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SHORT COMMUNICATION

In vitro evaluation of antioxidant and antibacterial properties of supercritical CO_2 extracted essential oil from clove bud (*Syzygium aromaticum*)

Misti Das[1] · Sutapa Roy[1,2] · Chandan Guha[2] · Asit Kumar Saha[1] · Mukesh Singh[3]

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Abstract

The antioxidant and antimicrobial activity of the essential oil extracted from *Syzygium aromaticum* was investigated. Extraction of oil was carried out following the standard methodology of supercritical CO_2 extraction. Antiradical and antimicrobial activity of extracted oil was studied. Chemical constituent of oil exhibited 65.3% eugenol. It was found that the extracted clove essential oil demonstrated high antioxidant contents which lead to free radical scavenging, reducing power, metal chelation and inhibition of lipid peroxidation. *Shigella dysenteriae* and *Staphylococcus aureus* showed susceptibility towards oil. Minimum Inhibitory concentration values for two tested bacteria are 3.0 and 2.5 mg/ml. Growth inhibition around well loaded with different concentration of oil further confirmed antimicrobial property. Additionally, clove essential oil also seen to be compatible to goat's erythrocytes. Findings suggest that clove essential oil may act as therapeutic molecules against diseases caused by pathogens and free radicals.

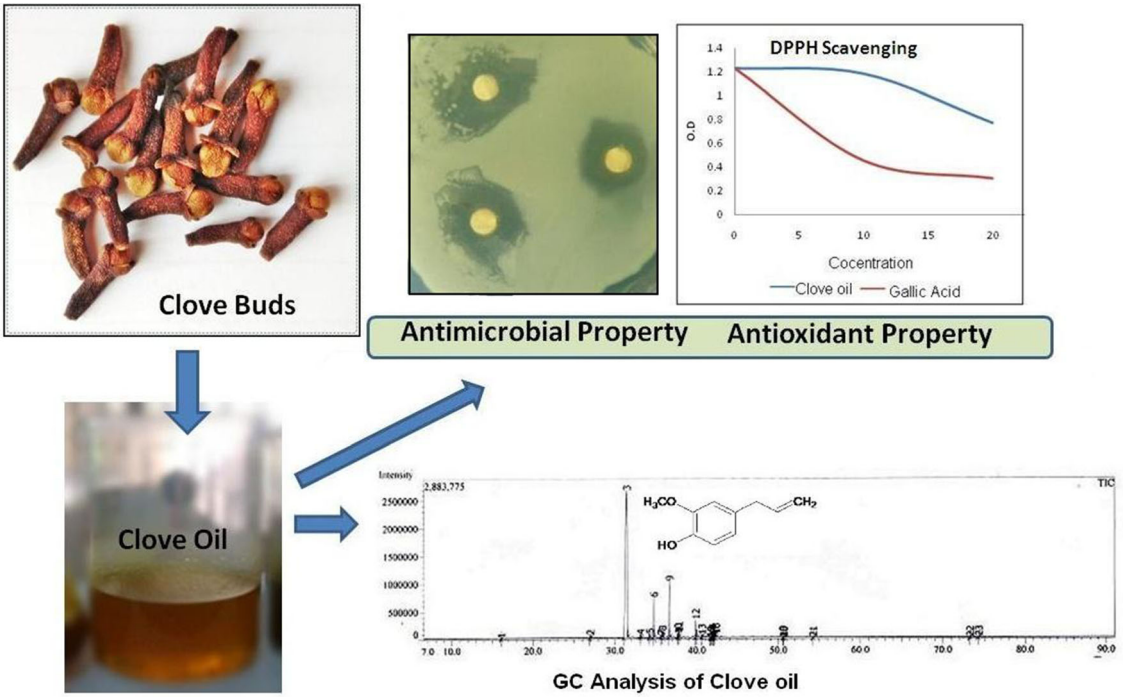
✉ Mukesh Singh
msingh006@gmail.com

[1] Department of Chemical Engineering, Haldia Institute of Technology, Haldia, West Bengal 721657, India

[2] Department of Chemical Engineering, Jadavpur University, Kolkata, India

[3] Department of Biotechnology, Haldia Institute of Technology, Haldia, West Bengal 721657, India

Graphic abstract



Keywords Antioxidant · Antimicrobial · *Syzygium aromaticum* · Essentials oil · Haemolysis

Abbreviations

EC	Effective concentration
GA	Gallic acid
h	Hour
ROS	Reactive oxygen species
SFE	Supercritical carbon dioxide extraction

One important cause of health disorder is oxidative stress which may lead to degenerative diseases like cancer, cardiovascular disease, ageing, immune system decline etc. (Halliwell et al. 1992). Oxidative stress arises when the antioxidant safeguarding system of human body fails to inhibit the overproduction of cell damaging reactive oxygen species (ROS) (Wang and Ballington 2007). Reports indicated that eating more antioxidant enriched foods leads to overcome immune deficiency and environmental stress factors (Willcox et al. 2009).

