

Constituents and Antibacterial Activity of Extract of Nagasari (*Mesua ferrea*) Leaves

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ABSTRACT

Nagasari (*Mesua ferrea*) had been widely known for medication use in South and South East Asia. This research aimed to identify the secondary metabolites and evaluate the antibacterial activity of ethanolic extract of leaves of *M. ferrea*. The dried leaves of *M. ferrea* were extracted with ethanol by remaceration method. The antibacterial activity was analyzed with disk diffusion method against *Escherichia coli* and *Bacillus subtilis*. The secondary metabolites were identified by the standard phytochemical screening method. The ethanolic extract of leaves of *M. ferrea* contained tannins, flavonoids, and terpenoids. Those metabolites might be responsible for its antibacterial activity against *E. coli* and *B. subtilis*.

Key words: *Mesua ferrea*, leaves, extract, antibacterial activity, secondary metabolites.

INTRODUCTION

Nagasari (*Mesua ferrea*, Clusiaceae) is the identity flora of Banyumas. In India, *M. ferrea* is considered as sacred plant and important component of some Ayurveda preparations (Suresh et al., 2014). Empirically, *M. ferrea* is used for anti inflammatory, antiseptic, tonic, carminative, cardio tonic, diuretic, antioxidant, immunostimulant, and antivenom. *M. ferrea* is also used for treatment of fever, influenza, asthma, and bronchitis. Almost all parts of plants of *M. ferrea* is utilized for medicinal purposes (Asif et al., 2017). In Baturraden, Central Java, decoction of leaves of *M. ferrea*, Adas, Dewandaru, Awar-awar, and Semanggi gunung is used for treatment of leukemia, mental illness, liver, dengue fever, and heart disease (Suparman et al., 2012).

Various extracts and isolates of *M. ferrea* collected in India, Thailand, and Malaysia have been reported active as antimicrobial agents for Gram-positive, Gram-negative bacteria, and also yeast (Chanda et al., 2013; Keawsaard and Kongtaweelert, 2012; Ullah et al., 2013). There are several secondary metabolites of *M. ferrea* that was responsible for its antimicrobial activity. Those metabolites are belongs to coumarin, xanthone, and triterpenoid derivatives (Roy et al., 2013; Suresh et al., 2014). In this research, we identify the secondary metabolites and evaluate the antibacterial activity of ethanolic extract of

leaves of *M. ferrea* from tropical region, Banyumas, Indonesia against *E.coli* and *B. subtilis*.

EXPERIMENTAL

2.1 Materials

Dried powder of leaves of *M. ferrea* and ethanol were used for preparation of the extract. Nutrient Agar (NA), distilled water, sterile NaCl, and tetracycline were used for antimicrobial assay. *E. coli* and *S. aureus* were used as the tested bacteria. Dragendorf's reagent, sulphate acid, chloride acid, and ferric chloride were utilized for phytochemical screening.

2.2 Preparation of leaves of *M. ferrea*

The leaves were collected from Notog, Banyumas. The identity of the plant was authenticated at Laboratory of Botany, Faculty of Biology, University of Jenderal Soedirman Purwokerto. The leaves were dried under direct sunlight. The dried leaves of *M. ferrea* were pulverized into fine powder with a simple grinding machine.

2.3 Extraction process

Powdered dried leaves of *M. ferrea* (500 g) were macerated with ethanol for 3 days. The process of maceration was repeated twice. Ethanol was then separated with rotary evaporator (Hoekou et al., 2017).

2.4 Phytochemical screening of ethanolic extract of leaves of *M. ferrea*

The group of secondary metabolites (flavonoids, alkaloids, steroids, saponins, terpenoids, and tannins) in ethanolic extract of leaves of *M. ferrea* were analyzed with standard phytochemical screening reported elsewhere (Fall et al., 2017; Jaradat et al., 2015).

2.5 Antimicrobial activity assay

The antimicrobial assay of ethanolic extract of *M. ferrea* was conducted following a method previously reported with a minor modification (Astuti et al., 2014). The ethanolic extract of *M. ferrea* was prepared into series of concentrations of 1000, 500, 250; 125, 62, 32, 15, 7.8, and 3.9 µg/ml. Sterile water and tetracycline were used as negative and positive control, respectively. The diameter of inhibition

zones were analyzed with one way ANOVA followed with Least Significance Differences (LSD) test at level of confidence of 95%. All the statistical analysis was performed with SPSS ver. 18.

RESULT AND DISCUSSION

The result of phytochemical screening of ethanolic extract of leaves of *M. ferrea* showed it contained tannins, flavonoids, and terpenoids (Table 1). Previously, mono- and sesquiterpenes in essential oils of *M. ferrea* collected in Thailand was majorly consisted of trans-caryophyllene, β -caryophyllene oxide, and α -humulene (Keawsaard and Kongtaweelert, 2012). Comprehensive reviews on phytochemistry of *M. ferrea* reported that this plant containing coumarins, xanthons, triterpenoids, flavonoids, glycosides, resins, and steroids, along with primary metabolites such as protein and fatty acids (Chahar et al., 2012; Naik and Indira, 2015).

