



ORIGINAL ARTICLE

Efficacy of Purple Chrysanthemum Ethanol Extract in Inhibiting *Staphylococcus aureus* and *Escherichia coli* Growth

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Abstract

Staphylococcus aureus, a Gram-positive bacterium, and *Escherichia coli*, a Gram-negative bacterium, are known to cause both enteric and systemic infections in humans. The improper use of antibiotics has often been associated with the emergence of antibiotic resistance. Purple chrysanthemum (*Chrysanthemum morifolium*) has shown significant potential as an alternative treatment, owing to its strong antioxidant, anti-inflammatory, and antibacterial properties. These properties are attributed to the presence of bioactive compounds such as polyphenols, flavones, essential oils, and other phytochemicals. This study aimed to identify secondary metabolites and evaluate the antibacterial activity of ethanol extracts from purple chrysanthemum (*Chrysanthemum morifolium*). The extraction process was conducted using Ultrasonic-Assisted Extraction (UAE), and the antibacterial activity was assessed using the disc diffusion method at extract concentrations of 12.5%, 25%, and 50%, with ciprofloxacin serving as the positive control. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenolics, and steroids in the ethanol extract of purple chrysanthemum. Importantly, all ethanol extracts demonstrated antibacterial activity. The highest inhibition zones were observed at a 50% concentration, with *S. aureus* showing an inhibition zone of 12.35 ± 0.4 mm, while *E. coli* exhibited an inhibition zone of 15.08 ± 0.22 mm.

Introduction

Various infections in humans and animals are caused by *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive coccus bacterium that frequently causes opportunistic infections and is a leading cause of superficial lesions, such as skin inflammation and ulcer infections [1]. On the other hand, *Escherichia coli* (*E. coli*) is a Gram-negative rod-shaped bacterium commonly found in the human gastrointestinal tract. It is the most common pathogen responsible for acute diarrhea in children and extraintestinal diseases, including pneumonia, bacteremia, and peritonitis [2,3]. The incidence of *S. aureus* bacteremia ranges from 9.3 to 65 cases per 100,000 people annually, depending on the region and population. Furthermore, methicillin-resistant *S. aureus* (MRSA) accounts for up to 28%, with 3.1% reported in Java and Bali [4,5]. Meanwhile, *E. coli* infections in high-income countries affect approximately 48 out of 100,000 people annually [6]. In developing countries, among children under 5 years of age, it is estimated that 196 million (95% UI 135–269) cases of *Enterotoxigenic Escherichia coli* (ETEC) and Shigella-induced diarrhea occur each year [7].

Antibiotics are widely used, particularly for treating infections; however, improper use can lead to antibiotic resistance [8]. This poses significant challenges in antibiotic therapy. For instance, *S. aureus* may resist daptomycin, an antibiotic that targets the cytoplasmic membrane of *S.*

aureus. The interaction of daptomycin with the cytoplasmic membrane is a Ca^{2+} -dependent process that leads to pore formation, intracellular ion leakage, and cell death in *S. aureus*. Resistance or reduced sensitivity to daptomycin may be associated with mutations in the *mprF* gene [9]. Similarly, *E. coli* can develop resistance to carbapenems. This resistance involves several mechanisms, including reduced antibiotic penetration through the outer membrane, active antibiotic efflux, modification of antibiotic targets through genetic mutations or post-translational changes, and enzymatic modification of antibiotics via catalytic enzymes [10–12].

