



Composition, Antioxidant, Antibacterial Activities and Mode of Action of Clove (*Syzygium aromaticum* L.) Buds Essential Oil

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Author's contribution

This work was carried out by the main author itself. He designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Subsequently, he managed the analyses of the study in accredited labs, literature searches, read and approved the final manuscript.

Original Research Article

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ABSTRACT

The use of natural antimicrobial compounds, especially extracted from plants, as food preservatives is nowadays widely used, since plant matrices possesses antimicrobial and antioxidant natural products to protect themselves from microbial infection and deterioration as well as for human health benefit properties. The present work was undertaken to examine the antioxidant and antimicrobial activities of clove (*Syzygium aromaticum* L.) buds essential oil. Clove essential oil (CEO) exhibited high amount of total phenolic compounds (TPC) with high radical scavenging activity toward DPPH, ABTS and linoleic acid radicals as well as iron chelating activity. The TPC of CEO was 845 mg GAE g⁻¹ demonstrated 509 µmol of TE g⁻¹ DPPH radicals scavenging activity and 713 µmol of TE g⁻¹ ABTS radicals scavenging activity. The antioxidant capacity of CEO exhibited 94% reduction when evaluated by β-carotene bleaching assay. The Reducing power activity related to iron chelating was 314 µmol of AAE g⁻¹. The composition of CEO exhibit high eugenol content (80.19%) over 16 identified components by GC-MS analysis. Furthermore, CEO exhibited antibacterial activity *in vitro* at low concentrations against tested food borne pathogens applying disc diffusion and microdilutions assays. The minimum inhibitory concentration values (MICs) for tested bacteria which were sensitive to CEO were in the range of 1400–3600 µg ml⁻¹. The phenolic components of CEO are

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most active and appear to act principally as membrane permeabilizers which established using staining-DNA fluorescence dye assay. Quantification of membrane permeabilization exudes a positive relationship between the cell membrane damaging and concentrations. Therefore, the CEO can be reliably used in commercial applications as antioxidant, antibacterial and flavoring agent in individual or in combination with common preservatives for controlling the undesirable organoleptic and microbial deteriorations in foodstuffs.

Keywords: *Syzygium aromaticum* L.; essential oil; total phenolic compounds; antioxidant activity; antibacterial activity; mode of action.

1. INTRODUCTION

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family Myrtaceae. Cloves are native to Indonesia and used as a spice in cuisines worldwide [1]. It is well known previously, as an anticarcinogenic [2], as a traditional remedy for asthma [3], disorder of digestive system [4], dental disorders, respiratory disorders, headaches and sore throat in many countries [5]. Besides the reported antimicrobial, antifungal and antiviral properties, the essential oil of *S. aromaticum* shows anti-inflammatory, and anesthetic activities [6]. The name of the main constituent of clove essential oil (CEO), is eugenol. Eugenol is a major volatile constituent (45–90%) of clove essential oil obtained through hydro-distillation of mainly (*S. aromaticum*) buds and leaves in addition to acetyleugenol, chavicol, acetyl salicylate and humulenes [1,2]. It is a remarkably versatile molecule incorporated as a functional ingredient in numerous products and has found applications in the pharmaceutical, agricultural, fragrance, flavour, cosmetic and various other industries. Its vast range of activities has been well-researched and includes antimicrobial, anti-inflammatory, antioxidant and anticancer activities. In addition, it is widely used in agricultural applications to protect foods from microorganisms during storage, which might have an effect on human health [7]. Also, eugenol has been classified as 'generally recognized as safe (GRAS)' by the U.S. Food and Drug Administration [8].

Interestingly, natural products have been used to treat microbial growth and numerous essential oils have demonstrated the ability to inhibit the growth of various pathogens. The effect of CEO and eugenol on the growth of Gram-positive (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes*) and Gram-negative (*Escherichia coli*, *Salmonella typhi*, *S. choleraesuis*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*) bacterial strains was investigated [9,10]. At 1000 ppm, eugenol inhibited the growth of the bacteria and complete inhibition was obtained against *P. aeruginosa* at a high concentration of 2000 ppm, which was high in comparison to ampicillin 1mgml^{-1} used as a positive control. The antibacterial activity of eugenol against various pathogens such as *E. coli*, *B. cereus*, *Helicobacter pylori*, *S. aureus*, *S. epidermidis*, *Streptococcus pneumoniae* and *S. pyogenes* was confirmed [11-14]. Moreover, the antimicrobial activity of incorporated eugenol (0.5%) in biofilm with more than 90% against two *P. aeruginosa* pathogens was emphasized. Comparing the antibacterial activity of eugenol to cinnamaldehyde, thymol, carvacrol against *E. coli*, Eugenol possessed the lowest antibacterial activity (MIC value: 1600mgl^{-1}), while their combinations had synergistic interactions resulting in MIC values of 400, 100, 100mgl^{-1} were recorded, respectively [15]. Additionally, the combination of cinnamate and eugenol produced a bactericidal synergistic