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ANTIMICROBIAL ACTIVITY OF EUGENOL DERIVATIVES

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Abstract: The antibacterial properties of the clove plant are due to the presence of eugenol, an aromatic phenolic compound. Eugenol was isolated from clove by stem distillation. The alkene group in eugenol was epoxidized resulting in the synthesis of epoxide-eugenol. The heterocyclic ring in epoxide was cleaved to a bromoalcohol derivative. The compounds synthesized epoxide-eugenol, bromo alcohol and euginol were tested for antimicrobial activity against *Staphylococcus aureus* (ATCC 25923). Epoxide-eugenol was found to be the most effective antimicrobial agent among the three compounds tested.

Keywords: Eugenol, epoxide, antimicrobial activity

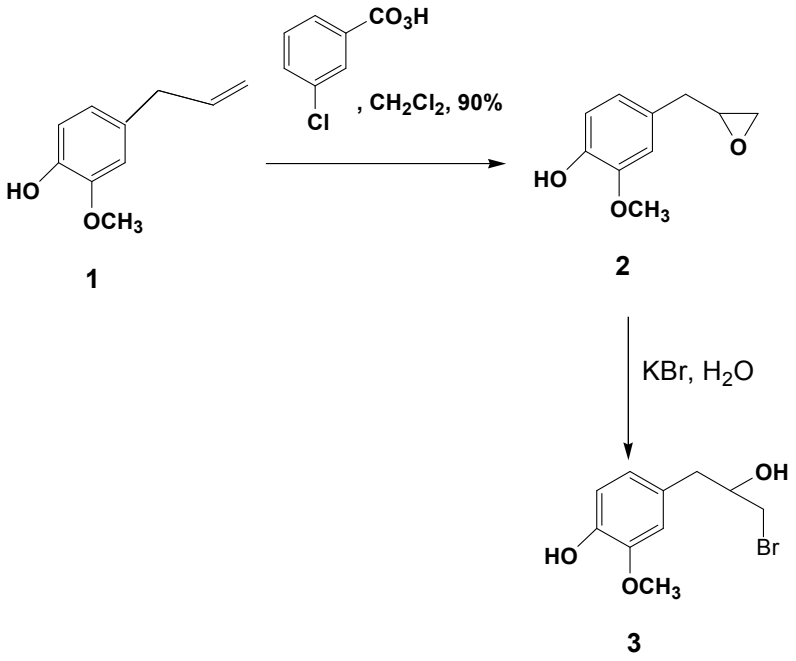
Introduction: Eugenol is an essential oil extracted from the dried flower buds of clove, *Eugenia caryophyllata*.[1] Eugenol is a clear to pale yellow oily liquid that can also be extracted from certain essential oils from nutmeg, cinnamon, and bay leaf and is slightly soluble in water and soluble in organic solvents. The main constituents of the essential oil extracted from *Eugenia caryophyllata* (clove) are carvacrol, thymol, eugenol and cinnamaldehyde. Clove is widely known to be cultivated in Indonesia, Sri-Lanka, Madagascar, Tanzania and Brazil. Cloves have shown to have a millennia-long history of safe use. The use of cloves as a breath freshener was documented in the 3rd Century B.C. Historical evidence shows that the Chinese required subjects awaiting an audience to chew cloves to mask breathe odors. Cloves have also been documented to be beneficial in treating dental disorders as described by Ancient Hindu texts. The medicinal uses of clove are known to include the following when used in combination form: fever reduction, decrease inflammation after tooth extraction (dry socket), in the treatment of premature ejaculation, to lessen the pain of anesthetic injections and as an ingredient in baking/cooking, perfumes, cigarettes, mouthwash, and toothpaste.[2,3,4] Eugenol has electron donating methoxy and hydroxy groups and an alkene moiety. We report herein a successful epoxidation of eugenol and conversion of the epoxide to a bromoalcohol derivative. In addition, the antimicrobial activities of these compounds are reported here.

Experimental: The following were conducted in our studies: extraction of Eugenol, synthesis of Epoxy-Eugenol, synthesis of Eugenol bromoalcohol and assessment of Antimicrobial Activity. The extraction of the essential oil, Eugenol was obtained by air-drying the leaves and stems of natural cloves and steam distillation was done to isolate eugenol from the oil. Identification of

essential oil components was accomplished by GC-MS by comparing their mass spectra with those in Wiley and NIST mass spectral databases resident in the system.

Epoxide Eugenol was synthesized by conversion of the alkene group in eugenol to an epoxide in good yield by stirring a solution of it in dichloromethane and 3-chloroperbenzoic acid. No isomerization of the double bond could be detected under this condition**.** The epoxide ring in (**2)** was opened by an aqueous solution of KBr to afford (**3**) (**Scheme 1).**

