# CHEMICAL COMPOSITION AND ACTIVITY OF ETHANOL EXTRACT FROM CLOVE BUDS (*SYZYGIUM AROMATICUM* L.)

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**TÓM TẮT**

## THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH CỦA DỊCH CHIẾT ETHANOL TỪ NỤ ĐINH HƯƠNG (*SYZYGIUM AROMATICUM* L.)

*Quá trình chiết nụ đinh hương (Syzygium aromatum L.) bằng kỹ thuật ngâm chiết với ethanol kết hợp siêu âm đã được thực hiện. Các yếu tố khác nhau ảnh hưởng đến hiệu suất chiết như nồng độ dung môi, loại nguyên liệu thô và tỷ lệ dung môi đã được nghiên cứu. Kết quả cho thấy hiệu suất chiết đạt 8,15 ± 0,12% trong điều kiện thích hợp nhất. Mẫu chiết xuất thu được trong điều kiện tối ưu có hàm lượng polyphenol tổng số là 384,8 ± 2,1 mg GAE/g dịch chiết. Phân tích thành phần dễ bay hơi của mẫu chiết bằng Headspace GC/MS đã phát hiện 34 hợp chất hóa học. Dịch chiết có khả năng chống oxy hóa mạnh chống lại gốc tự do DPPH với giá trị $IC_{50}$ là 3,61 ± 0,21 (µg/mL) và ức chế hai chủng vi khuẩn Escherichia coli và Staphylococcus aureus (giá trị MIC tương ứng là 6,25 mg/mL đối với cả hai chủng vi khuẩn được thử nghiệm).*

**Từ khoá:** *Nụ đinh hương, siêu âm, polyphenol, hoạt tính sinh học.*

## 1. INTRODUCTION

Cloves, scientifically known as *Syzygium aromaticum* L., are a type of plant from the Myrtaceae family. The dried flower buds have a distinct aroma. According to historical records, the emperors of China's Song Dynasty (960-1279 AD) forced their maids to suck on clove buds to keep their breath fresh and teeth healthy. At that time, cloves were mainly imported from the Moluccas and originated from Austronesian polities such as Java, Srivijaya, Champa, and Butuan [1]. Clove buds consist of eugenol, acetyl eugenol, *β*-caryophyllene, vanillin, crategolic acid, flavonoids such as eugenin, kaempferol, rhamnetin, and eugenitin, sesquiterpenes, triterpenoids such as oleanolic acid, stigmasterol, campesterol, tannins such as bicornin, gallotannic acid, methyl salicylate [2, 3]. Shan *et al.* determined the high polyphenol content of clove bud extract by HPLC analysis. The results showed that clove buds have a total polyphenol content equivalent to 14.38 g of gallic acid (converted)/100 g of dry ingredients. Moreover, clove bud extract contains relatively high flavonoid content compared to some surveyed herbs [4]. Recent publications have confirmed that cloves are important herbs with several benefits. Products made from essential oils and clove extracts have a broad scope of application due to their pharmacological effects and biological activities. The antioxidant activity of clove extract was screened using various

*in vitro* models, including *β*-carotene linoleate, ferric thiocyanate, and DPPH [5]. The strong antibacterial effect of aqueous extract from cloves has been successfully tested on three strains of microorganisms: *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* [6].

This study aimed to extract ethanol from clove buds using soaking extraction techniques combined with ultrasound. The extract's total polyphenol content and major chemical components were analyzed, and some biological activities of the extract (antioxidant and antibacterial) were investigated.

## 2. MATERIALS AND METHODS

### 2.1 Materials and chemicals

Clove buds were obtained from a farm in the Kulon Progo district of Yogyakarta, Indonesia, provided by Thanh Binh Pharmaceutical Company in Ho Chi Minh City. These buds are cleaned and chopped before being convection-dried at a temperature of 70°C until the humidity reaches less than 5%. After drying, the cloves are placed in a vacuum bag to preserve them for further research. Ethanol with 98% purity is provided by Chemsol in Vietnam. Various chemicals such as gallic acid, folin-ciocalteau, sodium carbonate, quercetin, aluminum chloride, sodium acetate, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and vitamin C are purchased from Sigma-Aldrich in the USA.

