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## Profile of Antibacterial Activities of Essential Oil and Oleoresin from Clove Buds against Several Food-Related Bacteria

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### Abstract

The oil from clove buds had a wide range of pharmacological action and bioactivities and is widely used in the medicine, food and flavouring industries. In this work, the antibacterial activity of the essential oil (EO) and oleoresin (OL) from clove buds against several food-related bacteria were evaluated based on Oxford cup method, the minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays, and the results showed that there is no obvious difference in antibacterial activity between essential oil and oleoresin, and they exhibited better antibacterial activity against test bacteria, and the antibacterial effects depended on its concentrations and action time. Kill-time assay also showed the essential oil and oleoresin from clove buds had a significant inhibitory effect on the growth rate of surviving *Staphylococcus aureus* and *Escherichia coli*.

**Keywords:** Antibacterial activity, Essential oil, Oleoresin, Clove buds.

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**Citation** | Xin-Ming Cao; Ting Liu; Qing-Ping Hu (2016). Profile of Antibacterial Activities of Essential Oil and Oleoresin from Clove Buds against Several Food-Related Bacteria. *Journal of Life Sciences Research*, 3(3): 40-43.

**DOI:** 10.20448/journal.504/2016.3.3/504.3.40.43

**ISSN(E) :** 2408-9184

**ISSN(P) :** 2518-0126

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**Contribution/Acknowledgement:** All authors contributed to the conception and design of the study.

**Funding:** This study received no specific financial support.

**Competing Interests:** The authors declare that they have no conflict of interests.

**Transparency:** The authors confirm that the manuscript is an honest, accurate, and transparent account of the study as planned; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained.

**Ethical:** This study follows all ethical practices during writing.

**Publisher:** Asian Online Journal Publishing Group

## 1. Introduction

Food poisoning and spoilage caused by microorganisms are the most important concerns of the food industry, and there has been a dramatic increase throughout the world in the number of reported cases of food-borne illness [1]. For many years, a variety of different chemical and synthetic compounds has been made to control microbial growth, and to reduce food poisoning and spoilage [2]. However, consumers have recently grown concerned about the side effects of synthetic chemicals and want safer materials for preventing and controlling pathogenic microorganisms in foods [3, 4].

Use of natural plant derived antibacterial can be effective in reducing the dependence, and help in controlling cross-contaminations by food-borne pathogens [5]. In addition, plants or their extracts can also be believed to be safe to humans [6-8]. The oil extracted from the dried flower buds of clove (*Syzygium aromaticum*), is used to relieve pain and to promote healing [9]. Clove oil has been listed as a 'Generally Regarded As Safe' substance by the United States Food and Drug Administration [10]. The clove essential oil exhibited a wide range of pharmacological action and bioactivities such as antioxidant [11, 12] antifungicidal [13] anticarcinogenic, anesthetic, repellent, and antiprotozoal effects [9, 14]. However, these informations are still limited; little work has been reported on the antibacterial properties of clove buds oleoresin obtained by organic solvent extraction on the growth of food-related bacteria, which would provide some foundational information for the developing and application of clove buds.

## 2. Materials and Methods

### 2.1. Plant Materials

Clove buds, which comes from Nanning City of Guangxi Province, were obtained as a commercial product from the local market on 2013. Dried clove buds were stored at  $-20^{\circ}\text{C}$  until analysis. The moisture content was 6.2% for clove buds, which was determined using a laboratory oven at  $110^{\circ}\text{C}$ .

### 2.2. Chemicals/Reagents

Nutrient agar (NA), nutrient broth (NB) and tryptone soy agar were from Beijing Aoboxing Bio-tech Co. Ltd. (Beijing, China). Other chemicals used were all of analytical grade.

### 2.3. Extraction of Clove Oil

The dried clove buds were ground and hydrodistilled for 4 h using a Clevenger-type apparatus. The oily layer was separated and dried with anhydrous sodium sulfate. The oil obtained was stored in tightly closed dark vials at  $4^{\circ}\text{C}$  until further analysis. The essential oil had specific clove aroma.

The dried clove buds were ground, and the oleoresin was extract by adding a certain amount of petroleum ether for 30 min. The extraction was carried out at room temperature and in absence of light and repeated three times. Then, the mixtures were filtered, and the supernatants were pooled, and vacuum-evaporated to dryness at  $20^{\circ}\text{C}$ . The oleoresin was obtained as a dark brown transparent liquid and had specific clove aroma and stored in tightly closed dark vials at  $4^{\circ}\text{C}$  until use.

### 2.4. Microbial Strains and Culture

Three Gram-positive strains were *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, and *Bacillus subtilis* ATCC 6051. Three Gram-negative bacteria were *Salmonella typhimurium* ATCC 19430, *Shigella dysenteriae* CMCC (B) 51252 and *Escherichia coli* ATCC 25922. The strains cultured at  $37^{\circ}\text{C}$  on NA or NB mediums.

