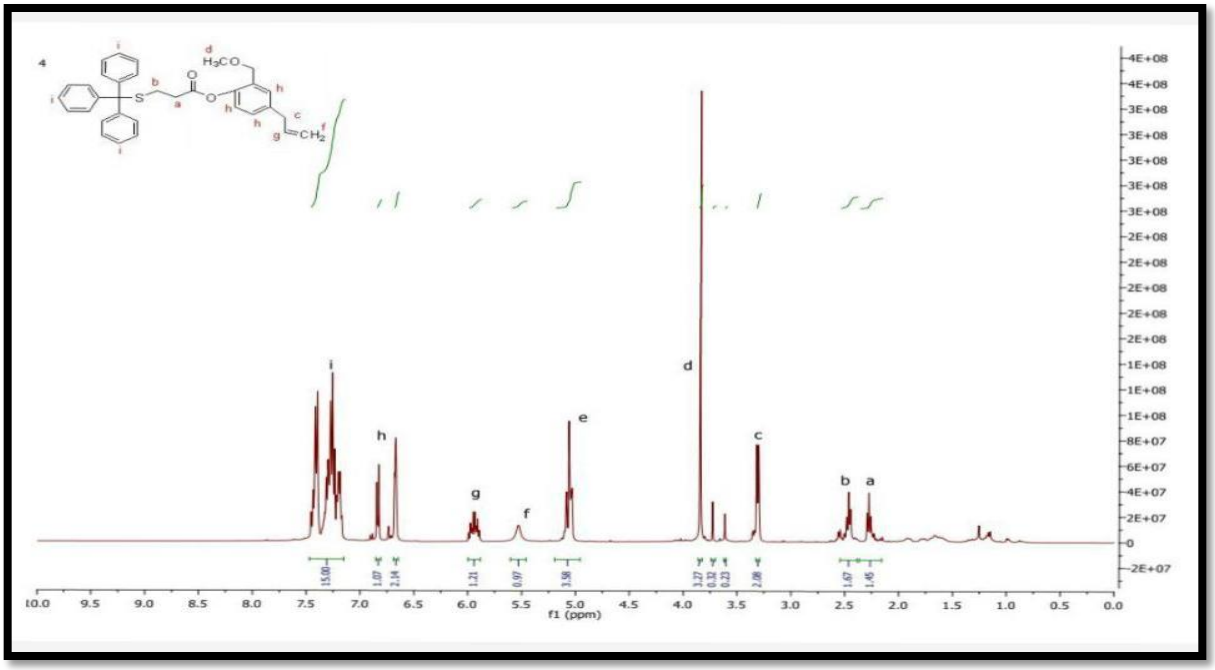

Figure (6): Experimental 1H-NMR for compound 3.

