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# STUDYING ANTIOXIDANT, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *Eleutherine bulbosa* BULBS

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**Abstract**

*In this investigation, the total polyphenol content (TPC) and total flavonoid content (TFC) of E. bulbosa bulbs were quantified using the Folin–Ciocalteu and aluminum chloride methods, respectively. The antioxidant capacity was evaluated using DPPH and ABTS radical scavenging assays. Additionally, the antibacterial and antifungal activities of fractionated extracts from E. bulbosa bulbs were assessed. Results showed that the TPC and TFC in the crude ethanol extracts of E. bulbosa bulbs were 93.51 ± 0.60 mg GAE/g and 32.25 ± 0.25 mg QE/g extract, respectively. The antioxidant activity of the ethyl acetate extract from E. bulbosa bulbs exhibited significant potency, with $IC_{50}$ values of 103.49 µg/mL and 141.25 µg/mL for the DPPH and ABTS assays, respectively. The ethyl acetate extract demonstrated the highest antibacterial activity against A. hydrophila, with the largest zone of inhibition measuring 26.51 ± 0.15 mm. It also indicated a potent antifungal effect of E. bulbosa bulb extracts, with 96.8% inhibition against B. dothidea at 312.5 µg/mL and a MIC value of 10,000 µg/mL. Therefore, the development of innovative food and pharmaceutical products derived from the bulbs of E. bulbosa is of considerable interest.*

**Keywords:** *Antioxidant activity, antibacterial activity, antifungal activity, Eleutherine bulbosa.*

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# NGHIÊN CỨU HOẠT TÍNH SINH HỌC CỦA CỦ SÂM ĐẠI HÀNH (*Eleutherine bulbosa*)

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**Tóm tắt**

*Trong nghiên cứu này, hàm lượng polyphenol tổng số (TPC) và flavonoid tổng số (TFC) trong cao chiết tổng từ củ Sâm đại hành đã được định lượng. Phương pháp trung hòa gốc tự do DPPH và ABTS được sử dụng để đánh giá khả năng kháng oxi hóa. Hoạt tính kháng khuẩn và kháng nấm của các cao chiết phân đoạn từ củ Sâm đại hành cũng đã được khảo nghiệm. Kết quả nghiên cứu cho thấy, hàm lượng TPC và TFC trong cao chiết tổng ethanol lần lượt là 93,51 ± 0,60 mg GAE/g và 32,25 ± 0,25 mg QE/g. Cao ethyl acetate thể hiện hoạt tính kháng oxi hóa mạnh với giá trị $IC_{50}$ = 103,49 µg/mL và 141,25 µg/mL tương ứng với phương pháp DPPH và ABTS. Hoạt tính kháng khuẩn tốt nhất được ghi nhận ở cao chiết ethyl acetate với vùng ức chế lớn nhất đối với chủng A. hydrophila (26,51 ± 0,15 mm). Kết quả nghiên cứu cũng đã chứng minh khả năng kháng nấm hiệu quả của cao ethyl acetate đối với B. dothidea, đạt 96,8% ở nồng độ 312,5 µg/mL và giá trị MIC đạt 10000 µg/mL. Do đó, việc phát triển các sản phẩm từ củ Sâm đại hành đang ngày càng thu hút sự quan tâm nghiên cứu.*

**Từ khóa:** *Kháng oxi hóa, kháng khuẩn, kháng nấm, Sâm đại hành.*

## 1. Introduction

Natural products are excellent candidates for alternative medicine in disease management. The bulbs of *Eleutherine bulbosa*, a notable member of the Iridaceae family, have significant therapeutic potential. Its red bulbs have long been used for the treatment of rheumatoid arthritis, dysentery, and cardiac diseases. The effectiveness and safety of traditional medicine are closely related to the chemical composition of the plant. The bulb is exceptionally rich in phytochemicals such as phenolic and flavonoid derivatives, naphthalene, anthraquinone, and naphthoquinone (Ieyama et al., 2011; Xu et al., 2014). Jiang et al. (2020) reported the isolation of six compounds from *E. bulbosa*, including three novel polyketides identified as eleubosas A-C. Additionally, our recent study identified eight bioactive compounds from the bulbs of *E. bulbosa*, namely eleutherin, gallic acid, chlorogenic acid, quercetin, kaempferol, rutin, epicatechin gallate, and myricetin (Kamarudin et al., 2021). Morphologically, *E. bulbosa* is an herbaceous, perennial flowering plant with a red bulb resembling an onion. The leaves are approximately 25 cm long, with five to six white flower petals arranged at the apex of the foliage. Chemical and pharmacological studies have revealed the antifungal, antiviral, and anticancer activities of its extracts (Alves et al., 2003; Ieyama et al., 2011). Generally, the extraction of phytoconstituents is considered the basic step in medicinal plant research, with crude extracts and fractions prepared and tested for antioxidant, antibacterial, and antifungal activities. Moreover, scientific evidence concerning the biological activities and potential uses of the bulb of *E. bulbosa* in Vietnam is scarce. Therefore, in this study, the bioactivities, including antioxidant, antibacterial, and antifungal activities, of different extracts from the bulb of *E. bulbosa* were investigated."