### 2.2. Ethanol extraction process from clove buds

After measuring the moisture content, a dry sample of the material was soaked in ethanol of varying concentrations for 24 hrs. at room temperature. This mixture was then sonicated at a frequency of 20 KHz for 10 min. The process was repeated thrice before pressing and removing the extract residue. The extract was centrifuged at 4000 *rpm* for 10 min. and filtered using Whatman filter paper (3 *μm*, 110 *mm*). The solvent of the extract was then removed on a rotary evaporator (45 $^{o}$C) to obtain a brown extract with a characteristic aroma [7].

Several effects of the extraction efficiency factors to determine the most suitable conditions for the extraction process were investigated. Specifically, the ethanol concentration ratio varied between 30-98%, and the ratio between the raw material and solvent (*m/v*) ranged from 1:25 - 1:5. The extraction efficiency was calculated using the formula:

$$\mathrm{H(extraction)} = \frac{m2}{m1}100\%$$

where $m_1$ is the weight of clove buds and $m_2$ is the volume of extract. (The weight of raw materials and products was calculated after deducting the amount of moisture).

### 2.3. Analysis methods

*2.3.1. Chemical composition analysis*

The total polyphenol content of a substance was analyzed using the method developed by Singleton *et al.* [8]. A reaction mixture was created by shaking 250 *μL* of the extract in 250 μL of water and 250 *μL* of folin-ciocalteu reagent together. Next, 250 *μL* of 10% $Na_2CO_3$ was added, and the mixture was incubated for 30 min. at 40 $^{o}$C in a thermostatic bath. The spectral absorbance of the reaction mixture was then measured at 765 *nm*. A standard curve equation to determine the polyphenol content was created using gallic acid as a standard. The polyphenol content was expressed as gallic acid equivalent in 1 g of extract (*mg* GAE/g extract).

The volatile composition of the ethanol extract from clove buds was analyzed

using a Headspace HS-20 instrument (Shimadzu, Japan). The sample was placed into the incubation chamber at 90 °C, and the equilibration time was 30 min. The sample injection temperature was set to 150 °C, and the sample transfer temperature was also 150 °C. The analysis was performed using a gas chromatograph GC-2030 (Shimadzu) coupled with MS-QP2020 (Shimadzu), which used a Rxi-5MS capillary column (length: 30 *m*, inner diameter: 0.25 *mm*, film thickness: 0.25 *µm*, Shimadzu, Japan). The thermal program was as follows: the column head temperature was held at 40 $^{o}$C for 3 min., then increased to 150 $^{o}$C at a rate of 5 $^{o}$C/min., further to 200 $^{o}$C at a rate of 10 $^{o}$C/min., at last to 280 $^{o}$C at a rate of 15 $^{o}$C/min. and held for 5 min. The ion source temp. was set to 250 $^{o}$C, and the interface temp. was also 250 $^{o}$C. Helium was used as the carrier gas with a 1.78 *mL*/min. flow rate. The stream was split at a ratio of 1:10 (split), and the column head pressure was set to 100 kPa.

*2.3.2. Analysis of antioxidant activity*

Antioxidant activity was measured using the DPPH method [9]. DPPH is a stable, purple free radical that reacts with antioxidant agents (donating H) and changes to a reduced form with a pale yellow color. The UV-Vis absorbance of the test sample was measured at a wavelength of 517 *nm* to determine the % inhibition (I). Based on the % inhibition at different concentrations of the test sample, the anti-oxidation ability of the sample was evaluated using the $IC_{50}$ value. The $IC_{50}$ value is defined as the concentration of a test sample that can inhibit 50% of free radicals. The lower the $IC_{50}$ value, the higher the activity of the sample, and vice versa. Vitamin C was used as the positive control in the test.

