CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

Research article

TOTAL PHENOLIC CONTENT, RADICAL SCAVENGING, AND ANTIBACTERIAL ACTIVITY OF THREE DIFFERENT FRACTIONS OF *PARIJOTO* FRUIT (*MEDINILLA SPECIOSA BLUME*)

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https://doi.org/10.34302/2025.17.3.5

Article history:
Received:
March 6th, 2025
Accepted:
Novembre 10th , 2025

Keywords:
Antibacterial activity;
DPPH radical scavenging activity;
Medinilla speciosa;
Antioxidant activity;
Phenoliccontent

ABSTRACT

Parijoto (*Medinilla speciosa Blume*), a traditional Indonesian medicinal plant, requires further scientific investigation. This study explored the total phenolic content, antioxidant activity, and antibacterial properties of three *Parijoto* fruit fractions. Understanding these variations helps identify the most potent fraction for functional food development. Methanol extract was fractionated into n-hexane (PNF), ethyl acetate (PEF), and methanol fractions (PMF). Total phenolic content was determined using the Folin-Ciocalteu method, and antioxidant activity via DPPH assay. Antibacterial activity against *E. coli* and *S. aureus* was assessed using the Kirby-Bauer disk diffusion method with varying fraction concentrations (30, 60, and 90% v/v). Chloramphenicol served as a positive control, and DMSO as a negative control. PNF exhibited the highest total phenolic content (146.29 ± 0.91 µg GAE/g) and highest antioxidant activity (IC_{50} 1.73 ± 0.09 µg/g), but the lowest antibacterial activity. Conversely, PEF demonstrated the strongest antibacterial activity against both bacteria, despite not having the highest phenolic content (68.83 ± 2.63 µg GAE/g) or antioxidant activity (IC_{50} 7.59 ± 0.42 µg/g). These results suggest that *Parijoto*'s antibacterial activity is not solely attributable to phenolic compounds. Other unidentified compounds may contribute to its antibacterial effects, highlighting the need for further biomolecular research to elucidate the underlying mechanisms.

1. Introduction

The microbiota balance in the human digestive system is influenced by diet, lifestyle and food hygiene(Wibawanti et al., 2021). When digestive problems arise, some people rely on microbiota, such as yogurt, which has beneficial medical effects (Pratama et al., 2018a). The continual use of antibiotics as a common

medication raises concerns about bacterial antibiotic resistance. *Escherichia coli* and *Staphylococcus aureus* are two bacterial species with some resistant strains because of the unwise use of antibiotics. *Escherichia coli* multidrug resistance has already been detected in Indian children (Feliatra et al., 2022). On the other hand, the first observed antibiotic resistance *in S. aureus* is penicillin-resistant *S. aureus* (PRSA) in the 1940s when the infection quickly emerged in hospitals (Craft et al., 2019). The rise of bacterial resistance takes the Indonesia government's attention because antibiotics are used widely as health therapy for humans, livestock, and cultivated fishes (Feliatra et al., 2022). The strategic solution is highly required to finalize the bacterial resistance in the interest of human health.

In recent years, people have started to follow the "back to nature" campaign by reducing chemical consumption. Researchers seek natural resources to be developed as herbal medicines. Several natural products as herbal medicines contain antimicrobial, anti-inflammatory, and immunomodulatory (Sarecka-Hujar & Szulc-Musioł, 2022). Natural products with various bioactive compounds are now commonly used as an alternative for health maintenance due to their minimum side effects compared to synthetic compounds in chemical drugs (Nurdyansyah & Widyastuti, 2019). The success of treatment with natural products is associated with its phytochemical constituents, such as phenolics, alkaloids, saponins, tannins, etc (Milanda et al., 2021). Phenolic compounds, including flavonoids, have a positive correlation to antioxidant and antibacterial activities (Al-Rajhi et al., 2022).

One of the local plants in Indonesia with flavonoid content is *parijoto* (*M. speciosa*). *Parijoto* is an endemic plant grown on the Mount Muria slope in Kudus, Central Java, Indonesia (Hanum et al., 2017). It has purplish red fruits with high anthocyanin, a water-soluble natural pigment that belongs to the flavonoid groups (Sa'adah et al., 2020). Flavonoids play a role as antioxidants to combat free radicals, whereas the tannin in *parijoto* shows antimicrobial activities through enzyme inhibition mechanisms and the formation of complexes with metal ions. This tannin improves the lipid profile and increases antioxidant activities (Hanum et al., 2017).