The purple chrysanthemum (*Chrysanthemum morifolium*) was chosen due to its potential as a herbal remedy and antibacterial activity [13]. This plant is also known to be non-toxic, biocompatible, and environmentally friendly, attributed to the polyphenols, flavones, essential oils, and other chemicals in the plant. Additionally, among the flavonoids found in *C. morifolium*, luteolin-7-glucoside and quercitrin are the most abundant, comprising 85.7% of the total flavonoids detected. According to the study by Hodaei et al. (2021), the *C. morifolium* extract demonstrated significant antibacterial properties against *S. aureus* and *E. coli* with minimum inhibitory concentration (MIC) values ranging from 10–20 mg/ml [14]. In a study by Felicia et al. (2020) investigating the antibacterial activity of a chrysanthemum leaf extract ointment against *Staphylococcus aureus*, it was found that ointments with concentrations of 12.5%, 25%, and 50% exhibited significant antibacterial activity. The highest inhibition zone was recorded at a 50% concentration, measuring 22.2 mm, while the 12.5% and 25% zones were 7.25 mm and 9.85 mm, respectively [15].

In the study by Boukhebt et al. analyzing the chemical composition and antimicrobial activity of *Chrysanthemum morifolium* essential oil, the agar diffusion method was employed against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The essential oil showed significant antibacterial effects with inhibition zones of 35 ± 1.2 mm against *Staphylococcus aureus* and 21 ± 0.87 mm against *Citrobacter freundii* but displayed resistance against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [16]. A study by Panda et al., which explored the antibacterial activity of *C. morifolium* leaf extract grown in vitro against human pathogenic bacteria, showed significant antibacterial activity against various bacterial strains, including *E. coli* and *S. aureus*, with measurable inhibition zones in agar diffusion tests [17].

In this study, the Ultrasonic-Assisted Extraction (UAE) method was employed for its numerous advantages, including reduced extraction time, enhanced yield, ease of use, versatility, and cost-effectiveness compared to other modern techniques. Building on this approach, the research aimed to identify the phytochemical compounds and evaluate the antibacterial efficacy of ethanol extracts from purple chrysanthemum (*Chrysanthemum morifolium*).

Materials and Methods

Plant Determination

The purple chrysanthemum (*Chrysanthemum morifolium*) flowers used in this study were collected and taxonomically identified by a qualified taxonomist at CV BIOVINA Laboratory. The identification process involved examining the morphological characteristics of the flower samples to confirm their scientific species name. The plant was classified as follows: it belongs to the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Asterales, and Family Asteraceae. The genus is *Chrysanthemum*, and the species is *Chrysanthemum morifolium* Ramat. The local name of the plant is "*Krisan Ungu*" (purple chrysanthemum).

Tools and Equipment

Blender, tray, basket, sieve, container, analytical balance, oven, stirring rod, filter paper, experimental glassware (test tubes, measuring cylinders, beakers, Erlenmeyer flasks), labeling paper, aluminum foil, tissue paper, incubator, micropipette, horn spoon, spirit lamp, inoculating

loop, tweezers, Laminar Air Flow (LAF) cabinet, autoclave, agar medium, vernier caliper, Ultrasonic-Assisted Extraction (UAE) device, rotary evaporator, porcelain dish, petri dish, paper discs, extract containers, and a camera.

Materials

Purple chrysanthemum (*Chrysanthemum morifolium*), distilled water, Nutrient Agar (NA), 96% ethanol, ciprofloxacin antibiotic, pure cultures of *Escherichia coli* and *Staphylococcus aureus*, sterile cotton, aluminum foil, Carboxymethyl Cellulose (CMC), and phytochemical test reagents (Wagner, Mayer, Dragendorff, concentrated HCl, concentrated H₂SO₄, FeCl₃, glacial acetic acid, and magnesium).

Sample Preparation

The samples of purple chrysanthemum (*Chrysanthemum morifolium*) from Tomohon City, North Sulawesi, at 780 meters above sea level. The flowers were cleaned under running water, air-dried for three days, and then placed in an oven at 60°C for 24 hours. The dried samples were ground using a blender and a 40-mesh sieve to obtain a fine, uniform dry simplicia powder.