To conduct the experiment, vitamin C and extract were mixed at concentrations within the survey limit range (*µg/mL*) with methanol. DPPH was dissolved in methanol solvent at a concentration of 1000 *µg/mL*. The reaction mixture consisted of 40 *µL* DPPH (1000 *µg/mL*) and 960 *µL* extract solution. The mixture was incubated in a dark chamber at room temperature for about 30 min., and then the spectral absorbance of DPPH at 517 *nm* was measured. The measurement process was repeated 3 times. The $IC_{50}$ value was calculated based on the percentage of inhibition and test sample concentration.

*2.3.3. Analysis of antibacterial activity*

The agar diffusion method to determine antibacterial activity described by Oonmetta-Aree *et al.* [16] was used. The study utilized bacterial strains such as *E.coli* and *S. aureus*, provided by the Quality Measurement Standards Technical Center 2 (Quatest 2). These strains were cultured and preserved at the laboratory of the International Analysis Center, Ho Chi Minh City University of Industry and Trade.

The experiment involved spreading 100 *µL* of bacterial solution (concentration 106 CFU/*mL*) evenly on Buffered Pepton Water (BPW) agar. The extract was dissolved in sterile distilled water at various concentrations (100.0, 50.0, 25.0, 12.5, and 6.25 *mg/mL*). Then, 50 *µL* of the extract was dropped several times into the agar holes (6 *mm* diameter) on the petri dish. The agar plate was incubated at 37 °C for 24 hrs. to produce Chloramphenicol (100 *ppm*). The negative control was sterile distilled water.

The ability of the extract to inhibit the bacteria was determined by measuring the diameter of the sterile ring created around the agar hole. The Minimum Inhibitory

Concentration (MIC) value of the extract was determined as the lowest concentration of the test solution capable of inhibiting the growth of microorganisms. The experiment was repeated three times, and the average diameter value was taken.

## 3. RESULTS AND DISCUSSION

### 3.1. Factors affecting extraction efficiency

According to survey data, the efficiency of the extraction process is significantly influenced by the concentration of ethanol used and the ratio of raw material to solvent. The relationship between these factors and the resulting performance is illustrated in *Figure 1*.

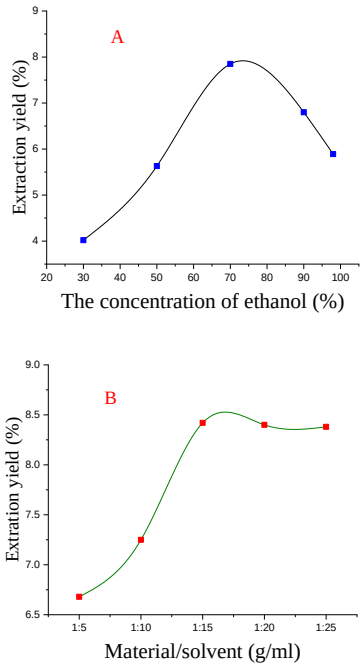


Figure 1. Effect of solvent concentration (A) and NL/DM ratio (B) on extraction efficiency

*Figure 1A* shows that the highest extraction efficiency (7.85%) is achieved at 70% ethanol concentration. This indicates that phenolic acid and flavonoid components make up a significant portion of the extract same as previous research by Lesmana D. and Afrendi E. [7,14]. The highest efficiency (8.05%) of *Figure 1B* was demonstrated at an NL/DM ratio of 1:15 (*m/v*). However, as the amount of solvent increases, the decreasing efficiency tends slightly because the diffusion of active ingredients in plant cells is more favorable with a larger amount of solvent. It is important to note that using a large amount of solvent can cause disadvantages during the solvent removal stage. Longer solvent removal times increase the amount of volatile substances lost due to evaporation or thermal decomposition of some gas-sensitive components in the extract mixture. After careful consideration, the most suitable conditions for the extraction process were identified as follows: 70% ethanol concentration, NL/DM ratio of 1:15, a soaking time of 72 hrs., and an ultrasound time of 30 min. Experimental verification at the optimal point resulted in an extraction efficiency of 8.15 ± 0.12%.

