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Antibacterial Activity of Ethyl Acetate Extract of Mango Bud (*Mangifera indica* L. var. Arum Manis) Against Multidrug-Resistant Bacteria

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ABSTRACT

Ethyl acetate extracts of *Mangifera indica* leaves had chemical constituents such as saponins, alkaloids, phenols, tannins, flavonoids and diterpenes. Purified extract of *Mangifera indica* leaves had antibacterial activity against *S. aureus* and *E. coli*. This research aimed to know the antibacterial activity of mango bud (*Mangifera indica* L. var Arum Manis) against *S. aureus, E. coli, P. aeruginosa* and their multidrug resistant spesies. Arum manis mango bud simplicia powder was extracted by maceration method used ethyl acetate solvent. This extract tested to flavonoids and alkaloids by qualitative methods. Antibacterial activity used disc diffusion method and the concentration of this extract such as 10, 20, 30, 40 and 50%. Data of antibacterial activity was analyzed by descriptively. Arum manis mango bud extract had antibacterial activity against all of tested bacteria with diameter of zone of inhibition at 6.76 – 12.20 mm. The arum manis mango bud extract contained flavonoids and alkaloids.

INTRODUCTION

The young mango leaves in India had used to eat and treat a several diseases such as burning sensation, diarrhea, dysentery, haemorrhoids, ulcer, kidney stone and wound (Khandare, 2016). These activity due its chemical constituents. *Mangifera indica* leaves ethyl acetate extracts from Mauritius had chemical constituents such as saponins, alkaloids, phenols, tannis, flavonoids and diterpenes. Phenol compounds characterized such as benzophenone derivates, flavonols, xanthones (as mangiferin) and gallotannis (Laulloo et al., 2018). Mangiferin is the most active constituent on mango leaves (Kumar et al., 2021). Mango bud of arum manis cultivar had the highest mangiferin content than other cultivar of mango that it was harvested at Manisi and Cipadung village, Bandung Indonesia (Cahyanto et al., 2020). Mango bud of arum manis cultivar, which had the highest mangiferin than other cultivar of mango, extracted by ethyl acetate solvent that it was antibacterial potential against bacteria particularly multidrug resistant bacteria. Mangiferin had antimicrobial activity through membrane disruption and damage to genetic materials (Kumar et al., 2021).

Purified extract of arum manis mango leaves had shown antibacterial activity against *S.*

aureus and *E. coli* at 6.25-100% concentration with diameter of inhibition zones at 13.18-19.93mm and 15.69-22.45mm. This extract had flavonoid content by thin layer chromatography (Mulangsri & Zulfa, 2020). Gabrol, a flavonoid in licorice, was showed rapidly to increase membrane permeability disruption against *methicillin resistant S. aureus* (Wu et al., 2019).

Bacteria, fungi, viruses and parasites caused nosocomial infection in human (Nimer, 2022). Nine percent infections are caused by bacteria and the other microorganism less contributing of infection. The mayor role bacteria of nosocomial infections were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Khan et al., 2015). Most prevalent bacteria were *S. aureus* as 14% from 55.2% Gram positive bacteria and *P. aeruginosa* and *E. coli* respectively 5% and 7% from 39% Gram negative bacteria (Nimer, 2022). Treatment of nosocomial infection is long term process and requires antibiotics. Inappropiate used of antibiotic can lead antibiotic-resistant microbia, so nosocomial infections can stimulate them. Strains of microbe, that commonly caused nosocomial infection, included of coagulase-negative staphylococci, methicillin-resistant *Staphylococcus aureus* (MRSA) and enterococci. Gram positive and Gram negative bacteria, the most prevalence caused nosocomial infections, were *S. cocci, S. aureus, K. pneumoniae, E. coli, P. aeruginosa* and *Enterobacter spp* (Nimer, 2022).

Hence, this research aims to know the antibacterial activity of ethyl acetate extract of mango bud (*Mangifera indica* L. var. Arum Manis) against multidrug-resistant bacteria. The multidrug-resistant bacteria used such as *MRSA, E. coli* and *P. aeruginosa.*

METHODS

Materials and tools

Arum manis mango bud obtained from Sukorejo; Kendal; Central Java which determined at Laboratorium Lingkungan Fakultas Biologi Universitas Jenderal Soedirman that its name spesies *Mangifera indica* L. var. Arum Manis. The other materials used such as ethyl acetate, dimethylsulfoxide (DMSO), t-butyl alcohol, NaCl physiologis, aquadest, Mg powder, Mayer and Dragendorff reagent. All of the bacterial strains (*S. aureus, MRSA, E. coli*, multidrug resistant *E. coli, P. aeruginosa*, multidrug resistant *P. aeruginosa*) obtained from Laboratorium Mikrobiologi Fakultas Kedokteran Universitas Muhammadiyah Semarang as isolate clinically, nutrient agar (*S. aureus*) and brain heart infusion agar media (*E. coli* and *P. aeruginosa*) for cultivated bacteria, Mueller Hinton agar media for antibacterial activity test.