Some previous studies have shown that *parijoto* has antibacterial activity. The n-hexane, ethyl acetate, and methanolic extract of *parijoto* showed antibacterial activities against *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa* (Milanda et al., 2021). However, the antibacterial activities of *parijoto* fraction with various solvents have not been reported. The fractionation with various polar to non-polar solvents is required to optimize the effect of specific bioactive compounds in natural products. So, this study aims to obtain the antibacterial activity of various fractions of *parijoto* methanolic extract to *E. coli* and *S. aureus*. The solvents used for fractionation are n-hexane, ethyl acetate, and methanol, respectively, representing non-polar, semi-polar, and polar solvents. This study provides new insights into the antibacterial activity of different solvent fractions of *parijoto* fruit, optimizing the extraction of bioactive compounds for targeted applications. Identifying the most effective fraction against *E. coli* and *S. aureus* supports the development of natural antibacterial agents for food and pharmaceutical use.

2. Materials and methods

2.1. Materials

2.1.1 Plant material and sample preparation

The *parijoto*'s fruit was obtained from the slopes of Mount Muria in Colo, Kudus, Central Java-Indonesia. The fruits collected from the *parijoto* plant grow naturally. The selected fruits, which are mature enough and marked with purplish red skin, were then extracted and fractionated in the Laboratory of Food Technology, Universitas PGRI Semarang, Central Java-Indonesia.

2.1.2 Chemical and reagents

Methanol, n-hexane, Ethyl Acetate, Folin-Ciocalteu Reagent, and Sodium Carbonate were purchased from Merck (Germany). Gallic acid and DPPH were purchased from Sigma Aldrich (Germany). Chloramphenicol was purchased

from Oxoid (UK). DMSO (dimethyl sulfoxide) and Nutrient Agar were purchased from Merck (Germany)

2.2. Methods

2.2.1. Extraction of parijoto's fruits

The extraction method was referred to Widyastuti & Nurdyansyah (2023) with some modifications. The *parijoto*'s fruits were separated from their branches, washed, and cut into small pieces. The fruits were then dried in dryer cabinet at 50°C for 48 hours. The dried fruits were mashed and sieved with 60 60-mesh sifter, and then the powder was extracted. A 100 g of powdered fruit was macerated in 1000 mL methanol (Merck, Germany) for 24 hours with twice re-maceration with the same solvent ratio. The macerates were collected and concentrated with a rotary vacuum evaporator (DLAB Scientific, China) at 40°C and 50 rpm. The crude *parijoto*'s methanolic extract (CPME) was ready for fractionation.

2.2.2. Fractionation of crude parijoto's methanolic extract

The fractionation procedure referred to Egua et al. (2014) with modifications. The CPME residue was dissolved in 500 mL aquadest and then fractionated by liquid-liquid fractionation with 500 mL n-hexane (Merck, Germany), ethyl acetate (Merck, Germany), and methanol (Merck, Germany). The solution was then evaporated with a rotary vacuum evaporator at 40°C. The fraction obtained, n-hexane (PNF), ethyl acetate (PEF), and methanol (PMF), were tested to evaluate the antibacterial activity of *parijoto*'s fruits to *E. coli* and *S. aureus*.

2.2.3. Measurement of total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu method according to the procedure described by Ghafoor et al. (2020) with modifications. The 500 µL of each PNF, PEF, and PMF were mixed with 400 µL of Folin-Ciocalteu reagent (Merck, Germany). The mixture was then incubated for 5 min at room temperature. After the incubation, 4 mL of 7% sodium carbonate (Merck, Germany) was added, and then distilled water was also added to the 10 mL final volume. The final mixture was incubated for 30 min at room temperature. The absorbances were measured at 730 nm with a UV-Vis spectrophotometer (Hitachi UH5300, Japan). Gallic acid (Sigma Aldrich, Germany) (25-150 µg/mL) was used as the standard for the calibration curve, so the total phenolic contents were expressed as gallic acid equivalents (µg GAE/mL).

2.2.4. Measurement of DPPH radical scavenging activity

The radical scavenging activity was analyzed with 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the method from Ghafoor et al. (2020) with modifications. Series of each fraction were prepared at 50, 100, 150, 200, and 250 µg/mL, then 4 mL of DPPH (Sigma Aldrich, Germany) was added, and the final volumes were made up to 5 mL. The mixtures were shaken gently or vortexed for 5 sec, then incubated for 30 min at room temperature in the dark. The absorbances were measured at 517 nm using a UV-Vis spectrophotometer (Hitachi UH5300, Japan). The DPPH radical scavenging activity was measured following the equation below. Then the IC_{50} was measured with the regression formula y = ax + b from the values of DPPH radical scavenging activities.

