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IN VITRO EVALUATION OF THE ANTIBACTERIAL POTENTIAL OF PSIDIUM GUAJAVA LEAF EXTRACT AGAINST *ESCHERICHIA COLI*: UNCOVERING THE ROLE OF PHYTOCHEMICAL COMPOUNDS

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Introduction: Urinary tract infections (UTIs) are prevalent among women and are commonly caused by *Escherichia coli*. Although antibiotics are effective, their misuse contributes to rising antimicrobial resistance. *Psidium guajava L.*, a traditional Indonesian medicinal plant, has shown promise as an alternative antibacterial agent. This study aimed to evaluate the inhibitory effect of *P. guajava* leaf extract against *E. coli*. **Methods**: Antibacterial activity was assessed using the agar diffusion method with *E. coli* ATCC 25922. Ethanolic extracts were obtained by maceration. Six treatment groups, including various concentrations of levofloxacin and dimethyl sulfoxide (DMSO), were tested to compare antibacterial effects. **Results**: Phytochemical screening revealed the presence of flavonoids, alkaloids, and tannins—compounds known for their antimicrobial activity. Inhibition zone diameters were measured and analysed using the Kruskal–Wallis one-way ANOVA. **Discussion**: The guava leaf extract demonstrated inhibitory activity against *E. coli*, likely due to its bioactive compounds. These constituents may exert antibacterial effects through disruption of nucleic acid synthesis, interference with membrane integrity, inhibition of energy metabolism, and prevention of biofilm formation. **Conclusion**: The ethanolic extract of *Psidium guajava* leaves exhibits significant antibacterial activity against *Escherichia coli*.

Keywords: Antibacterial; *Escherichia coli*; Inhibitory effect; Phytochemical screening; *Psidium guava* L extracts

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1. INTRODUCTION

Urinary tract infections (UTIs) are common infections that affect individuals of all ages and genders, presenting with varying clinical manifestations. UTIs are associated with significant morbidity and, in severe cases, may lead to mortality. Under normal conditions, the urinary tract is sterile and free from microbial colonization. However, pathogenic bacteria often originating from the gastrointestinal tract, can ascend and infect the urinary system. Infection typically occurs when bacterial virulence increases or the host's immune defenses are compromised, allowing bacterial colonization and proliferation within the urinary tract[1]. *Escherichia coli* is the most common cause of urinary tract infections, accounting for over 90 % of all urinary tract infections in young women. Signs and symptoms include frequent urination, dysuria, hematuria, piuria, and discomfort in the waist flanks, which indicate an ascending infection going to the upper urinary tract. [2]

Antibiotics remain the most effective treatment for bacterial infections, serving both preventive and therapeutic purposes 3. "The use of antibiotic medications can lead to numerous complications, with antibiotic resistance emerging as a significant global health threat. Initially confined to hospital settings, resistance has now become prevalent in the broader community, largely due to the inappropriate and excessive use of antibiotics. This issue is particularly pronounced in bacterial strains such as *Streptococcus pneumoniae, Staphylococcus aureus,* and *Escherichia coli*, which have demonstrated increasing resistance to commonly used antimicrobial agents.[4]When bacteria no longer respond to antibiotics, this is referred to as antibiotic resistance. Antibiotic resistance increases medical expenses, length of stay in hospitals, and death. [2.]

To evaluate historical trends in antimicrobial resistance, subgroup analyses conducted between 2000 and 2018 revealed a significant rise in ciprofloxacin resistance [5.] Given the rising incidence of bacterial resistance in Indonesia, innovative strategies are essential to curb the spread of antibiotic resistance. One promising approach involves the use of medicinal plants with demonstrated antibacterial activity. *Psidium guajava L.* (guava), a widely known therapeutic plant in Indonesia, is traditionally recognized for its distinctive pink fruit, strong aroma, and common use in treating ailments such as diarrhea and dengue fever. Various

phytochemical constituents of the guava plant, particularly flavonoids, tannins, and alkaloids, exhibit antibacterial properties, contributing to its potential in preventing infections caused by *Escherichia coli*. [6]