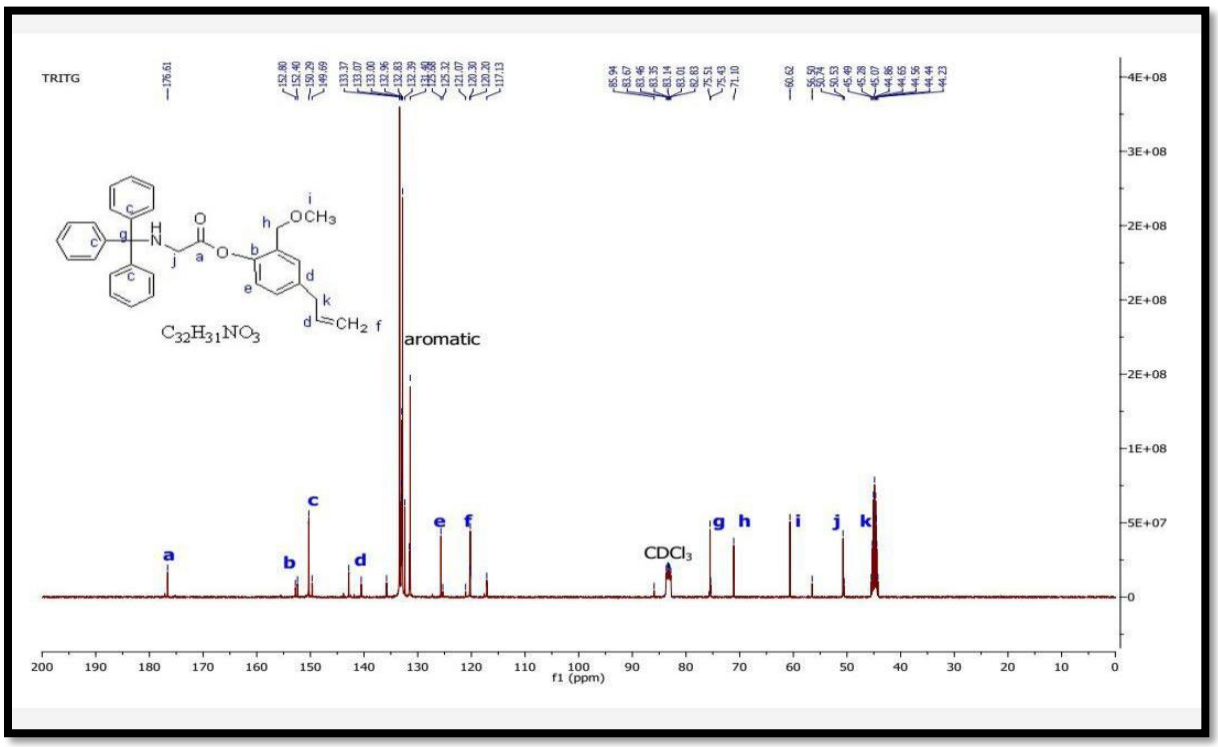

Figure (7): Experimental 13C-NMR for compound 2.

Discussion

Eugenol's chemical structure is related to phenol, maybe reaction with carboxylic group and formed esters,Therefore has demonstrated relevant biological potential with well-known antimicrobial and antioxidant action, and following reaction through Different Rf by TLC chromatography technique (thin layer TLC) is aluminum plate precoated with silica gel 60F254 was used for separation. When note difference between Rf for initial substance that are polar and product will be less polar evidence the product will formed.

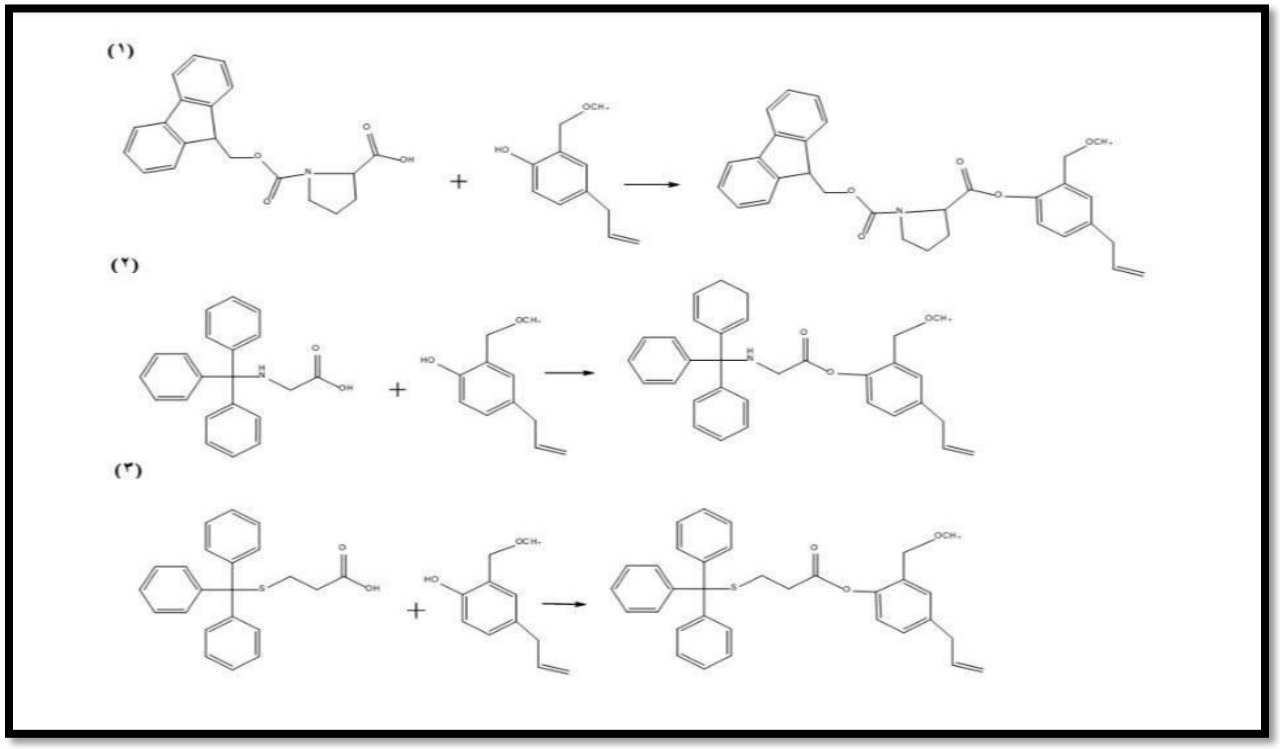

Figure (8): Eugenol derivatives that are prepared in this project.