Extraction Procedure

Extraction was performed using the UAE method, with 80 grams of the sample placed in an Erlenmeyer flask and 800 mL of 96% ethanol as the solvent. Ethanol 96% is often chosen as a solvent in extraction due to its ability to dissolve various bioactive compounds, including polar and non-polar ones. The ratio of sample to solvent was 1:10 (w/v). Extraction was performed for 30 minutes at 40°C, with an ultrasonic frequency of 40 kHz. The extract was then filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator. Extraction parameters, including a duration of 30 minutes, temperature of 40°C, ultrasonic frequency of 40 kHz, and a material-to-solvent ratio of 1:10, were optimized to achieve maximum extraction efficiency [18].

Phytochemical Screening

Qualitative phytochemical screening was conducted to identify secondary metabolite compounds present in the extract of purple chrysanthemum (*Chrysanthemum morifolium*). This screening included analyses of alkaloids, triterpenoids/steroids, tannins, flavonoids, saponins, and phenolics [19].

Antibacterial Activity Test

Before conducting the tests, all equipment was sterilized using an autoclave at 121°C for 30 minutes. The positive control solution was prepared from ciprofloxacin 0.5 g tablets. Ciprofloxacin was selected as the positive control in the antibacterial assay due to its broad-spectrum activity against Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, as well as several Gram-positive bacteria, including *Staphylococcus aureus*. This antibiotic works by inhibiting the enzymes DNA gyrase and topoisomerase IV, essential for bacterial DNA replication, thereby providing a consistent antibacterial effect [20]. Ciprofloxacin is also frequently used as a reference standard in research due to its proven efficacy, clinical relevance, and global acceptance as a positive control, facilitating the comparison of results across different studies. One ciprofloxacin tablet was crushed and dissolved in 50 mL of Carboxymethyl Cellulose (CMC). The negative control was prepared by dissolving 1 gram of CMC powder in 100 mL of 1% CMC solution. Test solutions with 12.5%, 25%, and 50% (v/v) were prepared by weighing 0.125 g, 0.25 g, and 0.5 g of the purple chrysanthemum ethanol extract, respectively, and dissolving each in 1 mL of CMC solution.

Basic media was prepared by weighing 2.8 g of nutrient agar (NA) and placing it in a petri dish. It was then mixed with 200 mL of distilled water and homogenized until boiling. The mixture was subsequently cooled to room temperature. The preparation of McFarland 0.5 standard solution was performed by mixing 10 mL of 1% H₂SO₄ solution with 0.05 mL of 1% BaCl₂

solution in a test tube. The turbidity of the McFarland solution was used as the standard for the turbidity of the bacterial test suspension.

The bacterial suspension was prepared by transferring the bacteria using an inoculating loop and then introduced into a test tube containing 10 mL of sterile 0.9% NaCl. Antibacterial activity was tested using the disc diffusion method (Kirby-Bauer disk diffusion). *Staphylococcus aureus* and *Escherichia coli* were obtained from the Clinical Microbiology Laboratory, Department of Pharmacy, Sam Ratulangi University, Manado, Indonesia. [21].

Observation and Measurement

Observations were made after a 24-hour incubation period. The clear zone diameters were then measured using a digital caliper. The data obtained from the antibacterial activity test were analyzed using the Statistical Package for the Social Sciences (SPSS) software with a One-Way ANOVA test to determine whether significant differences existed between the means of more than two groups. If the results were significant, a Post Hoc test was performed to compare pairwise groups and identify which groups differed significantly.

Results and Discussion

Sample Preparation

The plants of purple chrysanthemum (*Chrysanthemum morifolium*) underwent wet sorting to remove foreign materials from the flowers. A total of 693 grams of purple chrysanthemum flowers was obtained, while the resulting dry powder of the purple chrysanthemum sample yielded 81.6 grams of simplicia.

Extraction

Extraction separates a substance based on differences in solubility in two immiscible liquids, typically water and an organic solvent. A study by Coelho et al. (2022), which focused on optimizing and comparing conventional extraction methods with UAE, stated that the UAE method was 21% faster in performing extraction than conventional methods [22]. Another study by Mutia et al. (2019) suggested that the UAE method is more recommended due to its faster time efficiency and ability to increase the yield [23]. After evaporation, the extract was thick, weighing 12 grams, yielding 15%.