## 2. Experimental

### 2.1. Chemicals and reagents

Solvents utilized including *n*-hexane, ethyl acetate, and ethanol 96% were purchased from Chemsol company (Vietnam). DPPH and ABTS were supplied from Thermo Fisher Scientific, USA and AK Scientific, USA. Standard of gallic acid, quercetin, trolox, and cefixim were obtained from Sigma Chemical Co (St. Louis, MO, US).

Biological safety cabinet (Class II BSC, Esco, Indonesia), autoclave (HVE-50, Hirayama, Japan), centrifuge (Mikro 12-24, Hettich, Germany), were measured for antibacterial and antifungal activities.

### 2.2. Sample treatment and extraction

The bulbs of *Eleutherine bulbosa* were collected in January 2024 from a cultivated garden in Cho Moi district, An Giang province. The plant was authenticated by Nguyen Thi Kim Hue, and a voucher specimen (Elb032024) was deposited in the Department of Biology, College of Natural Sciences, Can Tho University. Air-dried and powdered red bulbs of *Eleutherine bulbosa* (3.0 kg) were extracted three times with 96% ethanol at room temperature. The solvent was then completely evaporated using a rotary evaporator under reduced pressure, yielding 270 g of residue. This residue was suspended in water and subsequently partitioned with *n*-hexane, ethyl acetate (EtOAc), and water, respectively. The resulting fractions were concentrated under reduced pressure to yield the corresponding solvent-soluble fractions: *n*-hexane (90 g), EtOAc (65 g), and water-soluble fraction (60 g)

### 2.3. Determinating total polyphenol content and total flavonoid content

The total polyphenol content (TPC) of the crude ethanol extract of *E. bulbosa* was measured using the Folin–Ciocalteu test, based on the protocol developed by Shehata et al.

(2021) with some modifications, using gallic acid as the standard. Briefly, 250 µL of the extract was mixed with 250 µL of distilled water and 250 µL of 10% aqueous Folin–Ciocalteu solution, then stirred and left to stand for 5 minutes. Subsequently, 250 µL of 10% aqueous $Na_2CO_3$ solution was added. The mixture was incubated at 40 °C for 30 minutes, and the absorbance was measured at a wavelength of 765 nm using a UV–Vis spectrophotometer (Jasco V-730 UV–Vis Spectrophotometer, Japan). The TPC of the plant extracts was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

The total flavonoid content (TFC) of the crude ethanol extract of *E. bulbosa* was determined following the procedure described by Shetty et al. (2016) with some modifications. A total of 1 mL of each extract was mixed with 1 mL of distilled water and 200 µL of 5% (w/w) $NaNO_2$. The mixture was incubated for 5 minutes before adding 200 µL of 10% (w/w) $AlCl_3$. After a 6-minute incubation at room temperature, 2 mL of NaOH 1M was added, and the volume was adjusted to 5 mL with distilled water. The absorbance was measured at 510 nm using a UV–Vis spectrophotometer (Jasco V-730 UV–Vis Spectrophotometer, Japan). A quercetin serial dilution was used to construct the TFC standard curve, with the results expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

### 2.4. Antioxidant activity

#### *2.3.1. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay*

In a 96-well microtiter plate, 50 µL of each sample was added to a methanol solution of DPPH at a concentration of $6 \times 10^{-5}$ M. After thorough mixing using a vortex mixer, the mixture was incubated for 30 minutes at room temperature. The absorbance was then measured at 517 nm. The DPPH radical scavenging activity was calculated as a percentage relative to the control. Vitamin C was used as a positive standard (Sharma & Bhat, 2009).

#### *2.3.2. The 2,2'-azinobis (3- ethylbenzothiazoline-6-sulfonic acid (ABTS) Assay*

The free radical 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) was generated by reacting ABTS solution in methanol (7 mM) with an aqueous potassium persulfate solution (2.45 mM). The resulting mixture was allowed to stand in the dark for 12-16 hours before use. For the aqueous extract, the ABTS solution was diluted with phosphate-buffered saline (PBS) (pH = 7.4) to achieve an absorbance of 0.700 ± 0.002 at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard to construct the calibration curve (Nenadis et al., 2004).