$$DPPH\ radical\ scavenging\ (\%) = \frac{[(Absorbance\ of\ control - Absorbance\ of\ sample)x\ 100]}{Absorbance\ of\ control}$$

2.2.5. Bacterial culture

The *E. coli* and *S. aureus* were obtained from Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada, Indonesia. The Preparation of bacterial suspensions was referred to Milanda et al. (2021) with modifications. The *E. coli* FNCC 0091 and *S. aureus* FNCC 0047 were sub-cultured in nutrient agar and incubated at 37°C for 18 to 24

hours. The bacterial suspension was diluted to reach 10^6 CFU/mL cell density. Both 10^6 CFU/mL *E. coli* FNCC 0091 and *S. aureus* FNCC 0047 were inoculated to nutrient broth at 37°C for 24 hours. The cultures were ready for the antibacterial activity test.

2.2.6. Antibacterial activity test

The test was performed in a completely randomized design to derive the optimal concentration of PNF, PEF, and PMF to inhibit the growth of *E. coli* and *S. aureus* following the Kirby-Bauer disk diffusion method. The variables for each fraction were 5% chloramphenicol (Oxoid, United Kingdom) as positive control, 0.5% DMSO (dimethyl sulfoxide) (Merck, Germany) as negative control, 30%, 60%, and 90% (v/v) of each fraction as treatments. The Kirby-Bauer method.

2.3. Data analysis

The data were replicated and presented as mean ± SD. The data was statistically analyzed with SPSS version 17 to perform one-way ANOVA analysis. Significant differences among means were determined by Duncan's multiple-range test. If the *p*-value was less than 0.05, it was considered as statistically significant.

3. Results and discussions

3.1. Drying Loss and Yield Extract

The amount of compound lost from *parijoto* fruit when it was heated for several hours at 105 °C was shown by the drying loss value (Hikmawanti et al., 2021). The drying process of *parijoto*'s fruits generates 85.49% drying loss. It can be known from the dry weight of the fruits, which is 322 g obtained from 2220 g of fresh fruits (Mona et al., 2022). The herbs, any plant parts for flavoring, medicine, food, or perfume, were mostly processed by drying to get shelf-stable products. The drying method preserves the quality of herbs by reducing moisture content, which leads to the inhibition of microbial growth and alteration of chemicals during storage (Thamkaew et al., 2021). The drying process in herbs, *parijoto*'s fruits can also generate a positive impact on its materials' quality. During the drying process, the water content was removed, but it can generate several value-added compounds in dried samples (Calín-Sánchez et al., 2020; Pratama et al., 2018b). The dried *parijoto*'s fruits were assumed to be more durable during the research.

The dried *parijoto*'s fruits were macerated with methanol as one of the universal solvents which have the capability to bind and dissolve chemical compounds from natural materials, including polar, semi-polar, or non-polar compounds (Nurdyansyah & Widyastuti, 2019). The extraction process of dried *parijoto*'s fruits generates 67,45% yields (Mona et al., 2022), then followed by liquid-liquid fractionation with n-hexan (PNF), ethyl acetate (PEF), and methanol (PMF). The fractionation yields of PNF, PEF, and PMF were 13.8%, 22.8%, and 45.4%, respectively (Mona et al., 2022). The yield of PMF was the highest among others, indicating that in this fraction may contain higher chemical compounds than the other fractions due to its ability to bind polar to non-polar compounds. The lowest yield was possessed by PNF which prefers to bond with non-polar compounds. The ethyl acetate, considered a polar solvent due to the presence of polar carbon-oxygen bonds, however, still could dissolve non-polar compounds since it also has alkyl groups which are non-polar in nature. So, PEF generates a higher yield than PNF but is still lower than PMF.

3.2. Total Phenolic Content (TPC) value

Phenolic compounds are among the valuable bioactive metabolites in plants (Elshahawy et al., 2022) that have many biological activities, related to antioxidant properties to free radicals neutralization, inhibition of pro-oxidant enzymes activity (cyclooxygenase or lipoxygenase), disruption of auto-oxidation chain reactions, and chelation of transition metal ions (Złotek et al., 2019). They are generated through photosynthesis in the plant as potential secondary metabolites. Their -OH (hydroxyl) groups are characteristically bound to proteins and other various compounds, and have the ability to exhibit several bioactivities, such as antioxidant and anticancer (Nam et al., 2017).