The developed techniques H1NMR,13C were used to to confirm the expected compounds.

A result of H1NMR eugenol analysis, absorved in position C1 peak CH at chemical shift 5.00 ppm ,positionC 2 peak CH at Chemical shift 4.98 ppm,position C3 =CH will be more deshield at chemical shift 5.92 ppm because presence double bond ,and exist CH2 at positionC4 at chemical shift 3.21ppm because aliphatic of ethyl group.

CH at position C6,C7,C8 indicate trisubtitute aromatic ring, position C9 has broad peak because presence of OH at chemical shift 5.35 ppm, exist CH2 in position C11 at chemical shift 4.80 ppm becase as allyllic compound ,OH postition C 12 at chemical shift 3.30 ppm.

A result of H1NMR L-pro-OH analysis , CH in position C1 at chemical shift 7.6 ppm ,CH in position C2 7.7ppm ,CH in position C3 at chemical shift 7 .8 ppm ,CH in position C4 at chemical shift 7.9 ppm ,indicate trisubstitute aromatic ring,H_C= a position C21 in chemical shift 6ppm because of vinyl compound, =CH2 in position C22 at chemical shift 5 ppm because of vinyl compound, CH2 in positionC7 at chemical shift 4.5 because aliphatic of ethyl group,H3CO in position 16 at chemical shift 3.8 ppm,CH2 in position C10 at chemical shift 3.4 ppm, CH2 in position in position 15 at chemical shift 2 ppm because saturated aromatic ring.

A result of H1NMR tritylglycin-OH analysis,NH position C1at chemical shift 2.5ppm has broad peak ,CH2 in position 10 at chemical shift 3ppm because secondary methyl group, H3CO in position 8 at chemical shift 3.5ppm, CH2 in position 4 ,7 at chemical shift 5ppm as vinylic group,= CH2 in position 12 at chemical shift 5.5ppm because vinylic group, CH2 in position C10 at chemical shift 6ppm, CH in position 6,7,8 at chemical shift 6.8 ppm indicate trisubstitute aromatic ring, three aromatic ring in position C1 at chemical shift 7.5 indicate trisubstitute aromatic ring.

A result of H1NMR tritylsulfanyl propanoic acid analysis,CH2 in position C5 at chemical shift 2ppm as secondary ethyl group,CH2 in position C4 at chemical shift 2.5ppm,CH2 in position C11 at chemical shift 3.3ppm,H3CO in position C9 at chemical shift 3.8ppm , =CH2 in position11 at chemical shift 5.5 ppm as vinylic group, H_C=CH at position C10 at chemical shift 6ppm as vinylic group, CH in position 6,7,8 at chemical shift 7ppm indicate trisubstitute aromatic ring,trisubstitute aromatic ring in position C1 at chemical shift 7.5ppm.

The result of eugenol 13C NMR analysis,C=H in position C1 at chemical shift 115ppm because double bond more deshield, CH in position C2 at chemical shift 136.5 ppm,CH2 in position 3 at chemical shift 39.8 ppm because aliphatic of ethyl group, CH in position C4 at chemical shift 132.5 ppm, CH in position C5 at chemical shift 129.5ppm ,CH in position C6 at chemical shift 115.7ppm,indicated trisubstitute aromatic ring , OH in position C7 at chemical shift 153.5 ppm,CH in position C8 at chemical shift 125ppm because allylic hydrogen, CH2 in position C9 at chemical shift 69.9ppm because secondary of methyl group ,OH in position C10 at chemical shift 58.9ppm.

The result of 13C NMR tritylglycin-OH analysis,C=O in position C4 at chemical Shift 180ppm because Carbonyl more deshield, O=CH in position C5 at chemical shift 155ppm because alph of carbonyl group, aromatic ring in position C1 at chemical shift 150ppm ,CH in position C10 at Chemical shift 140ppm , CH in positionC8 at chemical shift 125 ppm,=CH2 in position C13at chemical Shift 120ppm because of double bond more deshield, CH in position C8 at chemical shift 70ppm,OCH3 in position C10at chemical shift 60 ppm,CH2 in position C4at chemical shift 50ppm,CH2 in C 11at chemical shift 40ppm.

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