### 2.5. Antibacterial activity

In the present study, the antibacterial activity of extracts from *E. bulbosa* was assessed using the agar well diffusion method, with measurements taken of the diameter of growth inhibition zones and the minimal inhibitory concentration (MIC).

Antimicrobial activity was evaluated in triplicate using three standard bacterial strains obtained from the Research Institute for Aquaculture No. 2, Vietnam: *Aeromonas dhakensis*, *Aeromonas hydrophila*, and *Vibrio parahaemolyticus*, which are major pathogens in the aquaculture industry. The bacteria were cultured on tryptic soy agar (TSA, Acumedia, USA) for 24 hours at 37 °C. From each culture, a cell suspension was prepared in an aqueous 0.85% NaCl solution and adjusted to a 0.5 McFarland turbidity standard. A swab was then used to inoculate the bacteria onto the surface of Müeller-Hinton agar (Merck, Germany) plates (95 × 15 mm). Subsequently, 50 µL of each sample (dissolved in DMSO) was placed in 9 mm diameter wells punched into the agar medium. The plates were incubated at 37 °C

for 24 hours. After incubation, samples that produced inhibition zones around the wells were considered active. Cefixime (Sigma, USA) and DMSO were used as positive and negative controls, respectively.

To determine the minimal inhibitory concentration (MIC), a broth microdilution assay was conducted using Müeller-Hinton broth (MHB, Biolife, Italy) and standard bacterial inocula ($1.5 \times 10^6$ CFU/mL). Two-fold serial dilutions of the extracts were prepared, resulting in final concentrations ranging from 0.3 to 5.0 µg/mL. After incubation at 37 °C for 24 hours, the experiment was assessed by withdrawing 10 µL of sample from each well with no visible bacterial growth and subculturing it on TSA. The MIC was defined as the lowest concentration of the tested substance that prevented visible bacterial growth (Yeo et al., 2014).

### 2.6. Antifungal activity

Antifungal activities of the different plant extracts were assessed using the poisoned food technique as described by Adjou et al. (2012). Potato dextrose agar (PDA) medium was prepared, and the test samples were added to achieve final concentrations of 312.5, 625, 1250, 2500, 5000, and 10,000 µg/mL. Fungal strains were cut into small pieces approximately 6 mm in diameter. Using sterile forceps, these fungal pieces were placed in the center of Petri dishes containing the extract. The diameter of the mycelium was measured after 7 days of incubation at 28 °C. At the end of the incubation period, the plates were examined for any zones of inhibition. The diameter of the inhibition zones was measured and recorded. The percentage of inhibition of mycelial growth was calculated according to the following formula: (%) = (1 − dt/dc) × 100; where dc (mm) is the mean colony diameter in the control sets and dt (mm) is the mean colony diameter in the treatment sets. The Minimum Inhibitory Concentration (MIC) was determined as the least concentration that showed an inhibitory effect on test organism (Ye et al., 2013; Salama & Marraiki, 2010).

The fungi tested in this study were *Botryosphaeria dothidea* and *Lasiodiplodia theobromae* being known to cause serious diseases in agricultural regions of Vietnam.

### 2.7. Statistical analysis

Variation in the data was assessed using one-way analysis of variance (ANOVA). The results, derived from three independent experiments, are presented as mean ± SD, with n = 3. Statistical significance was determined based on a p-value of $< 0.05$.

## 3. Results and Discussion

### 3.1. Determinating total phenolic and flavonoid contents

The total polyphenol content (TPC) and total flavonoid content (TFC) of *E. bulbosa* were quantified. The regression equations for the standard curves of gallic acid and quercetin were $y = 0.0465x + 0.0688$ ($R^2 = 0.9967$) and $y = 0.0079x + 0.0883$ ($R^2 = 0.995$), respectively. These results indicate a strong linear relationship within the detection ranges.

In this study, the TPC and TFC in the crude ethanol extracts of *E. bulbosa* bulbs were 93.51 ± 0.60 mg GAE/g extract and 32.25 ± 0.25 mg QE/g extract, respectively.

The biological effects of many plant materials are influenced by their polyphenol and flavonoid content; thus, studying the variation in these compounds is crucial. Research has investigated the total phenolic content in *E. bulbosa* bulbs, which belong to the Iridaceae family. For instance, Morabandza et al. (2016) reported a total phenolic content of 27.12 ± 0.62 mg GAE/g extract in ethanol extracts of *E. bulbosa* bulbs. Additionally, Syariful et al. (2023) found a lower total phenolic content of 20.28 ± 3.22 mg GAE/g extract in *E. bulbosa* bulbs. Moreover, Islamudin et al. (2023) reported a TPC range of 12.56 to 45.82 mg GAE/g

extract. These results indicate that the ethanolic extract of *E. bulbosa* bulbs from Vietnam has a higher TPC compared to those reported in these studies.