Wijayanti & Ardigurnita (2018) reported that *parijoto*'s leaves extract possessed 3.95% of total polyphenols. These bioactive compounds provide effective antioxidant properties to counteract free radicals from lipid oxidations. Polyphenols have a protective ability to prevent oxidative stress that leads to organ damage and various degenerative diseases. Phytochemical screening of *parijoto*'s fruit fraction showed that *parijoto*'s n-hexane, ethyl acetate, and methanol fractions have polyphenolic compounds after the positive reaction of the sample's fraction with 1% $FeCl_3$. The *parijoto*'s fruits are also known to have flavonoids, saponins, and tannins based on phytochemical screenings (Syifaul Qulub et al., 2022).

The present study showed that the PNF has significantly highest total phenolic content ($p<0.05$) among the three fractions (**Table 1**). The lowest total phenolic content is possessed by PEF. The Total phenolic content of PMF is significantly higher than that of PEF but also significantly lower than that of PNF. This result indicates that the phenolic compounds of *parijoto*'s fruits are mainly non-polar because they were extracted to n-hexane as a non-polar solvent. The non-polar solvents are well known to be useful in the extraction and fractionation of polyphenols, flavonoids, terpenoids, fats, and oils (Abubakar & Haque, 2020).

Table 1. Total phenolic content of *parijoto*'s n-hexane (PNF), ethyl acetate (PEF), and methanol fraction (PMF) with gallic acids as standard

Samples	TPC (µg GAE/g)
PNF	146.29 ± 0.91^{c}
PEF	38.75 ± 1.70^{b}
PMF	68.83 ± 2.63^{a}

Note: different letters within column indicate a significant differences ($p < 0.05$)

The most polar solvent in this research is methanol. It is one of the universal solvents commonly used to extract bioactive natural products from plants. Methanol was expected to be the best solvent to extract both polar and non-polar compounds (El Houda Lezoul et al., 2020). The result showed that the most non-polar solvents obtained higher total phenolic compounds from *parijoto*'s fruits compared to ethyl acetate and methanol, which are more polar. Ethyl acetate with 0.228 polarities only obtained a few polyphenols from *parijoto*'s fruits, still lower than methanol, which is more polar with 0.762 polarities (Abubakar & Haque, 2020). These results indicate that parijoto fruit contains a high phenolic content, which may be linked to its strong antioxidant and antibacterial activities, as phenolic compounds are known for their antibacterial, antifungal, and antioxidant properties (Behiry et al., 2019).

3.3. DPPH radical Scavenging Activity

The Plant species have many antioxidant substances that are produced by different mechanisms. Several methods can be used to measure the antioxidant activity in the plant's extracts. One of the most common use methods is DPPH radical scavenging activity (Nam et al., 2017). The DPPH radical scavenging activity is based on the reduction of one-electron that reflects the free radical reducing activity of antioxidants. The antioxidant activities are highly correlated with the total flavonoid and phenolic content of the plant extract (Johari & Khong, 2019). The DPPH radical scavenging activity is commonly used for the measurement of the free radical scavenging potential of antioxidant molecules. This method is considered as one of the easy and standard colourimetric methods for the evaluation of antioxidant activities. The principle of the DPPH method is based on accepting a hydrogen atom from the scavenger molecules (antioxidant), resulting in a reduction of DPPH to DPPH2, and the purple colour changes into yellow colour. The colour change is monitored by a spectrophotometer and used for the determination of antioxidant properties (Mishra

et al., 2012). The IC_{50} or 50% inhibitory concentration values indicate the ability of the PNF, PEF, and PMF to inhibit or reduce free radicals by 50% (Nurdyansyah & Widyastuti, 2019).

The PNF exhibited the highest scavenging activity, as indicated by its lowest IC_{50} value of 1.73 µg/g which is required to inhibit 50% of free radicals. The PEF and PMF have IC_{50} at 4.13 and 7.59 µg/g, respectively (**Table 2**). PEF has significantly higher IC_{50} than PNF and lower than PMF. It indicates that PEF has scavenging activity lower than PNF but higher than PMF, based on its IC_{50}. The high radical scavenging activity possessed by PNF is due to its high total phenolic content, so it assumes that there is a correlation between total phenolic content and DPPH radical scavenging activity at PNF. Phenolic compounds as one of the secondary plant metabolites are synthesized by plant cells to act as antioxidant agents. These compounds could protect plants from the dangerous influences of oxidations (Elshahawy et al., 2022).