After considering and reviewing the various descriptions provided above, I, as a researcher, am interested in investigating the effects of guava leaf extract (*Psidium guajava L.*) as an inhibitor of *Escherichia coli* growth, with the aim of advancing the understanding of this medicinal plant's potential in combating *E. coli* infections. The objectives of this study are threefold: (1) to evaluate in vitro the ability of the phytochemical components in guava leaf extract to suppress *Escherichia coli* bacterial growth; (2) to identify the phytochemical profile of guava leaf extract (*Psidium guajava L.*); and (3) to determine the concentration of guava leaf extract (*Psidium guajava L.*) capable of inhibiting the development of *Escherichia coli* in vitro.

2. MATERIALS AND METHODS

This is an experimental study utilizing a posttest-only control group design. The *Psidium guajava L.* leaf extract was prepared in the Biology Laboratory at the Faculty of Pharmacy, Universitas Sumatera Utara. Phytochemical screening of the extract was performed in the same laboratory. The investigation of the antibacterial activity of Psidium guajava leaf extract against *Escherichia coli* growth was conducted in the Microbiology Laboratory at the Faculty of Medicine, Universitas Sumatera Utara. The study was carried out from July to October 2021.

The sample for this study consists of *Escherichia coli* bacteria exposed to various concentrations of Psidium guajava leaf extract. *Escherichia coli* ATCC 25922 was obtained from the Microbiology Laboratory at the Faculty of Medicine, Universitas Sumatera Utara, while the *Psidium guajava L.* leaves were sourced from a garden in Tanjong Morawa.

In this study, six treatments were administered using *Psidium guajava L.* leaf extract at concentrations of 12.5%, 25%, 50%, 75%, levofloxacin as the positive control, and dimethyl sulfoxide (DMSO) as the negative control. These six treatments were repeated four times, resulting in a total of 24 samples.

Ethical approval

The Research Ethics Committees had approved the study, which had been approved by the Research Ethics Committees of

Universitas Sumatera Utara, Indonesia (approval number: 945/KEPK/USU/2021).

Collection of plant material

The fresh leaves of *Psidium guajava L.* were collected from a garden located at Tanjong Morawa, Medan, Indonesia, in July 2021. The plant specimen was identified and authenticated at the Herbarium Medanese Laboratory, Universitas Sumatera Utara. After identification, the plant material was processed for extraction.

Preparation of the plant extract

The leaves were thoroughly washed with running tap water and shade-dried for 24 hours. Subsequently, the leaves were further dried in a drying cabinet with a 40-watt lamp for 5 days. Once dried, the leaves were crushed into small pieces and then ground into a fine powder using a grinder. The powdered leaves were stored in plastic bags for future use.

A total of 500 grams of dried powdered leaves were macerated by adding 5 liters of 96% ethanol to the container, which was then sealed tightly and soaked for 24 hours, with occasional stirring. After 24 hours, the extract solution was filtered through Whatman paper No. 1. This process was repeated twice. The combined filtrates were collected and evaporated using a rotary vacuum evaporator. The remaining extract was further concentrated by placing it in a glass bottle in an oven, yielding a viscous extract. The extract was then stored in the refrigerator at 4 °C for future use

Assessment of antibacterial activity

Escherichia coli ATCC 25922, obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara, was used in this study. The antibacterial activity of the leaf extracts was evaluated using the agar-well diffusion method. Petri dishes containing Muller Hinton agar were inoculated with a 24-hour-old culture of *Escherichia coli*. Wells, approximately 6 mm in diameter, were bored into the agar using a sterile cork borer, and varying concentrations of the extract were added to each well. The extract was dissolved in dimethyl sulfoxide (DMSO) at concentrations of 12.5%, 25%, 50%, and 75%. Levofloxacin was used as a positive control, and DMSO served as the negative control. The plates were then incubated at 37°C for 24 hours in an upright position. The antibacterial activity was assessed by measuring the diameter of the inhibition zone around the well using a caliper.