### 3.2. Results of chemical composition analysis of the extract

The extract samples were analyzed under ideal conditions using the folin-ciocalteu method to determine their polyphenol content. The calculation was based on the gallic acid standard curve equation. The results indicated that the total polyphenol content of the ethanol extract sample from clove buds was 384.8 ± 2.1 *mg* GAE/g extract.

Table 1. Some chemical components of ethanol extract from clove buds

| Peaks | Chemical compounds | Molecular formula | Retention time (min.) | Ratios (%) |
|---|---|---|---|---|
| 8 | Furfural | $C_5H_4O_2$ | 5.195 | 0.43 |
| 15 | Allylphenol | $C_9H_{10}O$ | 18.692 | 0.6 |
| 18 | Eugenol | $C_{10}H_{12}O_2$ | 21.677 | 41.58 |
| 21 | Caryophyllene | $C_{15}H_{24}$ | 23.525 | 5.84 |
| 22 | alpha.-Humulene | $C_{15}H_{24}$ | 24.474 | 0.61 |
| 24 | Eugenolacetate | $C_{12}H_{14}O_3$ | 27.358 | 2.98 |
| 28 | Caryophyllene oxide | $C_{15}H_{24}O$ | 27.358 | 0.39 |

The headspace GC/MS technique was used to identify volatile compounds in the extract. The results confirmed the presence of 34 different compounds, including several that play important roles in biological effects and plant flavors, such as eugenol (subs. 18), eugenol acetate (subs. 24), and caryophyllene (subs. 21). This confirms that the ultrasound-assisted extraction technique not only produces extracts with a higher total polyphenol content than the traditional method but also minimizes the loss of volatile components by performing the extraction and solvent evaporation processes at temperatures below 45 °C. *Table 1* and *Figure 2* provide spectral signals and characteristics of some typical compounds.

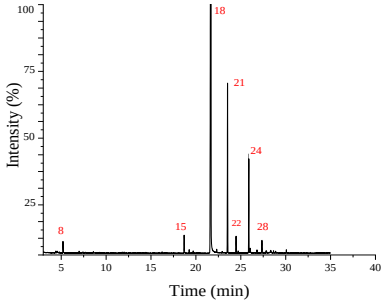


Figure 2. GC - headspace chromatogram of ethanol extract from clove buds

### 3.3. Results of analyzing the antioxidant activity of the extract

The neutralization of DPPH free radicals is an old method used to determine antioxidant activity. This method is based on the ability to convert to DPPH-H, which is the reduced form of DPPH radicals when receiving hydrogen from antioxidants. The amount of free radicals of DPPH decreases proportionally to the extract concentration as the antioxidant content increases.

*Table 2* shows the results of the antioxidant activity test. The ethanol extract from clove buds showed an inhibition of 85,788 ± 0.7% of free radicals at a concentration of 0.8 *µg/mL*. The ability to remove DPPH free radicals is proportional to the extract concentration. As the concentration increases from 1.6 *µg/mL* to 8.0 *µg/mL*, the ability to remove free radicals gradually increases from 30,434 ± 0.25% to 85,788 ± 0.7%.

Table 2. Results of analyzing the antioxidant activity of the extract

| Entry | Conc. of extract (µg/mL) | Inhibition I (%) | $IC_{50}$ (µg/mL) |
|---|---|---|---|
| 1 | 1.6 | 30.434 ± 0.25 | |
| 2 | 3.2 | 48.848 ± 0.8 | |
| 3 | 4.8 | 62.375 ± 0.5 | **3.61 ± 0.21** |
| 4 | 6.4 | 73.597 ± 0.4 | |
| 5 | 8.0 | 85.788 ± 0.7 | |

The ethanol extract from clove buds was compared with vitamin C as a positive control for its ability to neutralize DPPH free radicals (*Figure 3*). The results indicated that all concentrations fell within the survey limits. The DPPH neutralization efficiency of the extracts was slightly lower than that of vitamin C. Based on the standard curve, the $IC_{50}$ value for the ethanol extract from clove buds was calculated to be 3.61 *µg/mL*, while vitamin C had an $IC_{50}$ value of 3.316 *µg/mL*. Additionally, recent publications have shown that the ethanol extract from clove buds exhibits outstanding antioxidant effects compared to other plant extracts. For example, a streptocaulon leaf extract (*Streptocaulon juventas*) demonstrated the highest antioxidant ability at 72.07% at a concentration of 500 *µg/mL* [10], and guava leaf extract neutralized 74.4% of free radicals at a concentration of 100 *µg/mL* [11]. This suggests a linear relationship between the antioxidant effect and the total polyphenol content in plant extracts, which is consistent with previous reports by Paixao N.[12] and Stratil P.[13].