Rahmi et al. (2021) investigated the ethanol extracts of *E. bulbosa* bulbs from three different locations in Indonesia and reported a lower total flavonoid content, ranging from 5.04 ± 0.39 mg QE/g extract to 7.59 ± 0.04 mg QE/g extract. Similarly, Munaeni et al. (2019) found a lower flavonoid content in ethanol extracts of *E. bulbosa* bulbs, with a value of 6.61 ± 0.00 mg QE/g extract. Such variability may be attributed to differences in geographical origins or extraction methods.

### 3.2. *In vitro* antioxidant activity results

The free radical scavenging activities of all extracts, as measured by the DPPH and ABTS methods, are presented in Table 1.

**Table 1. $IC_{50}$ values of different extracts**

| Plant Extracts | $IC_{50}$ (μg/mL) | |
|---|---|---|
| | **DPPH** | **ABTS** |
| Crude | 165.66 ± 14.35 | 181.58 ± 22.35 |
| *n*-Hexane | 282.23 ± 16.29 | 360.09 ± 20.57 |
| Ethyl acetate | 103.49 ± 9.73 | 141.25 ± 20.32 |
| **Positive Control** | | |
| Vitamin C | 3.98 ± 0.74 | |
| Trolox | | 3.41 ± 0.07 |

All the extracts exhibited concentration-dependent DPPH radical scavenging activities which were in the following order: ethyl acetate extract > crude extract > *n*-hexane extract. Ethyl acetate extract *E. bulbosa* bulbs exhibited good scavenging potential with $IC_{50}$ = 103.49 μg/mL and 141.25 μg/mL for DPPH and ABTS, respectively. The ethyl acetate extract likely had the strongest antioxidant activity due to its ability to selectively extract compounds with high antioxidant potential. Many antioxidants, such as flavonoids, phenolic acids, and tannins, are polar or semi-polar and are more efficiently extracted by ethyl acetate than by non-polar solvents like *n*-hexane.

Several studies have demonstrated that phenols possess significant antioxidant capacity (Sultana et al., 2007; Islam et al., 2016). The results indicate that *E. bulbosa* bulbs exhibit notable antioxidant potential, consistent with their high total phenolic content (TPC) and total flavonoid content (TFC).

### 3.3. *In vitro* antibacterial activity results

Bacterial infections are a major cause of mortality in aquaculture. Due to increasing bacterial resistance to commercial standard and reserve antibiotics, there is a growing need to identify new substances with antibacterial activity against pathogenic bacteria (Mundt et al., 2003). Medicinal plants may offer promising alternatives for treating fish pathogens in aquaculture (Turker et al., 2009). In this study, extracts of *E. bulbosa* bulbs were screened for antibacterial activity against three bacterial strains commonly found in aquaculture: *Aeromonas hydrophila*, *Aeromonas dhakensis*, and *Vibrio parahaemolyticus* (Buller et al., 2004). The results presented in Table 2 indicate that all extracts exhibited varying degrees of antimicrobial activity against the tested microorganisms. The disc diffusion method results showed that the ethyl acetate extract of *E. bulbosa* bulbs demonstrated the strongest antibacterial activity against the fish pathogenic bacteria compared to the other extracts, with the most notable activity observed against *A. hydrophila* (26.51 mm inhibition zone). The

ethyl acetate extract of *E. bulbosa* bulbs may serve as a potential alternative to traditional antibiotics in the aquaculture sector.