Phytochemical screening

The phytochemical screening shown in the table involves qualitative tests to identify various bioactive compounds in plant extracts using specific chemical reagents. Flavonoids are detected using a combination of magnesium powder, alcohol, and hydrochloric acid, which indicates their presence through a color change (typically amil or reddish). Alkaloids are identified using Dragendorff's, Bouchardat's, and Meyer's reagents, each forming characteristic precipitates when alkaloids are present. Tannins are tested using ferric chloride ($FeCl_3$), which yields a blue-black or greenish color upon reaction. Saponins are identified by their ability to produce stable froth when the extract is vigorously shaken with hot water. Glycosides are detected through the Molisch test followed by the addition of sulfuric acid (H_2SO_4), leading to a reddish-violet ring at the interface. Lastly, triterpenes and steroids are revealed by the Liebermann-Burchard test, which gives a color reaction indicating the presence of sterol or triterpene structures. These tests help confirm the phytochemical constituents responsible for potential medicinal properties of plant extracts.

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 25. Data were analyzed using Kruskal-Wallis One Way ANOVA statistical. The data were analyzed using SPSS, starting with the Shapiro-Wilk test for normality ($p > 0.05$ indicates normal distribution) and Levene's test for homogeneity ($p > 0.05$ indicates homogeneous variance). If both criteria were met, One-Way ANOVA ($p < 0.05$ indicates significant difference) was applied, followed by a post hoc test to identify which groups differed. If assumptions were not met ($p < 0.05$), the Kruskal-Walli's test was used as an alternative.

3. RESULT

Upon completion of the guava leaf extract preparation, it is imperative to systematically identify and analyze the bioactive compounds present in the extract, which may contribute to its potential antibacterial activity. Accordingly, a comprehensive phytochemical analysis was performed in the Biological Laboratory of the Universitas Sumatera Utara School of Pharmacy, employing a six-parameter approach and a qualitative assay method, utilizing a range of specific reagents. The findings from the phytochemical screening are detailed in the parameters listed in Table 1 below.

Table 1 Phytochemical test of guava leaf extract *(Psidim guajava L.)*

Compound	Reagent	Interpretation of Results
Flavonoid	Mg powder + Amil Alcohol + HCl	+
Alkaloid	Dragendroff	+
	Bouchardat	+
	Meyer	+
Tannin	$FeCl_3$	+
Saponin	Hot water/ whipped	-
Glycosides	Molish + H_2SO_4	+
Triterpene/ Steroid	Lieberman-Bourchat	+

The diameters of the inhibition zones of *Escherichia coli* that have been given *Psidium guajava L.* leaf extract at concentrations of 12.5%, 25%, 50%, and 75% for the positive control and negative control are shown in Table 2.

Table 2 : The diameter of inhibition zone of ethanolic leaf extract *Psidium guajava L.*

Repetition	*C - DMSO*	*Extract 12.5%*	*Extract 25%*	*Extract 50%*	*Extract 75%*	*C + Levofloxacin*
I	0	17.60	19.85	19.20	20.50	53.55
II	0	17.55	18.45	19.95	20.70	50.55
III	0	17.70	18.50	19.65	20.30	49.50
IV	0	17.80	19.00	20.50	21.20	50.02
Mean	**0**	**17.6625**	**18.95**	**19.825**	**20.675**	**50.905**
Mean ± SD	**0**	**17.6625 ± 0.11086**	**18.95 ± 0.6493**	**19.825 ± 0.5454**	**20.675 ± 0.3862**	**50.905 ± 1.8147**