It is possibly that those groups of metabolites were responsible for the antibacterial activity of the ethanolic extract of *M. ferrea*. A study has reported a strong relationship between the content of flavonoids with the antimicrobial activity of extracts of *M. ferrea* collected from Quang Nam, Vietnam (Phuong and Nhat, 2015). n-Hexane and methanolic extracts of *M. ferrea* that contained flavonoids and triterpenoids showed a better antimicrobial activity against Gram- positive and Gram-negative bacteria than those of aqueous extract that did not contain those two metabolites (Rawat and Upadhyaya, 2013).

Table 1. The result of screening of phytochemicals in ethanolic extract of leaves of *M. ferrea*

Group of Metabolites	Reagents	Results		Conclusion
		Positive (Fall et al., 2017; Jaradat et al., 2015)	Our study	
Alkaloid	Dragendrof's	Formation of orange precipitate	Brown precipitate was formed	Negative
Saponin	-	Formation of stable froth	No froth was observed	Negative
Phenolic	FeCl ₃	Formation of purple color	Purple hue was formed	Positive
Tannin	FeCl ₃	Formation of dark green color	Dark green hue was formed	Positive
Flavonoid	HCl	Formation of red color	Red hue was	Positive

Terpenoid	H ₂ SO ₄	Formation of brownish red color	formed Brownish red hue was formed	Positive
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The antimicrobial activity of ethanolic extract of leaves of *M. ferrea* was shown by their diameters of inhibition zone against *E. coli* and *B. subtilis* (Table 1). Based on those diameter zones, all tested concentrations of ethanolic extract possessed antibacterial activity against *E. coli*, shown by a significantly differences between those samples and negative control (DMSO). The ethanolic extract of leaves of *M. ferrea* at concentration of 250, and 125 µg/ml showed a comparable diameter of inhibition with that of tetracycline (positive control). At higher concentration (1000 and 500 µg/ml), their antibacterial activity against *E. coli* were better than tetracycline, with the mean of diameter of inhibition zone were 2.5 and 2.13 cm, respectively.

Ethanolic extract of leaves of *M. ferrea* also showed the antibacterial activity against *B. subtilis*. All tested concentrations showed inhibition zones with diameter of inhibition zones varied from 0.7-1.27 cm. Statistical analysis showed that ethanolic extract of *M. ferrea* from concentration of 7.82 µg/ml started to exhibit the growth of *B. subtilis*. Somehow their antibacterial activity against *B. subtilis* was weaker than that of tetracycline.

The ethanolic extract of leaves of *M. ferrea* was more potent against *E. coli* (a Gram-negative bacterium) than against *B. subtilis* (a Gram-positive one). It is shown by the significance difference of their diameter of inhibition zones at all concentrations used in this study. This result exhibited that apparently the Gram-negative bacteria was more sensitive to the ethanolic extract of leaves of *M. ferrea*. Our result was similar with a study reported earlier, that the MIC of methanolic extract of seed of *M. ferrea* against *E. coli* and *B. subtilis* were 10 and 80 µg/ml, respectively (Rawat and Upadhyaya, 2013). Another report stated that the diameter of inhibition zone of 10% ethanolic extract of bark of *M. ferrea* against the growth of those two bacteria was 11.50 and 9.33 mm, respectively (Phuong and Nhat, 2015).

Gram-positive and Gram-negative bacteria were differed by the structure of their cell wall (Navarre and Schneewind, 1999). The antimicrobial compounds in ethanolic extract of *M. ferrea* might affect the outer structure of the bacteria. Our results suggested that phenolics were the main compound that was responsible for the antibacterial activity *M.*

ferrea. There were two groups of phenolic compounds in our study, they were tannins and flavonoids. We referred to the previous report exhibited that Gram-negative bacteria were more susceptible to phenolic compounds from berries than the Gram-positive one (Puupponen-Pimia et al., 2001).

Tabel 1. The diameter of inhibition zone of ethanolic extract of leaves of *M. ferrea* against the tested bacteria

Concentration (µg/ml)	Inhibition zone diameter (cm) against	
	<i>E. coli</i>	<i>B. subtilis</i>
1000	2.50±0*	1.27±0.06*
500	2.13±0.06*	1.17±0.06*
250	1.80±0*^	1.10±0*
125	1.70±0*^	1.22±0.24*
62.50	1.61±0.10*	1.04±0.03*
31.25	1.57±0.06*	1.12±0.16*
15.63	1.37±0.33*	0.94±0.12*
7.82	1.60±0*	0.93±0.06*
3.91	1.40±0*	0.70±0.56
Positive control	2.00±0	2.00±0
Negative control	0.80±0	0.80±0

*showed that the given group was significantly different from the negative control, ^ showed that the given group was not significantly different from the positive control. Analysis was conducted at level of confidence of 0.95.

CONCLUSION

The ethanolic extract of leaves of *M. ferrea* contained tannins, flavonoids, and terpenoids. Those compounds might be responsible for its antibacterial activity against *E. coli* and *B. subtilis*.

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