**Table 2. Zone of inhibition and MIC of extracts**

| **Extracts** | | **Diameter of growth inhibition zones at different concentrations** | | | | | **MIC mg/mL)** |
|---|---|---|---|---|---|---|---|
| **Extracts (mg/mL)** | | **0.3125** | **0.625** | **1.250** | **2.500** | **5.000** | |
| **Crude extract** | *A. hydrophila* | 14.23±0.28$^{e}$ | 15.57±0.38$^{d}$ | 16.45±0.50$^{c}$ | 17.33±0.14$^{b}$ | 18.25±0.54$^{a}$ | MIC≤0.3125 |
| | *A. dhakensis* | 15.66±0.15$^{e}$ | 16.63±0.24$^{d}$ | 17.08±0.45$^{c}$ | 18.81±0.19$^{b}$ | 20.26±0.28$^{a}$ | MIC≤0.3125 |
| | *V. parahaemolyticus* | 14.43±0.17$^{e}$ | 15.66±0.22$^{d}$ | 16.68±0.29$^{c}$ | 17.71±0.12$^{b}$ | 18.86 ±0.45$^{a}$ | MIC≤0.3125 |
| ***n*-Hexane extract** | *A. hydrophila* | 10.13±0.26$^{d}$ | 10.27±0.10$^{d}$ | 11.33±0.21$^{c}$ | 12.60±0.24$^{b}$ | 14.54±0.29$^{a}$ | MIC≤0.3125 |
| | *A. dhakensis* | 13.25±0.25$^{c}$ | 10.86±0.19$^{d}$ | 16.23±0.28$^{b}$ | 17.80 ±0.33$^{a}$ | 10.79±0.25$^{d}$ | MIC≤0.3125 |
| | *V. parahaemolyticus* | 12.95±0.30$^{d}$ | 13.65±0.20$^{c}$ | 14.67±0.18$^{b}$ | 14.83±0.34$^{b}$ | 15.23±0.28$^{a}$ | MIC≤0.3125 |
| **Ethyl acetate extract** | ***A. hydrophila*** | **19.93±0.25$^{d}$** | **22.67±0.28$^{c}$** | **24.86±0.24$^{b}$** | **26.33±0.30$^{a}$** | **26.51±0.15$^{a}$** | **MIC≤0.3125** |
| | ***A. dhakensis*** | **18.24±0.28$^{e}$** | **19.66±0.34$^{d}$** | **21.34±0.16$^{c}$** | **25.10±0.30$^{a}$** | **24.68±0.16$^{b}$** | **MIC≤0.3125** |
| | ***V. parahaemolyticus*** | **21.23±0.13$^{c}$** | **21.44±0.24$^{c}$** | **22.43±0.24$^{b}$** | **22.30±0.20$^{b}$** | **23.63±0.38$^{a}$** | **MIC≤0.3125** |
| **Control (µg/mL)** | | **8** | **16** | **32** | **64** | **128** | **MIC (µg/mL)** |
| **Cefixim** | *A. hydrophila* | 22.83±0.58$^{c}$ | 23.00±1.20$^{c}$ | 26.67±2.31$^{b}$ | 30.97±1.53$^{a}$ | 31.16±0.47$^{a}$ | MIC≤8 |
| | *A. dhakensis* | - | - | - | 8.97±0.48$^{b}$ | 15.03±0.56$^{a}$ | MIC=64 |
| | *V. parahaemolyticus* | - | - | - | 10.54±0.14$^{b}$ | 12.75±0.62$^{a}$ | MIC=64 |

*Note: (-): no inhibitory effect. Values are presented as means ± SE (n = 3). Different letters (a, b, c, d, e) in the same row show sinificant difference at the level of 0.05. The results were statistically analyzed using Minitab 16 software (ANOVA-Turkey's).*

Previous studies have demonstrated antibacterial activity of *E. bulbosa* bulbs extracts. For instance, Munaeni et al. (2019) reported that an extract of *E. bulbosa* bulbs could inhibit *Vibrio parahaemolyticus* at a concentration of 10 mg/mL, resulting in the largest zone of inhibition (11.83 ± 0.06 mm). In another study, Munaeni et al. (2017) found that ethanol extracts of *E. bulbosa* bulbs significantly inhibited the growth of *Vibrio harveyi* in a dose-dependent manner compared to chloramphenicol. Additionally, Jiang et al. (2020) investigated the antimicrobial activity of active fractions extracted from *E. bulbosa* bulbs against pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The antibacterial activity of *E. bulbosa* bulbs extracts has been attributed to secondary metabolite compounds, including flavonoids and phenolic compounds, which are known to inhibit bacterial growth (Negi et al., 2012).

### 3.4. *In vitro* antifungal activity results

Plant extracts have demonstrated exceptional biological activities against microorganisms (Arif et al., 2009). In this study, *E. bulbosa* bulbs extracts were screened for antifungal activity against two strains of fungi including *Botryosphaeria dothidea* and *Lasiodiplodia theobromae*, which commonly occur in agriculture sectors (Chen et al., 2016; Xing et al., 2023). As shown in Table 3, all *E. bulbosa* bulbs extracts used in this study exhibited varying degrees of antifungal activity against the *Botryosphaeria dothidea* and *Lasiodiplodia theobromae* strains and increased linearly with increase in concentration of samples. However, the ethyl acetate extract of *E. bulbosa* bulbs was the most effective among the extracts tested. In fact, this extract exhibited potent antifungal effect against *B. dothidea* with the percentage inhibition from 96.8% at the concentration of 312.5 µg/mL and an MIC value of 10,000 µg/mL. *E. bulbosa* is one of the most popular nutritional and medicinal plants, rich in a variety of secondary metabolites, including polyphenols.