Phytochemical Screening

Phytochemical screening was conducted to provide an overview of the compounds' classes in the extract. These compounds, such as alkaloids, flavonoids, saponins, tannins, and phenols, generally possess significant biological activity and may function as antimicrobial or anti-inflammatory agents [24].

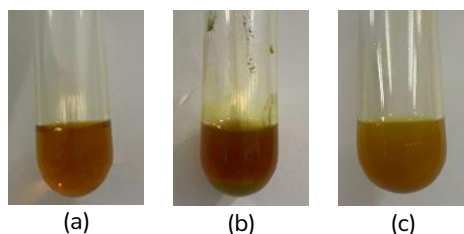


Figure 1. (a) Mayer's test, (b) Wagner's test, (c) Dragendorff's test.

Figure 1 shows the analysis of alkaloid compounds in the purple chrysanthemum extract, where Mayer, Wagner, and Dragendorff reagents were added to identify the presence of alkaloids in the plant extract based on the color changes that occurred with these three reagents. The results indicated that purple chrysanthemum contains alkaloids. In Figure 1a, the appearance of

a reddish-brown precipitate indicates a positive result for the Wagner test. Figure 1b shows the formation of a white precipitate in the Mayer test, while Figure 1c demonstrates the formation of a red or orange precipitate in the Dragendorff test [25].

Figure 2a presents the analysis of flavonoid compounds, where the diluted purple chrysanthemum extract was treated with concentrated HCl to convert flavonoids into their flavilium salt form. The results indicated the presence of flavonoid compounds, marked by a color change to red. Figure 2b shows the saponin test, where the extract was heated to achieve a more homogeneous mixture. The results indicated that the extract does not contain saponins, as evidenced by the absence of stable foam formation. Figure 2c demonstrates the tannin compound analysis, where FeCl₃ was added to identify tannin compounds. The results revealed that the extract contains tannins, as indicated by a color change to a bluish-black hue [26].

Figure 2d presents the analysis of steroid compounds. The diluted purple chrysanthemum extract was treated with glacial acetic acid to produce acetyl derivatives. Subsequently, H₂SO₄ was added to hydrolyze compounds containing hydroxyl (-OH) groups in the steroids. The results revealed the presence of triterpenoids in the extract, indicated by a color change to reddish-purple [27]. Figure 2e shows the phenolic compound analysis, where FeCl₃ was added to detect the presence of phenolic groups. The reaction between FeCl₃ and the hydroxyl (-OH) group in phenolic compounds forms a colored complex. The results confirmed that the extract contains phenols, as indicated by a color change to dark green.

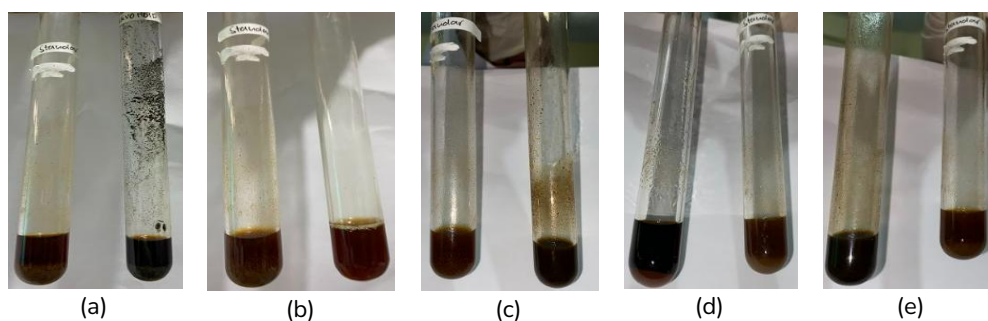


Figure 2. (a) Flavonoid's test (b) Saponin's test (c) Tannin's test (d) Steroid's test (e) Phenolic's test.