Synthetic antioxidants are being used as dietary supplements, food preservatives, anti-ageing ingredient in cosmetics, diesel oil preservatives, etc. (Sherwin 1990; Andreassi and Andreassi 2004). Different research works have been assumed that inappropriate dosing of most commonly used synthetic antioxidants cannot provide complete protection against ROS attack in body due to their carcinogenic and toxic behaviour (Dundar and Aslan 2000; Halliwell 2007). Therefore, utilization of antioxidant components from natural sources can help in removing free radicals and even without altering the endogenous antioxidative defence system (Song et al. 2010).

Clove (*Aromaticum syzigium*) is widely found as evergreen bushes in India and it neighbouring countries. Clove oil has several therapeutic usages because of high antioxidant and antimicrobial properties (Bezerra et al. 2017). Comparing with antiradical effect of natural antioxidants clove oil ranks as high as ascorbic acid (Chaieb et al. 2007). The essential oil contains eugenol, sesquiterpenes, diterpenes, aldehydes, ketones, aromatics etc. (Nieto 2017). Phenolic compounds of essential oils have unique characteristics in having both antioxidant and antimicrobial property (Velioglu et al. 1998). Accordingly uses of essential oils are directed worldwide for multidimensional applications in medicine, aromatherapy, fragrance and food industries, in perfumery, cosmetics, etc. (Sacchetti et al. 2005; Wei and Shibamoto 2010). Essential oil from clove may vary in its chemical compositions and flavour depending on different factors like part of plants used to extract oil, methods of extraction, seasoning, genetic factors, soil etc. Good quality and highest yields are obtained from clove buds.

Table 1 Composition of clove essential oil from clove buds as analyzed by GC–MS

Components	Content (%)
Eugenol	65.3303
β-caryophyllene	14.782
Eugenol acetate	14.55
α-Humulene	1.43
α-Cubene	1.4025
β-Cedrene	1.097
Caryophyllene oxide	1.0944
Farnesene	0.1576
β-Cubene	0.1474
α-Caryophyllene	0.013
Humulene	0.005
Humulene epoxide	0.013
Caryophylenoxide	0.023
2′,3′,4′-Trimethoxyacetophenone	0.013
Delta-Cadinene	0.002
Cembrene	0.001

The objective of the present work was to investigate the antioxidant and antibacterial properties of the clove essential oil extracted from buds using supercritical fluid extraction technique (SFE). Antibacterial property of extracted oil was evaluated against *Shigella dysenteriae* and *Staphylococcus aureus*. Biocompatibility of extracted oil was tested on goat's erythrocytes.

The matured buds of clove were obtained from Kolkata (West Bengal, India). Dried powder particles of clove buds with average diameter of 0.64 mm were used for SFE of oil. The SFE set up (Model No: CSL/SCF/1L2/400) used in this study was supplied by Chemtron Science Laboratories Pvt. Ltd., Navi Mumbai, India. Extraction of oil from 250 gm of ground buds was carried out at 40 °C and pressure of 20 MPa maintaining sufficient CO_2 flow for a period of 210 min. Clove oil extracted was further analyzed by Gas Chromatography and identification of each component was done by Mass Spectroscopy using SHIMADZU GCMS-QP2010 SE model. Different MS library used were WILEY, NIST and SHIM.

Phenol, antioxidant content, lipid peroxidation and antiradical assays of extracted clove oil were measured following the methodology of Dutta and Singh (2011). Reducing power and metal chelating capacity of oil was measured following the methodologies of Oyaizu (1986) and Dinis et al. (1994). GA was used as standard antioxidant for all assay systems.

In vitro antimicrobial property of clove oil was tested against *S. dysenteriae* and *S. aureus* by performing well diffusion assay (Datta et al. 2011). MIC was estimated for the tested bacteria. Each freshly grown bacterial strain (1×10^5 cells) was inoculated in nutrient broth supplemented with 0–10 mg/ml of clove oil and allowed to grow for 24 h at 37 °C. Thereafter, evaluation of MIC value was done (Singh 2016).

Hemolytic assay was done to check the biocompatibility of extracted clove oil on goat erythrocytes (Goswami et al. 2015).

All experiments were carried out in triplicate. Data points were represented by the mean of the measured values. Statistical analysis was carried out using MS-Excel and online software GraphPad.

Essential oil extracted from clove buds by SFE process yielded 45.5 ml/1 kg. GC–MS analysis demonstrated various important components in the extracted oil (Table 1). Eugenol is reported as the major component of clove oil (Khalil et al. 2017). The percentage yield of eugenol was 65.3%. Literature confirms that clove oil obtained by SFE gives the highest percentage of eugenol compared to other extraction procedures (Wenqiang et al. 2007). Extraction by this method is quick because of little viscosity and high diffusivity of the supercritical fluid and thus ideal extraction method.