The presence of phenolic compounds in the ethyl acetate extract likely contributes to its potent inhibitory effect against fungal strains. Phenolic compounds are well-documented for their biological activity and antimicrobial properties, suggesting that the antifungal

activity of the ethyl acetate extract may be due to these compounds (Bansal et al., 2013). Several mechanisms have been proposed to explain the antimicrobial effects of polyphenols, including their ability to neutralize bacterial toxins, inhibit biofilm formation, reduce adhesion to host ligands, inhibit enzymes, interact with eukaryotic DNA, and disrupt membranes (Barile et al., 2007).

In a study conducted by Kusuma et al. (2010), eleutherin isolated from *E. bulbosa* bulbs was reported to significantly inhibit *Trichophyton mentagrophytes* in an agar diffusion assay. Similarly, Alves et al. (2003) investigated the growth of *Cladosporium sphaerospermum*, a pathogenic fungus affecting plants and marine species, using extracts from *E. bulbosa* bulbs. Their study demonstrated potent inhibitory activity of the extract in a bioautography assay.

**Table 3. The percentage of inhibition and MIC values of different extracts**

| **Extracts and isolated compounds** | | **The percentage of inhibition at different concentrations** (%) | | | | | | **MIC** (µg/mL) |
|---|---|---|---|---|---|---|---|---|
| Extracts (µg/mL) | | 312.5 | 625 | 1250 | 2500 | 5000 | 10000 | |
| Crude extract | *B. dothidea* | 39.1±3.0$^{d}$ | 48.1±6.8$^{cd}$ | 57.2±7.1$^{bc}$ | 62.2±6.6$^{bc}$ | 69.7±6.8$^{b}$ | 100.0±0.0$^{a}$ | 10000 |
| | *L. theobromae* | 49.0±3.2$^{e}$ | 53.9±4.8$^{de}$ | 60.8±3.5$^{cd}$ | 71.5±5.9$^{c}$ | 94.0±4.9$^{b}$ | 100.0±0.0$^{a}$ | 10000 |
| *n*-Hexane extract | *B. dothidea* | 30.9±2.0$^{e}$ | 33.4±0.7$^{d}$ | 36.6±2.1$^{d}$ | 53.4±1.8$^{c}$ | 69.2±1.3$^{b}$ | 100.0±0.0$^{a}$ | 10000 |
| | *L. theobromae* | 18.3±2.4$^{e}$ | 39.1±2.1$^{d}$ | 42.4±2.8$^{d}$ | 59.1±4.7$^{c}$ | 69.8±4.1$^{b}$ | 80.6±4.8$^{a}$ | >10000 |
| Ethyl acetate extract | *B. dothidea* | 96.8±1.6$^{b}$ | 97.7±0.2$^{ab}$ | 97.7±0.2$^{ab}$ | 98.4±1.4$^{ab}$ | 99.2±1.4$^{ab}$ | 100.0±0.0$^{a}$ | 10000 |
| | *L. theobromae* | 80.5±2.1$^{b}$ | 84.4±2.1$^{ab}$ | 84.3±2.1$^{ab}$ | 87.5±2.5$^{a}$ | 88.7±2.6$^{a}$ | 100.0±0.0$^{a}$ | 10000 |
| Control compound (µg/mL) | | 312.5 | 625 | 1250 | 2500 | 5000 | 10000 | |
| Metiram complex | *B. dothidea* | 25.6±8.5$^{d}$ | 65.7±4.7$^{c}$ | 81.4±7.4$^{b}$ | 97.5±0.2$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 5000 |
| | *L. theobromae* | 17.1±4.5$^{f}$ | 25.7±2.3$^{e}$ | 46.8±5.1$^{d}$ | 57.9±1.9$^{c}$ | 79.6±3.2$^{b}$ | 100.0±0.0$^{a}$ | 10000 |

*Note: Values are presented as means ± SE (n = 3). Different letters (a, b, c, d, e) in the same row show sinificant difference at the level of 0.05. The results were statistically analyzed using Minitab 16 software (ANOVA- Turkey's).*

## 4. Conclusion

In the present study, the crude ethanol extract of *E. bulbosa* bulbs was found to contain high amounts of total polyphenols and total flavonoids. The ethyl acetate extract demonstrated significant antioxidant, antibacterial, and antifungal activities, indicating its potential as a source of bioactive metabolites for medicinal and pharmaceutical applications. Consequently, further research on the isolation of bioactive components from this plant is ongoing in our laboratory.