Table 2. The IC_{50} of DPPH radical scavenging activity of *parijoto*'s n-hexane (PNF), ethyl acetate (PEF), and methanol fraction (PMF) with gallic acids as standard

Samples	IC_{50} (µg/g)
PNF	1.73 ± 0.09^{c}
PEF	4.13 ± 2.01^{b}
PMF	7.59 ± 0.42^{a}

Note: different letters within column indicate a significant differences ($p < 0.05$)

The PEF has higher DPPH scavenging activity than PMF shown by their $IC_{50\ value}$. From this result, it is known that the DPPH scavenging activity of the non-polar fraction is higher than the polar fraction. The PNF as the most non-polar fraction, has the highest scavenging activity and the PMS as the most polar fraction has the lowest scavenging activity. The bioactive compounds that are responsible as antioxidants might be more bonded to non-polar solvents than polar solvents. So, the non-polar fraction provides stronger antioxidant activities than the polar fraction.

3.4. Antibacterial activity against *E. coli*

The correlation between phenolic compounds and the antibacterial activity of plant extract is still being discussed among researchers (Belhaoues et al., 2020). However, antibacterial activity may not be solely attributed to phenolic compounds, as other bioactive compounds, such as flavonoids, alkaloids, and terpenoids, could interact synergistically to enhance the antibacterial effects of the fractions or extracts (Mohammed et al., 2021; Villa et al., 2013). In this study, all three fractions have tighter inhibition zones than positive control and there are significant differences ($p<0.05$) between positive control and all different fractions.

Table 3 shows that PEF has the highest antibacterial activity against *E. coli* due to its wide inhibition zone. *Escherichia coli* in this study was known to be more sensitive to PEF, than PMF, and the last to PNF seen from the inhibition zones. The PNF with the highest total phenolic content and radical scavenging activity turns out to have the lowest antibacterial effect to *E. coli*. It is suggested that maybe there are some other bioactive interactions that are responsible for the antibacterial activity of *Parijoto*'s fractions. The antibacterial activity of *Parijoto*'s fractions is affected not only by its phenolic and antioxidant compounds but also by other compounds, which will have to be studied in the next studies.

The highest concentration of Parijoto fruit extract fraction exhibited the widest inhibition zone, showing a significant ($p<0.05$) antibacterial effect compared to lower concentrations. However, its inhibition zone is still significantly tighter than that of the positive control, which used chloramphenicol as an antibiotic. This result indicates that some non-polar compounds in *Parijoto*'s fruit, which are extracted by ethyl acetate, are more responsible

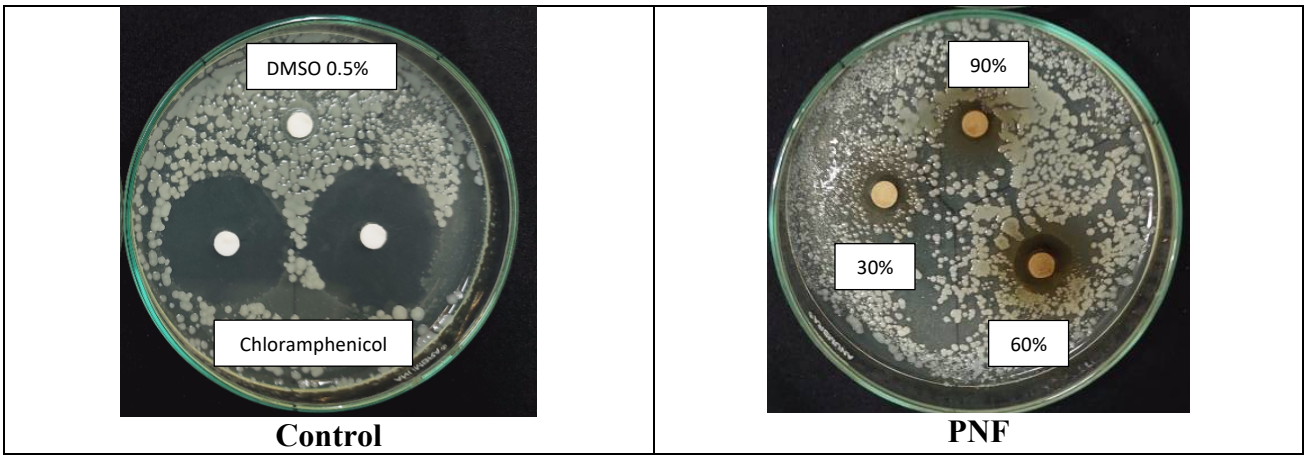
for its antibacterial activity compared to more non-polar compounds in PNF or more polar compounds in PMF. As the most non-polar fraction, PNF has the lowest antibacterial activity against *E. coli* and PMF, as the most polar fraction, has higher antibacterial activity than PNF but it is still lower than PEF. So, the PEF is the most potent fraction of *Parijoto*'s fruit to hamper *E. coli*'s growth and the most potent concentration is in 90% with 11.16 mm of inhibition zone's diameter.