Antibacterial Activity Test

The antibacterial test was conducted using the disc diffusion method, which offers several advantages, including cost efficiency, flexibility, and allowing visible organism growth. The agar medium that had been treated was first incubated for 24 hours to observe bacterial colony growth and determine the bacterial spread pattern on the paper disc. After incubation, the inhibition zone was measured using a digital caliper. The inhibition zone (clear zone) was calculated by summing the horizontal and vertical diameters and then dividing by two. Figure 3a presents the results of the antibacterial activity test against *S. aureus*, and Figure 3b shows the results for *E. coli*, with calculations based on the formed inhibition zones.

In Table 1, the results of the inhibition zone diameter measurements against *S. aureus* and *E. coli* are presented based on antibacterial activity assessed using the disc diffusion method. The clear zone formed around the disc indicates the ability of the extract and antibiotics to inhibit bacterial growth. Data interpretation was performed by measuring the diameter of the inhibition zone around the disc using a digital caliper.

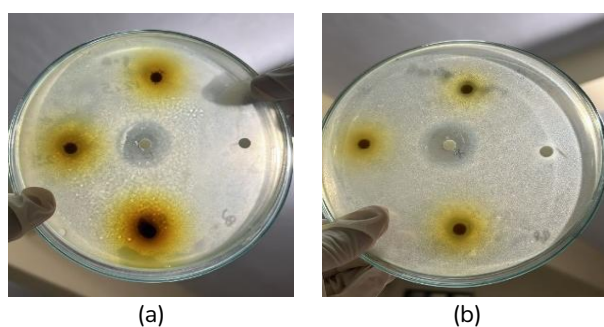


Figure 3. (a) *Staphylococcus aureus*, (b) *Escherichia coli*.

Based on Table 1, both *S. aureus* and *E. coli* at all three concentrations exhibited antibacterial activity, indicated by the formation of a clear zone. The antibacterial activity is classified into four levels: weak, moderate, strong, and very strong. The activity is considered weak if the inhibition zone diameter is <5 mm, moderate between 5-10 mm, strong between 10-20 mm, and strong if >20 mm [28]. For both bacteria, the 12.5% concentration exhibited moderate inhibition, while the 25% and 50% concentrations were categorized as strong, and ciprofloxacin exhibited very strong activity.

Table 1. Antibacterial Activity of Ethanol Extract of Purple Chrysanthemum Flowers Against *Staphylococcus aureus* and *Escherichia coli*.

Bacteria	Test Solution	Diameter of the Inhibition Mean (mm)	Std. Deviation
<i>S. aureus</i>	Concentration 12.5%	9.83	0.4
	Concentration 25%	10.61	0.37
	Concentration 50%	12.35	1.05
	Ciprofloxacin (+)	26.91	0.17
	CMC (-)	0	0
<i>E. coli</i>	Concentration 12.5%	9.3	1.62
	Concentration 25%	10.65	0.7
	Concentration 50%	15.08	0.22
	Ciprofloxacin (+)	26.86	1.56
	CMC (-)	0	0

The size of the inhibition zones formed in *Staphylococcus aureus* and *Escherichia coli* was directly proportional to the increase in extract concentration. This indicates that antibacterial activity increased with higher extract concentrations as the number of active antibacterial compounds increased. The increased concentration of these antibacterial compounds is thought to enhance their penetration, potentially disrupting the microbial cell's metabolic system and leading to cell death [29]. The highest concentration of extract that inhibited the growth of both bacterial strains was 50%, while the lowest concentration with minimal inhibition was 12.5%. This suggests that as the extract concentration increases, the inhibitory effect strengthens, as evidenced by the larger clear zone or inhibition zone diameter.