Scheme 1



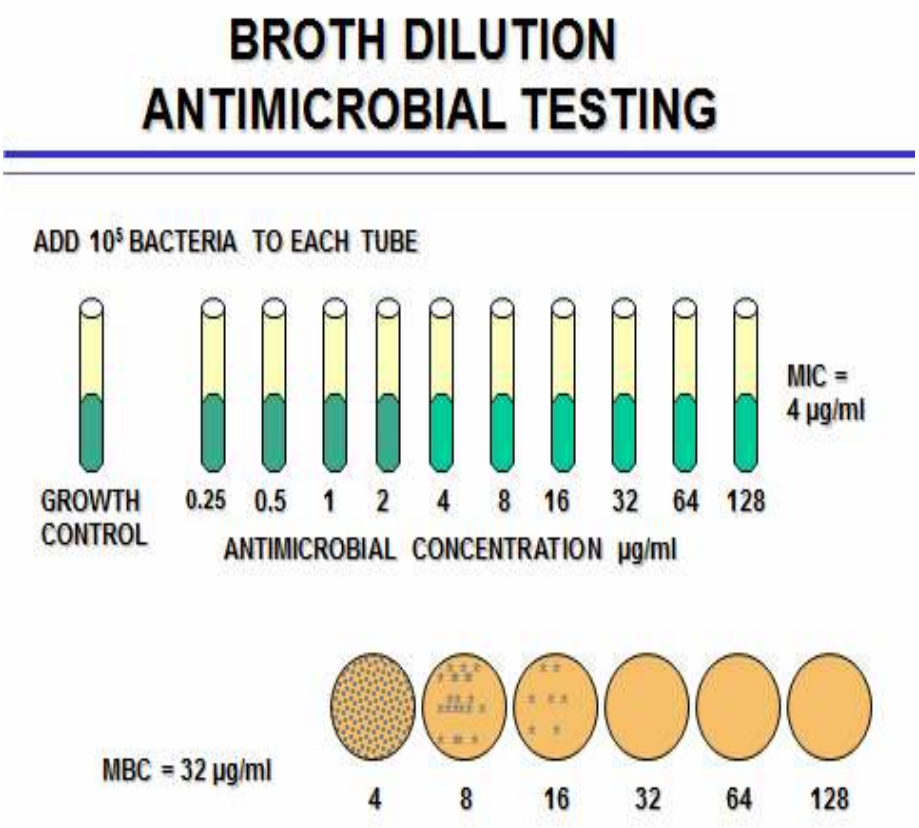
Assessment of antimicrobial activity was done using the principles of minimum inhibitory concentration (MIC), lowest concentration that results in inhibition of visible growth, minimum bactericidal concentration (MBC) and lowest concentration that kills at least 99.9% of the original inoculum. The materials and reagents used in the assessment of antimicrobial activity were the following: Mueller-Hinton broth (M-H broth), McFarland turbidity standards, clean acid-washed borosilicate glass tubes, pipettes, 5% sheep blood agar (SBA) plates, Eugenol, Epoxide-eugenol, Bromo alcohol and dimethyl sulphoxide (DMSO, 0.5%). The bacterial strain used in the assessment was Staphylococcus aureus (ATCC 25923) obtained from a commercial supplier, Thermo Fisher Scientific Remel Products Lenexa, KS.

MIC/MBC was prepared by method of Inoculum and Staphylococcus aureus (ATCC 25923) was subcultured onto SBA and incubated overnight at 35°C. A tube containing 2 mL M-H broth was inoculated with 8 bacterial colonies from the agar plate and the turbidity was adjusted to match a 0.5 McFarland standard (approximately 108 CFU/mL). After standardizing the bacterial inoculum to a 0.5 McFarland standard, it was diluted in M-H broth yielding a bacterial concentration of approximately 1 x 106 CFU/mL. Broth macrodilution and inoculation was conducted. The broth macrodilution method was used for the determination of MIC (NCCLS 1990).

Eugenol(**1**), epoxide-eugenol (**2**) and bromo alcohol (**3**) were each dissolved in dimethyl sulphoxide (DMSO, 0.5%) before use in preparing a twofold serial dilution in 1 mL M-H broth. Aliquots of 1 mL of the dilute inoculum were added to all tubes. The final inoculum size was approximately 5 x 105 CFU/mL. Control tests were carried out in parallel, using DMSO and doxycycline hyclate (Pfizer Inc, NY) for comparison and all tubes were incubated for 20 hours at 35°C. Quantitation of the Inoculum was conducted from the final dilution of inoculum and four serial tenfold dilutions were performed in M-H broth. In duplicate, 0.1 mL of the dilution were spread evenly on SBA using a sterile bent glass rod and the plates were incubated overnight at 35°C.

Determination of MIC was done after overnight incubation. The MIC of the test bacteria was determined by visual inspection of the tubes. All tubes without growth were vortexed to resuspend the bacteria and reincubated for an additional 4 hours at 35°C. The tubes were visually inspected again and MIC determined.

Determination of MBC was done after the 4 hour additional incubation. Visually clear tubes were vortexed and 0.1 mL aliquots were spread evenly on SBA using a sterile bent glass rod and plates were incubated overnight at 35°C



Results: The MIC was determined as the lowest concentration of test agent that prevented visible growth of bacteria. The MBC was determined by comparing bacterial growth on test plates with the quantitative subculture. The MBC was determined as the lowest concentration of test agent that produced a 99.9% kill or a three log10 reduction in bacterial growth.

The Minimum Inhibitory Concentrations (MIC) for epoxide-euginol, bromo-alcohol and euginol were 57 µg/mL, 115 µg/mL, and 115 µg/mL respectively (**Table 1).** The Minimum

Bactericidal Concentrations (MBC) were 115 μg/mL, 230 μg/mL and 230 μg/mL respectively (**Table 1**).

Table 1. MIC (μg/mL) and MBC (μg/mL) of Expoxide-Eugenol, Bromo-alcoholand Eugenol to *Staphylococcus aureus*

Compounds	**Antimicrobial Activity Present**	**Minimum Inihibitory Concentration (MIC)**	**Minimum Bactericidal Concentration (MBC)**
Eugenol (**1**)	Yes	115	230
Epoxide-Eugenol (**2**)	Yes	57	115
Bromo-Alcohol (**3**)	Yes	115	230
Doxycline (Control)	Yes	1.2	2.4

Conclusion: Epoxide-eugenol was found to be the most effective antimicrobial agent among the three compounds tested. Further study is needed to ascertain if epoxide-eugenol has the same safety as eugenol which has been used as an antimicrobial agent for decades

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