Table 3. The inhibition zone diameters of *Parijoto*'s n-hexane (PNF), ethyl acetate (PEF), and methanol fraction (PMF) against *E. coli* FNCC 0091

Group of Treatment	Inhibition Zone (mm)		
	PNF	PEF	PMF
Chloramphenicol (Positive control)	27.06 ± 0.97^{e}	26.78 ± 1.02^{e}	26.66 ± 0.49^{e}
DMSO 0.5% (Negative control)	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}
T1 (30% of *Parijoto*'s fraction)	1.3 ± 0.32^{b}	7.18 ± 0.24^{b}	6.40 ± 0.01^{b}
T2 (60% of *Parijoto*'s fraction)	3.22 ± 0.30^{c}	10.13 ± 0.25^{c}	7.91 ± 0.57^{cd}
T3 (90% of *Parijoto*'s fraction)	4.63 ± 0.65^{d}	11.16 ± 0.77^{d}	8.27 ± 0.21^{d}

Note: different letters within column indicate a significant differences ($p < 0.05$)

The inhibition zones at *E. coli* cultures in positive and negative control, as well as also PNF, PEF, and PMF treatment are shown in **Figure 1**. It shows that DMSO 0.5% as a negative control did not inhibit the growth of *E. coli*, but chloramphenicol did as a positive control. It also shows that 90% *Parijoto*'s fractions inhibit the growth of *E. coli* better than the 30 and 60% fractions, which can be known from the diameter of inhibition zones.



Control **PNF**

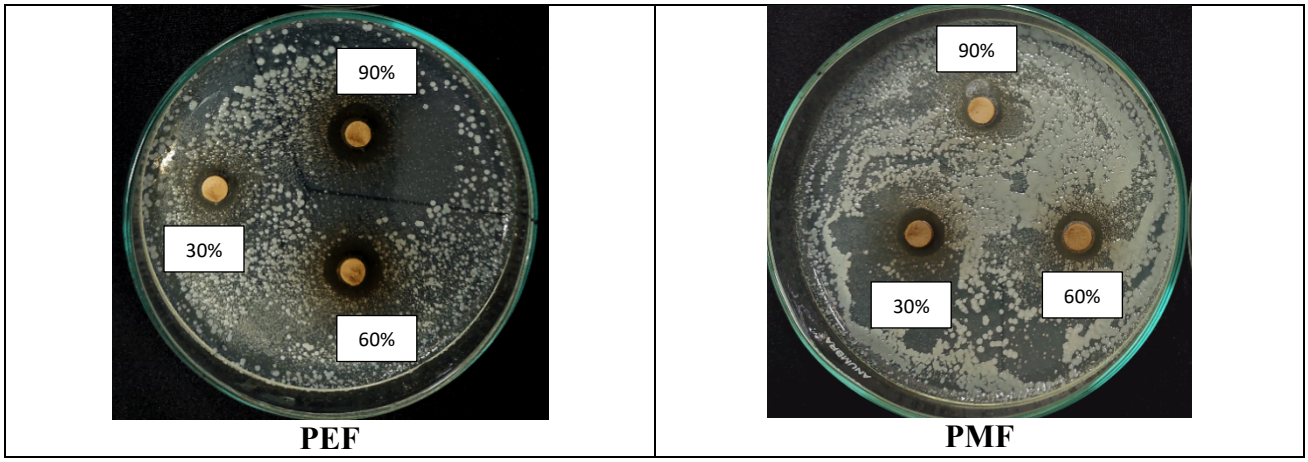


Figure 1. The inhibition zone of *E. coli* with *Parijoto*'s n-hexane fraction (PNF), ethyl acetate fraction (PEF), methanol fraction (PMF), chloramphenicol as positive control, and DMSO 0.5% as negative control.

3.5. Antibacterial activity against *S. aureus*

Staphylococcus aureus is a Gram-positive bacteria that is responsible for some major infectious diseases. It has evolved to resistant strain to some antibiotics, like methicillin and others, forming methicillin-resistant *S. aureus* (MRSA). The resistant strain of *S. aureus* is 10-fold more infectious than all multi-drug-resistant Gram-negative bacteria (Craft et al., 2019). However, some researchers suggested that Gram-positive bacteria, like *S. aureus,* are more sensitive to the presence of bioactive compounds in plant extracts (Jurić et al., 2021).