There is a difference in the inhibition zones between *Staphylococcus aureus* and *Escherichia coli*. The main reason for this difference lies in the structure of the cell wall. *E. coli*, a Gram-negative bacterium, has a thin peptidoglycan layer and is covered by an outer membrane, making it more susceptible to damage by antibacterial compounds. In contrast, *S. aureus*, a Gram-positive bacterium, has a thicker cell wall due to its rigid peptidoglycan layer containing teichoic acids. The mechanism of action of antibacterial compounds involves the inhibition of topoisomerase II (DNA gyrase) and topoisomerase IV enzymes in bacteria, which impede bacterial growth. The sensitivity levels of both bacterial types also differ. Gram-positive bacteria tend to be more

sensitive to changes in physical and chemical environments than Gram-negative bacteria, which produce exopolysaccharides, such as gel-like alginate, and form biofilms [30].

S. aureus can develop resistance through various mechanisms, including horizontal gene transfer of resistance genes encoded by mobile genetic elements such as plasmids, transposons, and integrons. Strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*) are known to modify the penicillin-binding protein (PBP2a), rendering beta-lactam antibiotics ineffective. Similarly, *E. coli* possesses a complex resistance mechanism, including the production of β -lactamase enzymes such as Extended Spectrum Beta-Lactamase (ESBL), which can degrade antibiotics, reduce outer membrane permeability, and increase efflux pump activity that expels antibiotics from the bacterial cell [31].

The compounds present in the ethanol extract of purple chrysanthemum flowers, such as alkaloids and flavonoids, offer unique mechanisms of action. Alkaloids have the ability to inhibit bacterial cell wall synthesis by interfering with peptidoglycan components, disrupting membrane permeability, interfering with DNA function, and inhibiting protein synthesis. This activity leads to inhibited bacterial growth or even bacterial cell death [32]. On the other hand, flavonoids exhibit antibacterial properties through different mechanisms, such as binding to bacterial proteins and inhibiting enzymatic activities required in metabolic processes. The lipophilic nature of flavonoids also allows them to damage bacterial cell membranes by penetrating the lipid bilayer, disrupting bacterial cell function [33].

Based on the average diameter of the inhibition zones, the largest zones for both bacteria were observed in the positive control group, ciprofloxacin, followed by the 50% extract concentration, which was categorized as strong. This study demonstrates that the ethanol extract of purple chrysanthemum flowers has promising potential as an antibacterial agent against *S. aureus* and *E. coli*, although its inhibitory effect, as measured by the inhibition zone diameter and statistically, was not as effective as ciprofloxacin. The in vitro results in this study show promising antibacterial activity against *S. aureus* and *E. coli*. However, it should be noted that in vitro conditions do not reflect the complex biological environment in the human body. This study did not evaluate the stability of the *Chrysanthemum morifolium* flower extract. Therefore, further research is needed to assess the stability of the active compounds in this extract, including optimal storage conditions and stability over time.

Conclusions

This study focused on the identification of secondary metabolites and the antibacterial activity testing of the ethanol extract of purple chrysanthemum flowers (*Chrysanthemum morifolium*) sourced from Kaskasen, North Tomohon District, Tomohon City, North Sulawesi, Indonesia. Phytochemical analysis revealed the presence of secondary metabolites, such as alkaloids, flavonoids, tannins, phenolics, and steroids. The ethanol extract of purple chrysanthemum flowers exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. At 12.5%, the inhibition against both bacteria was categorized as moderate, while at 25% and 50%, the inhibition was categorized as strong. The highest inhibition was observed at a concentration of 50% compared to other concentrations of the ethanol extract. This study did not consider the effects of immune factors, metabolism, and bioavailability of active compounds, which may influence their clinical efficacy. Additionally, the study employed a limited concentration range for testing. Therefore, further research is needed, including in vivo studies and pharmacokinetic studies, to confirm the therapeutic potential of these compounds. Furthermore, in vivo studies with a broader concentration range are also required.

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