The tools used in this research such as glassware, micropipette, jar for maceration, rotary evaporation (Heidolph), Laminar Air Flow (Airtech), autoclave (All american model 1925x), incubator (Binder CO_2 incubator).

Sample preparation

Arum manis mango bud were sorted based on color of bud such as reddish, brownish, brownish yellow, brownish green and the top level part. The result of sortation washed and dried by oven at 50℃. The dried leaves were ground became powder (simplicia powder).

The simplicia powder used extraction with ethyl acetate solvent by maceration method. The maceration method was done by (Ministry of Health of the Republic of Indonesia, 1986) but its done only 5 days for duration total of maceration. One thousand eight hundred grams of simplicia powder soaked with ethyl acetate solvent 13.5L for 3 days at room temperature and manual stirring occasionally. After 3 days, it filtered and collected the macerate (macerate I). Their pulp soaked again with 4.5L solvent for 2 days. After 2 days, it filtered and collected the macerate (macerate II). The macerate I and II were mixed and evaporated their solvent by rotary evaporator at 50℃ until became thick extract. Yields extract counted by comparing obtained extract and simplicia powder.

Phytochemical screening of extract

50 mg Arum manis mango bud ethyl acetate extracts were dissolved in 10 mL ethanol pa and filtered. The solution was added Mg powder and t-butyl alcohol. Then add a few drops of concentrated HCl solution through the tube wall. The formation of red, yellow or orange color on the t-butyl alcohol layer indicates the presence of flavonoid compounds (Sarker et al., 2006).

Arum manis mango bud ethyl acetate extracts were heated in a test tube with 10 mL of 1% hydrochloric acid for 30 minutes in a boiling water bath. Then filtered using filterred paper then divided into two tubes A and B equally.

Tube A is added with 3 drops of Dragendorf reagent, and tube B is added with 3 drops of Mayer's reagent. The positive result of alkaloids is indicated by the formation of precipitates in the two reagents (Sarker et al., 2006).

Antibacterial activity test

Antibacterial activity test was done using disk diffusion method (Balouiri et al., 2016). The concentration of arum manis mango bud were 10%, 20%, 30%, 40% and 50% (eq 1, 2, 3, 4 and 5mg/disk; one disk contained 10µL of sample) and tested against *S. aureus, methicillin resistant S. aureus (MRSA), E. coli, E. coli multidrug resistant, P. aeruginosa,* and *P. aeruginosa multidrug resistant* bacteria (all of bacteria cultured by Laboratorium Mikrobiologi Fakultas Kedokteran Universitas Muhammadiyah). The positive control was used chloramphenicol (30µg/disc) for bacterial strain such *S. aureus* and *E. coli*, tigecycline (15µg/disc) for MRSA and *E. coli multidrug resistant* bacteria then ciprofloxacin (5µg/disc) for *P. aeruginosa,* and *P. aeruginosa multidrug resistant* bacteria. The negative control used DMSO (the solvent of samples). After overnight (24 hours) incubation at 37℃, the observation of the inhibition zone was showed or not. The diameters of inhibition zones are measured with caliper on the undersurface of petridish. This step repeated triplicate.

Method and result analysis

The data of antibacterial assay result were analyzed descriptively with the observation of the inhibition zone formed. The diameters of inhibition zones are measured and averaged ± SD.

DISCUSSION

The determination of mango plant was done by Laboratorium Lingkungan Fakultas Biologi Universitas Diponegoro Jenderal Soedirman Purwokerto with its certificate number B/168/UN.23.6.10/TA.00.01/2022. Result of plant determination was Anacardiaceae family which was name spesies as Mangifera indica L with Arum manis variety. The plants and mango bud show at **Figure 1**. The result of extraction obtained 62 grams extracts (3.44% yields). The arum manis mango bud ethyl acetate extracts has blackish green colour and very thick show at **Figure 2** also it has bitter taste and mango odor. This color of ethyl acetate extract was caused by

A **B**

Figure 1. *Mangifera indica* L. var. Arum manis plants; tree (A) and bud (B)

pigment content. Green pigment colour is presence chlorophyll content. Ethyl acetate solvent can dissolve chlorophyll although it is slightly soluble (Kim et al., 2020).