In this study, for PNF and PMF, the inhibition zones are wider than the inhibition zones which form in the *E. coli* cultures. However, for PEF, the inhibition zones are tighter than in *E. coli* cultures. These results assume that *S. aureus* is more susceptible than *E. coli* in the treatment of PNF and PMF, but it is not in the treatment of PEF. There are no differences in the order of sensitivity to Parijoto fruit extract fraction from *E. coli* cultures. *Staphylococcus aureus* is most sensitive to PEF with the widest inhibition diameter of 9.81 mm at 90% PEF concentration and most potent to PNF with a range of inhibition diameter 3.8 to 4.95 mm. The higher concentration of Parijoto fruit extract fractions inhibited the growth of *S. aureus* more than the lower concentrations (Table 4).

Table 4. The inhibition zone diameters of *Parijoto*'s n-hexane (PNF), ethyl acetate (PEF), and methanol fraction (PMF) against *Staphylococcus aureus* FNCC 0047

Group of Treatment	Inhibition Zone (mm)		
	PNF	PEF	PMF
Chloramphenicol (Positive control)	$21.83 \pm 2{,}75^{d}$	20.6 ± 2.15^{e}	22.9 ± 4.55^{e}
DMSO 0.5% (Negative control)	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}
T1 (30% of *Parijoto*'s fraction)	3.8 ± 0.37^{b}	7.1 ± 0.27^{b}	6.4 ± 0.23^{b}
T2 (60% of *Parijoto*'s fraction)	4.21 ± 0.78^{bc}	8.28 ± 0.24^{c}	8.28 ± 0.51^{c}
T3 (90% of *Parijoto*'s fraction)	4.95 ± 0.45^{c}	9.81 ± 0.78^{d}	9.24 ± 0.69^{cd}

Note: different letters within column indicate a significant differences ($p < 0.05$)

All three concentrations of Parijoto's fraction, 30, 60, and 90%, significantly ($p<0.05$) inhibit the growth of *S. aureus* compared to negative control. But the inhibition zones which are formed after the treatment were still significantly tighter than chloramphenicol as positive control. The PEF showed the strongest inhibition activity than PNF and PMF. It assumed that ethyl acetate as solvent succeeds to bind the compounds which are responsible for *Parijoto* fruit's antibacterial activities. However, the diameter of inhibition zones at PMF treatment are slightly similar to PEF, which at 60% concentration, both showed the same diameter of inhibition zone that is 8.28 mm. This result suggested that PEF and PMF may have similar antibacterial activity against *S. aureus,* so the non-polar and polar compounds which bond to ethyl acetate and methanol are more effective in inhibiting the growth of *S. aureus* than compounds which bond to n-hexan with a tighter range of diameters at 3.8 to 4.96 mm.

The inhibition zones at *S. aureus* cultures in positive and negative control, also PNF, PEF, and PMF treatment (**Figure 2**). The negative control showed no inhibition of *S. aureus* growth, while chloramphenicol, used as the positive control, exhibited the strongest antibacterial effect, as indicated by the widest inhibition zones. It is also seen that the 90% Parijoto fruit extract fraction showed more inhibition of growth of *S. aureus* than 30% and 60% fractions concentrations which can be known from the diameter of inhibition zones.