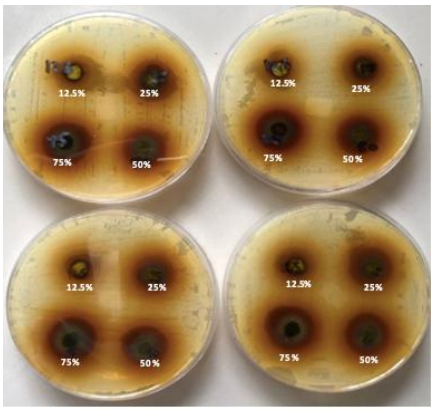

Figure 1. Inhibition zone of *Psidium guajava* extract

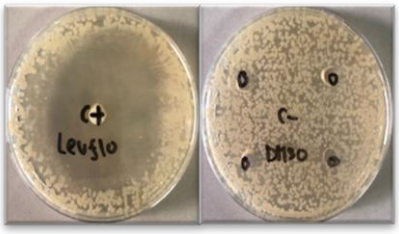

Figure 2. Inhibition zone of control positive and negative

Table 2 presents the measurements of the inhibitory zone of guava leaf extract on the growth of *E. coli* bacteria, highlighting the effects of extracts at concentrations of 12.5%, 25%, 50%, and 75%. The data suggest that the inhibitory zone increases with higher concentrations of the extract. Although the difference in the inhibitory zone between concentrations was only one millimeter, it is evident that concentration plays a significant role in the inhibition of bacterial growth. However, when compared to some positive controls, the inhibitory zones induced by the guava leaf extract at varying concentrations were smaller and less pronounced. This indicates that while guava leaf extract contains bioactive compounds capable of suppressing *E. coli* growth, its effectiveness is less than that of the positive controls.

The Kruskal-Wallis one-way ANOVA test revealed a significance value of 0.000 ($P < 0.05$), indicating a statistically significant difference in the inhibitory effects of the various concentrations of *Psidium guajava L.* leaf extract on the growth of *Escherichia coli*.

4. DISCUSSION

The ethanol extract of *Psidium guajava L.* leaves demonstrates inhibitory effects on the growth of *Escherichia coli*. This assertion is further supported by the identification of active compounds within the extract, as evidenced by the results of the phytochemical tests. These tests revealed the presence of alkaloids, flavonoids, glycosides, tannins, and triterpenes, all of which contribute to the antibacterial activity. Specifically, flavonoid compounds play a crucial role in combating bacterial growth through several mechanisms, including inhibition of nucleic acid synthesis, disruption of cytoplasmic membrane function, interference with energy metabolism, prevention of biofilm formation and bacterial attachment, formation of pores in cell

membranes, alteration of membrane permeability, and attenuation of acoustic signaling. These multifaceted actions underscore the potential of *Psidium guajava L.* leaf extract as a natural antibacterial agent [7]. Flavonoid chemicals can cause bacterial cell damage and denature proteins, thereby preventing bacterial growth.[8]

In alkaloid compounds, there are nitrogen-containing base groups that can interact with amino acid compounds found in bacterial cell walls and DNA. The reaction generates structural alterations and amino acid configurations in bacteria, leading to a genetic imbalance in the DNA chain. This imbalance causes harm and encourages the destruction or lysis of bacteria, ultimately leading to their death.[9] Furthermore, the OH group in the alkaloid structure can boost efficacy in suppressing bacterial growth through protein denaturation, resulting in increased cell membrane permeability. Because bacterial cell membranes have increased permeability, internal components leak to the outside, causing the bacteria to die gradually. [10]

Tannins have antibacterial actions, including activating enzyme-inactivated cell membranes and cell walls [8.] Tannins are water-soluble chemical compounds found in a broad variety of plants that have potent antibacterial activities. There are several ways that tannins stop microbes from growing. These include the removal of iron through iron classion, the inhibition of oxidative phosphorylation, the elimination of compounds essential for microbe growth, and the inhibition of enzymes required in the extracellular cytoplasmic membranes.[11]