### 2.5. Oxford Cup Method

The essential oil and oleoresin was dissolved in ethanol and sterilized by filtration through  $0.22\ \mu\text{m}$  Millipore filters. Antimicrobial tests were then carried out by the Oxford cup method using  $100\ \mu\text{L}$  of suspension containing  $1 \times 10^7$  colony forming units (CFU)/mL of bacteria spread on nutrient agar (NA) medium. Oxford cups were placed on the inoculated agar, and then  $100\ \mu\text{L}$  of sample was added. The diameter of inhibition zone (DIZ) was measured after 24h of incubation at  $37^{\circ}\text{C}$ , and ethanol was used as a negative control.

### 2.6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC) Assay

MIC and MBC were determined according to the method described by Diao, et al. [15] with minor modifications. Briefly, stock solution of samples was prepared in ethanol. Two fold serial dilutions of extracts were filtered through  $0.22\ \mu\text{m}$  Millipore filters and prepared in sterile NB medium. To each tube,  $50\ \mu\text{L}$  of the inoculum containing approximately  $1 \times 10^7$  CFU/mL microorganisms were added. The tubes were then incubated at  $37^{\circ}\text{C}$  and examined for evidence of the growth. The MIC was determined as the lowest concentration of extracts that demonstrated no visible growth for incubating for 24 h, while the MBC was the lowest concentration of the test extracts that showed no visible growth in the culture incubating at  $37^{\circ}\text{C}$  for 48 h.

### 2.7. Kill-time Analysis

The assay was performed by the method described by Muroi and Kubo [16] with some modifications. The effects of the clove oil with different concentrations ( $0.5\times$ ,  $1\times$  and  $2\times$ MIC) on the growth of tested bacteria were studied. Fifty microliters of the clove oils filtrating through  $0.22\ \mu\text{m}$  Millipore filters was added to 4.9 mL of the sterile NB medium, and then mixed with  $50\ \mu\text{L}$  of a 10 h culture of tested bacteria ( $1 \times 10^7$  CFU/mL). The cultures were incubated at  $37^{\circ}\text{C}$  and shaken with a rotary shaker at 120 rpm. At selected time intervals, samples from test culture were taken and the absorbance at 600 nm ( $\text{OD}_{600}$ ) was measured.

### 3. Results and Discussion

#### 3.1. DIZ of Samples

The DIZ values of the essential oil and oleoresin against tested strains were presented in Table 1. The essential oil and oleoresin showed varying degrees of antibacterial activity against tested Gram-positive bacteria and Gram-negative bacteria. The DIZ values of the essential oil and oleoresin against all tested bacterial strains were in the range of 11.3-25.2mm and 11.3-27.1 mm respectively, and the DIZ was the maximum value for *S. typhimurium*, followed by for *S. dysenteriae*, the lowest for *L. monocytogenes*. For essential oil and oleoresin, the differences in antibacterial activity may be associated with their bioactive constituents, the genetic characteristics and growth characteristics of different bacteria.

#### 3.2. MIC and MBC of Samples

Table 2 showed that the MIC and MBC values of essential oil and oleoresin for tested bacterial strains were in the range of 0.313-0.625 mg/mL and 0.313-1.25 mg/mL, respectively. There is no difference in MIC values except for *S. dysenteriae* and *S. typhimurium* and in MBC except for *L. monocytogenes* and *E. coli* for essential oil and oleoresin which was different from the results of DIZ assay, which was result from difference in cultural conditions. Some studies reported that the Gram positive bacteria were more inhibited than the Gram-negative ones because of the structural differences in the outer layers of Gram-negative and Gram-positive bacteria [17, 18]. However, this result was not found in the present study.

#### 3.3. Kill-Time Analysis

*S. aureus* is well-known for being resistant towards some antibiotics and for its production of several enterotoxins that cause many enteritis types [19] and *E. coli* is a common quality control bacterium in food inspection. Therefore, they were selected as the model organisms for further study the effect of the essential oil and oleoresin of clove buds on the viable counts of tested bacterial pathogen in the present study. The effect of the essential oil and oleoresin on the optical density (OD) values of *S. aureus* and *E. coli* at 600 nm is shown in Figure 1. As observed in Figure 1, no obvious difference in the viable counts of tested bacteria was found between the essential oil and oleoresin. OD values of the control and treatments had no obvious change within 2 h after treating. Thereafter, the OD values of control significantly and rapidly increased, indicating that *S. aureus* and *E. coli* entered upon logarithmic phase. Compared to the control, *S. aureus* and *E. coli* treated with the essential oil at the 0.5× and 1×MIC showed a slow increase during 12 h of incubation, but OD values were far below the control; while the OD values of treatment at 2×MIC had no change. These results showed the antibacterial activity of clove buds essential oil and oleoresin and showed a significant inhibitory effect on *S. aureus* and *E. coli*, supporting the results stated above, and showed that the treatment time and concentration of the essential oil had great influences on antibacterial effects.