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## References

Adjou, E. S., Kouton, S., Dahouenon-Ahoussi, E., Sohounhloue, C. K., & Soumanou, M. M. (2012). Antifungal activity of *Ocimum canum* essential oil against toxinogenic fungi isolated from peanut seeds in post-harvest in Benin. *International Research Journal of Biological Sciences*, *1*(7), 20–26.

Alves, T.M.A., Kloos, H., & Zani, C.L. (2003). Eleutherinone, a novel fungitoxic naphthoquinone from *Eleutherine bulbosa* (Iridaceae). *Memorias do Instituto Oswaldo Cruz*, *98*(5), 709 –712.

Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R. S., Lavekar, G. S., & Dabur, R. (2009). Natural products – antifungal agents derived from plants. *Journal of Asian Natural Products Research*, *11*(7), 621–638. https://doi.org/10.1080/10286020902942350.

Bansal, S., Choudhary, S., Sharma, M., Kumar, S.S., Lohan, S., Bhardwaj, V., Syan, N., & Jyoti, S. (2013). Tea: a native source of antimicrobial agents. *Food Research International*, *53*, 568-584.

Barile, E., Bonanomi, G., Antignani, V., Zolfaghari, B., Sajjadi, S.E., Scala, F., & Lanzotti, V. (2007). Saponins from *Allium minutiflorum* with antifungal activity. *Phytochemistry*, *68*, 596-603.

Buller, N. B. (2004). *Bacteria from fish and other aquatic animals: A practical identification manual.* CABI Publishing. UK.

Chen, L., Wang, Q., Chen, H, Sun, G. W., Liu, H. X., & Wang, H. K. (2016). *Agrobacterium tumefaciens*-mediated transformation of *Botryosphaeria dothidea*. *World Journal of Microbiology & Biotechnology*, *32*(106), 1-5.

Ieyama, T., Gunawan-Puteri, M.D., & Kawabata, J. (2011). *α*-Glucosidase inhibitors from the bulb of *Eleutherine americana*. *Food Chemistry*, *128(2)*, 308-11. https://doi.org/ 10.1016/j.foodchem.2011.03.021.

Islam, M. N., Kabir, M. S. H., Kader, S. M. A., Mahmudul H., Samrat, E. K., Imran H., Jony, M. N. H., Chowdhury, M. S., Abul H., & Rahman, M. M. (2016). Total phenol, total flavonoid content and antioxidant potential of methanol extract of *Boehmeria platyphylla* D Don leaves. *World Journal of Pharmaceutical Research*, *5*(5), 334–344.

Islamudin, A., Angga, C. N., Riki R. M., Nur, M. Z., Iswahyudi, I., Hajrah, H., Niken, I., Arifuddin, M., Siska, S., Supandi, S., & Neneng, S. S. A. (2023). Optimization of microwave-assisted extraction on polyphenol metabolite from *Eleutherine bulbosa* (Mill.) urb. bulbs using response surface methodology. *Journal of Advanced Pharmaceutical Technology & Research* , *14*(2), 113-118.

Jiang, H., Man, W. J., Hou, A. J., Yang, L., Xing, X. D., Yan, M. L., … Yang, L. (2020). The chemical constituents from the active fractions of *Eleutherine bulbosa* with their antimicrobial activity. *Natural Product Research*, *34*(12), 1743–1749. https://doi.org/10.1080/14786419.2018.1530229

Kamarudin, A.A., Sayuti, N.H., Saad, N., Razak, N.A.A., & Esa, N.M. (2021). *Eleutherine bulbosa* (Mill.) urb. bulb: Review of the pharmacological activities and its prospects for application. *International Journal of Molecular Sciences*, *22*(13), 6747. https://doi.org/10.3390/ijms22136747.

Kusuma, I.W., Arung, E.T., Rosamah, E., Purwatiningsih, S., Kuspradini, H., Astuti, J., Kim, Y.U., & Shimizu, K. (2010). Antidermatophyte and antimelanogenesis compound from *Eleutherine americana* grown in Indonesia. *Journal of Natural Medicines*, *64*, 223–226.

Morabandza, C. J., Okiemy-Akieli, M. G., Okiemy, E., Andzi-Barhé, T., & Ongoka, P.R. (2016). Total phenols, total flavonoids content; antioxidant and antifungal activities of ethanolic and aqueous extracts of *Eleutherine bulbosa* (Iridaceae). *World Journal of Pharmaceutical Sciences*, 252-255.