Terpenoids have toxic chemical effects on both gram-positive and gram-negative bacterial cell walls. Terpenoids interact with proteins on cell membranes and intracellular components, causing disruptions in the *Escherichia coli* bacteria's membrane structure. This creates structural and functional problems with the cell membrane. Damage to the cell membrane causes the cytoplasm to coagulate, increasing the membrane's permeability, leading to leakage of intracellular components and a decrease in ATP generation.[12] Triterpenoids have the ability to suppress bacterial growth. The last compound is saponins, which have the ability to inhibit the growth of gram-positive bacteria. Plants, specifically the roots, skin, leaves, seeds, and fruit, contain saponin compounds that serve as a self-defense system.[8]

This study diluted guava leaf extract into four concentrations: 12.5 %, 25 %, 50%, and 75%. Each increase in concentration leads to an increase in the inhibition zone. The results of this study agree with

Yustina's research on the phytochemical activity and antibacterial effects of guava leaf extract, which suggests that the antibacterial activity goes up as the concentration of guava leaf goes up.[13] The researchers conclude in this study that the higher the concentration, the greater the activity of bacterial inhibitors. This may be because the higher the concentration of extracts, the higher the content of anti-bacterial compounds, so that they can inhibit bacterial growth more maximally.

Another study only uses concentrations starting from 25 %, 50%, 75%, and 100% with solid dilution methods[13], so that researchers conduct trials again with different methods where it is easier, ergonomic and measurement is not difficult, namely with well diffusion methods and the use of lower concentrations of 12.5 % and the results are enough to provide data that a concentration of 12.5 % can also be effective and have bacterial growth-inhibiting activity.[14]

The difference in the effect of extract concentration and the amount of bland zone in this study compared to other studies may be due to differences in the content of metabolite compounds in guava leaf extract, depending on the location, condition, and weather of the plant's growth. A plant's content of metabolite compounds is influenced by several internal and external factors. Genes influence internal factors, while external factors such as light, temperature, humidity, pH, nutrient content in the soil, and the height of different locations certainly influence different conditions.[14]

In 2014, research at Aristyanti discovered that chemical levels in the soil, specifically sodium, potassium, and calcium, can influence the levels of flavonoid metabolite compounds.[15] Siswoyo's 1999 research indicates that high calcium administration in plants leads to increased levels of flavonoid compounds. Calcium chemicals act as enzyme activators, especially those related to proteins, so they will further facilitate the process of forming secondary metabolites that are specific reactions.[16] The difference in soil pH from one place to another also has the potential to affect the content of secondary metabolite compounds in the extract. This is due to the fact that low-pH soils and waters typically experience nutrient deficiency and low productivity levels.[15] The study by Kulsum et al. (2019) also stated that the maceration time has an influence on the content of substances that dissolve in solvents. Longer extraction times allow for more substances to be extracted. However, there is a maximum time limit for the solvent's ability to extract the contents of a dissolved material.

The optimum time for ethanol solvents is 72 hours, or 3 days.[17]

The data follows a normal distribution if the significance value is greater than 0.05 in the normality test. However, the homogeneity test revealed a significance value less than 0.05, indicating that the data is not homogeneous, meaning the data groups originate from populations with different variances. As a result, the assumptions for performing a one-way ANOVA were not met, prompting the use of the Kruskal-Wallis test. The Kruskal-Wallis test yielded a significance value of 0.001 ($p < 0.05$), indicating a statistically significant difference in the inhibitory effects of varying concentrations of guava leaf extract on the growth of *Escherichia coli* bacteria.

5. CONCLUSIONS

In conclusion, the ethanolic extract of *Psidium guajava L.* leaves demonstrates antibacterial activity against the growth of *Escherichia coli* bacteria. Phytochemical analysis, conducted using six parameters, identified the presence of compounds with potential antibacterial properties, including flavonoids, tannins, alkaloids, and triterpenes. Furthermore, the results indicate that the inhibitory effect increases with the concentration of the extract, with higher concentrations leading to larger inhibition zones.

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