### 4. Conclusions

In conclusion, the work showed that there is no obvious difference in antibacterial activity between essential oil and oleoresin from clove buds, and they exhibited better antibacterial activity against test bacteria, and the antibacterial effects depended on its concentrations and action time. Kill-time assay also confirmed the essential oil and oleoresin had a significant inhibitory effect on *Staphylococcus aureus* and *Escherichia coli*. However, further research on the chemical compositions, mechanisms of action, and the toxicological effect of essential oil and oleoresin from clove buds is still necessary to fully evaluate the potential of clove buds in foods and medicines.

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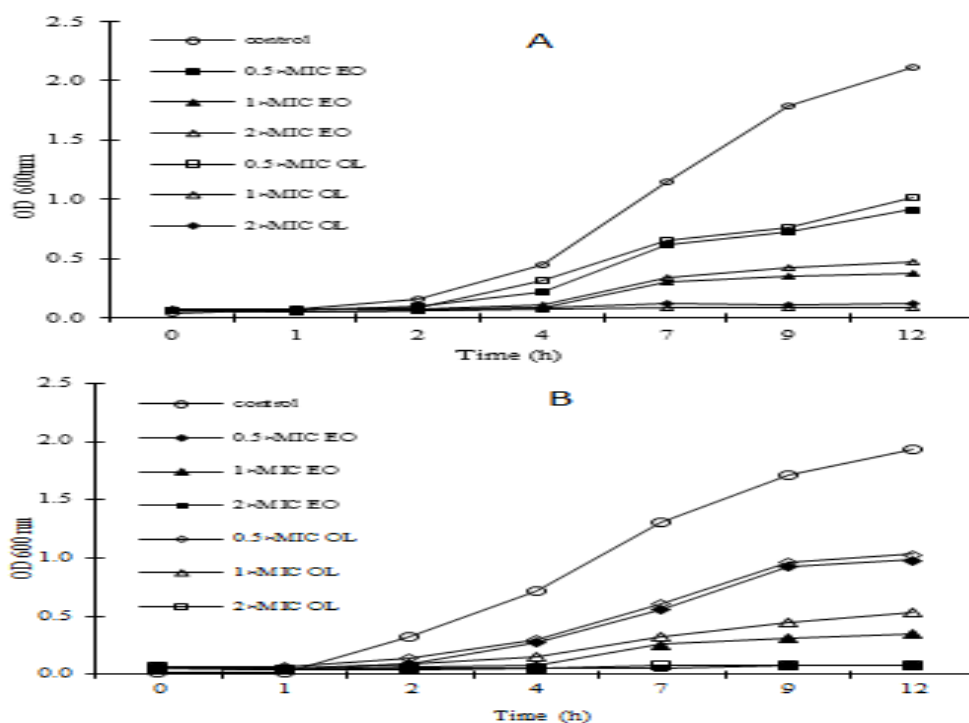
**Table-1.** DIZ value of the essential oil and oleoresin of clove buds

	DIZ (mm)	
	Essential oil	Oleoresin
Gram-positive		
<i>S. aureus</i>	11.4±0.3 d	14.3±0.2 b
<i>B. subtilis</i>	16.5±0.1 c	15.0± 0.2 b
<i>L. monocytogenes</i>	11.3±0.5 d	11.3±0.3 c
Gram-negative		
<i>E. coli</i>	15.2±0.4 c	12.3±0.4 c
<i>S. dysenteriae</i>	18.1±0.4 b	16.3±0.5 b
<i>S. typhimurium</i>	25.2±0.3 a	27.1±0.6 a

<sup>a</sup> Values represent means of three independent replicates ± SD. Different letters within a column indicate statistically significant differences between the means ( $p < 0.05$ ) for DIZ.

**Table-2.** MIC and MBC value of essential oil and oleoresin of clove buds

	essential oil (mg/mL)		oleoresin (mg/mL)	
	MIC	MBC	MIC	MBC
Gram-positive				
<i>S. aureus</i>	0.625	1.25	0.625	1.25
<i>B. subtilis</i>	0.313	0.313	0.313	0.313
<i>L. monocytogenes</i>	0.313	0.313	0.313	0.625
Gram-negative				
<i>E. coli</i>	0.625	0.625	0.625	1.25
<i>S. dysenteriae</i>	0.313	0.625	0.625	0.625
<i>S. typhimurium</i>	0.313	0.625	0.625	0.625



**Fig-1.** Effect of the essential oil and oleoresin on the viability of the tested *S. aureus* (A) and *E. coli* (B)