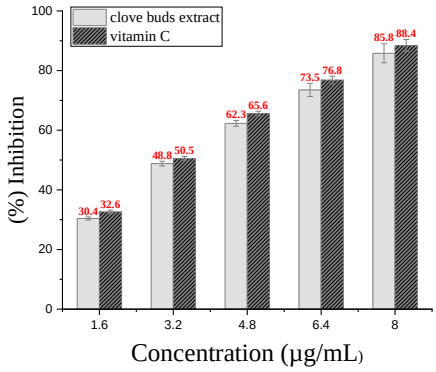


Figure 3. DPPH antioxidant capacity of extract and vitamin C

### 3.4. Results of analyzing the antibacterial activity of the extract

The study found that the ethanol extract obtained from clove buds has an antibacterial effect on both *E. coli* and *S. aureus* bacterial strains. Both strains' resistance level depends on the extract concentration used. The inhibitory effect of the extract was measured by the antibacterial ring diameter for each tested concentration, the results of which are listed in *Table 3.*

*Table 3.* Antibacterial circle diameter (mm) of extract at various concentrations for bacterial strains

| Entry | Conc. of extract (mg/mL) | *E.coli* | *S. aureus* |
|---|---|---|---|
| 1 | 100 | 17.4 ± 0.45 | 19.2 ± 0.25 |
| 2 | 50 | 13.4 ± 0.34 | 15.8 ± 0.2 |
| 3 | 25 | 8.82 ± 0.22 | 9.28 ± 0.52 |
| 4 | 12.5 | 6.39 ± 0.38 | 6.90 ± 0.32 |
| 5 | 6.25 | 0 | 0 |
| 6 | Chloramphenicol (100 ppm) | 8.42 ± 0.2 | 9.12 ± 0.32 |

Data obtained from the study indicated that the ethanol extract from clove buds was more effective in inhibiting the growth of *S. aureus* at nearly all concentrations. The largest inhibition zone in the *S. aureus* strain reached 19.2 ± 0.25 *mm*, while in the *E. coli* strain, it was 17.4 ± 0.45 *mm*. The inhibitory effect of Chloramphenicol against the corresponding bacterial strains was measured at 8.42 *mm* and 9.12 *mm*, respectively, equivalent to the extract at a concentration of 25 *mg/mL*. Furthermore, the study also revealed the minimum inhibitory concentration (MIC) value at 6.25 *mg/mL* for both tested bacterial strains.

The antibacterial activity of the clove bud ethanol extract in this study was slightly less effective than that of the clove juice extract used in the publication by Shehu I., *et al.* [15]. According to the study, the antibacterial circle diameter of the aqueous extract at a concentration of 100 *mg/mL* reached 20 *mm* for *E. coli* and 21 *mm* for *S. aureus*.

## 4. CONCLUSION

Ethanol was used to extract compounds from clove buds (*S. aromaticum*), using a soaking technique combined with ultrasound. Chemical composition analysis revealed that the extract had a total polyphenol content of 384.8 ± 2.1 *mg* GAE per gram of extract. *In vitro* testing of ethanol extracts from clove buds demonstrated that they possess strong antioxidant properties (85.788 ± 0.74% resistance to DPPH free radicals at a concentration of 115.2 *µg/mL* and an $IC_{50}$ value of 3.61 ± 0.21 *µg/mL*). Furthermore, the extract exhibited a relatively good inhibitory effect on strains of *E.coli* and *S.aureus*. These results indicate that the ethanol extract from clove buds has potential applications in the pharmaceutical and cosmetic industries.

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