Clove essential oil has been reported to exhibit high antioxidant and antimicrobial activity (Chaieb et al. 2007). Phenol and total antioxidant content in oil was 200 and 380 µg/ml GA equivalents respectively. High value of antioxidant in clove oil is positively correlated to phenolic substances in it (d'Avila Farias et al. 2014). The EC_{50} value for DPPH Scavenging activity of oil is 40 ug/mL. The antioxidant activity in clove oil is linked with the synergistic interaction of phenolic compounds and secondary metabolites present in the oil (Chaieb et al. 2007; d'Avila Farias et al. 2014). Metal chelating activity of oil showed it role in reduction of ROS production in Fenton reactions. The EC_{50} value for inhibition of lipid peroxidation by clove oil and GA are 25 and 28 ug/ml respectively (Table 2).

The antibacterial activity of clove oil against selected human pathogenic bacteria namely *S. dysenteriae* and *S. aureus* was studied. MIC values for two bacteria are 3.0

Table 2 EC_{50} values of different assay system conducted on clove oil

Sample	DPPH	Reducing power	Metal chelating	Lipid peroxidation
Clove oil	40.28	27.144	62.33	25.11
Gallic acid	6	26.04	42.33	25

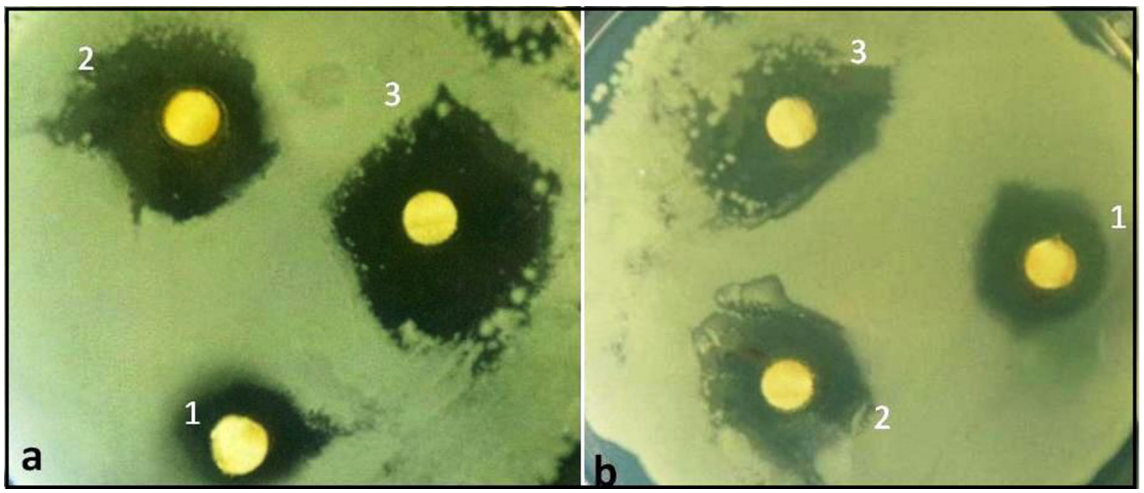


Fig. 1 Antimicrobial activity of clove oil against *S. dysenteriae* and *S. aureus* by well diffusion assay. (colour online)

and 2.5 mg/ml. Antimicrobial property was also demonstrated by well diffusion assay. Inhibition of bacterial growth around the well loaded with oil demonstrates antimicrobial property (Fig. 1). The results are very encouraging especially for *S. aureus* compared to *S. dysenteriae*. The present result corroborate with previously published articles which demonstrated high susceptibility of gram positive bacteria compared to gram negative one (Radünz et al. 2019; Singh et al. 2016). The effectiveness of clove essential oil as antimicrobial agent in traditional medicine is well documented which supports the present results. Antimicrobial activity of essential oil depends upon its composition, functional groups and synergistic effect of all components.

Haemolysis may occur due to excess of antioxidant. Therefore to check the oil biocompatibility, haemolysis assay was performed. Haemolysis of goat's blood in presence of clove oil for 1 h ranged from 5 to 25% for concentration < 100 ug/ml to 1 mg/ml.

The present study evidently indicates that clove essential oil has powerful antioxidant activity against various oxidative systems in vitro. In fact in all the oxidative systems tested clove oil has antioxidant activity comparable to that of GA. Antimicrobial property of clove oil was also promising against both gram positive and gram negative bacteria. Haemolysis result confirmed biocompatibility of clove oil. Therefore, clove essential oil may used as a source of natural antioxidants and possible food supplement, with potential application in the pharmaceutical industry.

Compliance with ethical standards

Conflict of interest Authors declared no conflict of interest.

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