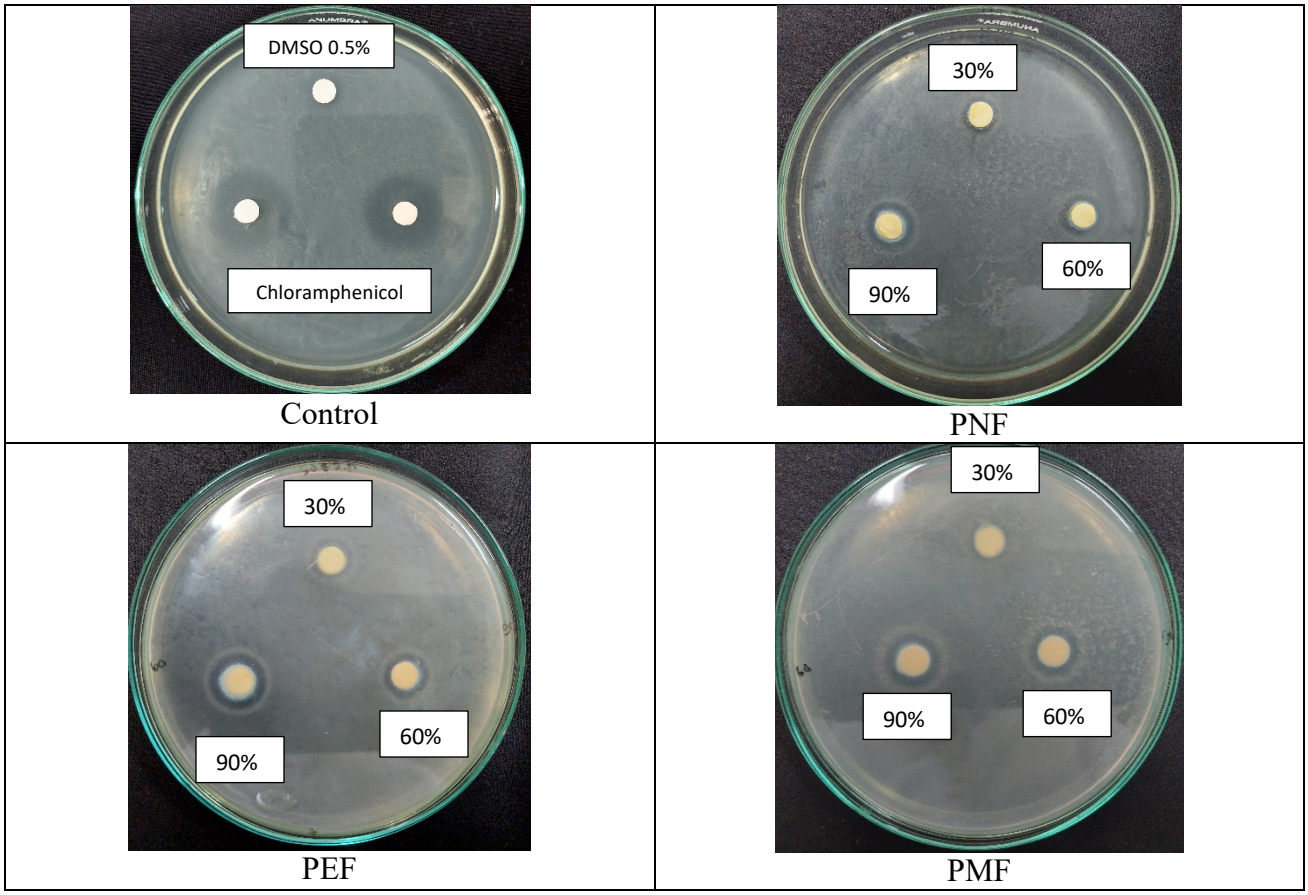


Figure 2. The inhibition zone of *S. aureus* with *Parijoto*'s n-hexane fraction (PNF), ethyl acetate fraction (PEF), methanol fraction (PMF), chloramphenicol as positive control, and DMSO 0.5% as negative control.

This study indicated that the Parijoto fruit extract fractionation was one of the techniques to determine the potency of Parijoto as a local plant in Central Java to combat the bacterial activities like *E. coli* and *S. aureus* which have pathogenicity effect to human. Antibacterial activity is mostly assumed to be correlated with total phenolic content and antioxidant activities in most plant extracts and fractions.

However, in this study, PNF with highest total phenolic content and radical-scavenging activity turns out as the lowest antibacterial activity against both *E. coli* and *S. aureus*. This indicated that the antibacterial activity of Parijoto fruit extract fractions not only correlated to phenolic compounds which leads to antioxidant activities but there might be many other interactions of Parijoto's bioactive compounds to positively induce its antibacterial activity. It needs some research to follow up the reasons why the Parijoto fruit extract fractions with high phenolic content and radical-scavenging activity turns out as the weakest one to inhibit *E. coli* and *S. aureus*. It may require advance molecular analysis to study about biomolecular interactions of Parijoto fruit extract fractions to know the right pathway which leads to its antibacterial activity

4. Conclusions

This study indicated that the Parijoto's fruit extract fractionation was one of the techniques to determine the potency of Parijoto as a local plant in Central Java to combat the bacterial activities like E. coli and S. aureus which have pathogenicity effect to human. The antibacterial activity was mostly assumed to be correlated with total phenolic content and antioxidant activities in most plant extracts and fractions. The present study showed that the n-hexane fraction (PNF) possesses highest total phenolic content and DPPH radical-scavenging activity turns out as the lowest antibacterial activity against both E. coli and S. aureus. This indicated that the antibacterial activity in Parijoto's fruit extract may be correlated not only with total phenolic content but also correlated with other factors and metabolic pathways.

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Acknowledgments

We would like to say thanks a lot to the Institution of research and community services of Universitas PGRI Semarang for financial assistance in this research. We also thank to all laboratory members of Department of Food Technology, Universitas PGRI Semarang for all the helpful hands during the research. This research is partially supported by Food Safety Scientific Consortium through the Research Implementation Agreement Contract No. 369/UN7.A/HK/X/2024.