Munaeni, W., Widanarni, Munti Y., Mia, S., & Aris, T. W. (2019). Phytochemical analysis and antibacterial activities of *Eleutherine bulbosa* (Mill.) Urb. extract against *Vibrio parahaemolyticus*. *Asian Pacific Journal of Tropical Biomedicine*, *9*(9), 397-404. https://doi.org/ 10.4103/2221-1691.267669.

Munaeni, W., Pariakan, A., Abidin, L.B., & Yuhana, M. (2017). *In vitro* phytochemical and inhibitory potential test of Bawang Hutan bulb extract (*Eleutherine palmifolia*) on *Vibrio harveyi*. *Microbiology Indonesia*, *11*, 75-80.

Mundt, S., Kreitlow, S., & Jansen, R. (2003). Fatty acids with antibacterial activity from the cyanobacterium Oscillatoria redekei HUB 051. *Journal of Applied Phycology*, *15*, 263-276.

Nenadis, N., Wang, L. -F., Tsimidou, M., & Zhang, H.-Y. (2004). Estimation of scavenging activity of phenolic compounds using the ABTS+ assay. *Journal of Agricultural and Food Chemistry*, *52*(15), 4669-4674. https://doi.org/10.1021/jf0400056.

Negi, P.S. (2012). Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. *International Journal of Food Microbiology*, *156*(1), 7-17. https://doi.org/10.1016/j.ijfoodmicro.2012.03.006.

Rahmi, M., Helmina, W., Wahyudin, B. J., Kartini, Finna, S., Muhammad, F., & Abdul, W. (2021). Standardization of *Eleutherine bulbosa* urb. bulbs and total flavonoid content from three locations in Kalimantan, Indonesia. *Pharmacognosy Journal*, *13*(1),73-80. https://doi.org/10.5530/pj.2021.13.11.

Salama, H. M., & Marraiki, N. (2010). Antimicrobial activity and phytochemical analyses of *Polygonum aviculare* L. (Polygonaceae), naturally growing in Egypt. *Saudi Journal of Biological Sciences*, *17(*1*)*, 57-63.

Sharma, O. P. & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, *113*(4), 1202-1205. https://doi.org/10.1016/j.foodchem.2008.

Shehata, M. G., Awad, T. S., Asker, D., El Sohaimy, S. A., Abd El- Aziz, N. M., & Youssef, M. M. (2021). Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts. *Current Research in Food Science*, *4*, 326–335. https://doi.org/10.1016/j.crfs.2021.05.001

Shetty, S. B., Mahin-Syed-Ismail, P., Varghese, S., Thomas-George, B., Kandathil-Thajuraj, P., Baby, D., Haleem, S., Sreedhar, S., & Devang-Divakar, D. (2016). Antimicrobial effects of *Citrus sinensis* peel extracts against dental caries bacteria: An *in vitro* study. *Journal of Clinical and Experimental Dentistry*, *8*(1), e71–e77. https://doi.org/10.4317/jced.52493

Sultana, B., Anwar, F., & Przybylski, R. (2007). Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating. *Food Chemistry*, *104*(3), 997–1005. https://doi.org/10.1016/j.foodchem.2006.12.061

Syariful, A., Akhmad, K., Nurdinah, Nur'afia1., Yonelian, Y. (2023). Standardization of *Eleutherine bulbosa* urb. bulbs extract from Lampo, Donggala, Central Sulawesi. Natural Sciences, *12*(1), 29–34.

Turker, H., Yıldırım, A. B., & Karakaş, F. B. (2009). Sensitivity of bacteria ısolated from fish to some medicinal plants. *Turkish Journal of Fisheries and Aquatic Sciences*, *9*, 181-186.

Xing, Q., Zhou, X., Cao, Y., Peng, J., Zhang, W., Wang, X., Wu, J., Li, X., & Yan, J. (2023). The woody plant-degrading pathogen Lasiodiplodia theobromae effector LtCre1 targets the grapevine sugar-signaling protein VvRHIP1 to suppress host immunity. *Journal of Experimental Botany*, *74*(8), 2768-2785. https://doi.org/10.1093/jxb/erad055.

Xu, Q. -L., He, C. M., Zeng, L,, Zhang, H., & Wang, H. F. (2014). Research Advances on Chemistry and Pharmacology of *Eleutherine bulbosa*. *Forestry Science and Technology of Guangdong Province*, *30*, 82–89.

Ye, C., Dai, D., & Hu, W. (2013). Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.). *Food Control*, *30*, 48-53.

Yeo, Y. L., Chia, Y. Y., Lee, C. H., Sow, H. S., & Yap, & W. S. (2014). Effectiveness of maceration periods with different extraction solvents on *in-vitro* antimicrobial activity from fruit of *Momordica charantia* L. *Journal of Applied Pharmaceutical Science*, *4*, 16-23.