Bitter taste was caused by phenols, flavonoids, cathecins and caffeins content (Drewnowski, 2001). Mango odor was caused by essential oil content such as α-gurjunene, trans-caryophyllene, α-humulene, α-selinene and camphor which were bacteriostatic potential (Kumar et al., 2021). Ethyl acetate solvent can dissolve of phytochemical such as flavonoids, alkaloids, steroids, terpenoids (Oladoye et al., 2022) and mangiferin 3-methyl ether as derivate of mangiferin (Kumar et al., 2021) that was found in mango trees.

The results of phytochemical screening arum manis mango bud ethyl acetate extracts show at **Table 1**. Flavonoids and alkaloids compounds were tested in arum manis mango bud ethyl acetate extracts. The presence of flavonoids in this extracts was showed light green colour while the alkaloids was showed white and brown precipitate by Mayer and Wagner reagents.

Figure 2. Apperance of arum manis mango bud ethyl acetate extracts

Table 1. The results of phytochemical screening from arum manis mango bud ethyl acetate extract

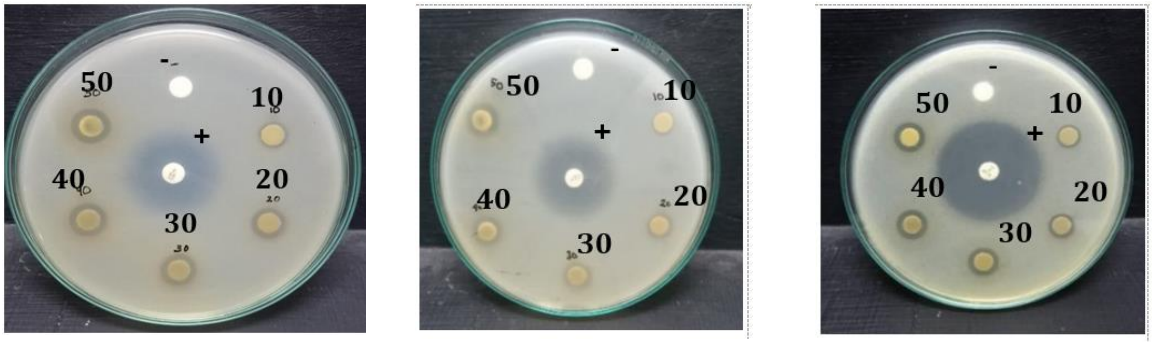
Sample	Result
Arum manis mango bud ethyl acetate extracts	
Flavonoids	+
Alkaloids	+

Note: (+) = presence bioactive compounds

The green and blue color occasionally appearance from the result of Shinoda test which was presence of flavonoids (Pant et al., 2017). Mango leaves ethyl acetate extract by Soxhlet method was contained alkaloids, flavonoids, phenols, saponin and tannins (Laulloo et al., 2018). Flavonols are type of flavonoid which was contained in ethyl acetate extract from mango leaves such as kaempferol; quercetin; quercetin 3-O-glucoside; quercetin pentoside; quercetin carboxylic acid; epicatechin gallate hexamalonate; quercetin 3-O-rhamnoside; rhamnetin; rhamnetin hexoside (Kumar et al., 2021).

is clear area of media around paperdisc which is not grow of bacteria.

The extracts was had antibacterial activity by zone of inhibition formed but the results against *P. aeruginosa* multidrug resistant was formed lightly cloudy zone of inhibition at all concentrations. Lightly cloudy zone of inhibition showed an area of growth with less turbid if it compared with outside area of zone of inhibition. Lightly cloudy zone of inhibition showed slowed down of bacteria growth. The slowed bacteria growth could cause by nutrient and ambient temperature factors (Qiu et al., 2022). In this case, the slowed bacteria growth, was not caused by its factors but it was caused by intrinsic factors on *P. aeruginosa* multidrug resistant. This intrinsic factors were permeability of outer membrane limited, efflux pump and producing enzyme for inactivated antibiotics (Pang et al., 2019). The slowed bacteria growth could caused by transformation of phenotype which was mediated by their pre-existing genetic repertoire such on *Salmonella enterica* (Pontes & Groisman, 2019). The arum manis mango bud ethyl acetate extracts had not been able to inhibit of *P.*



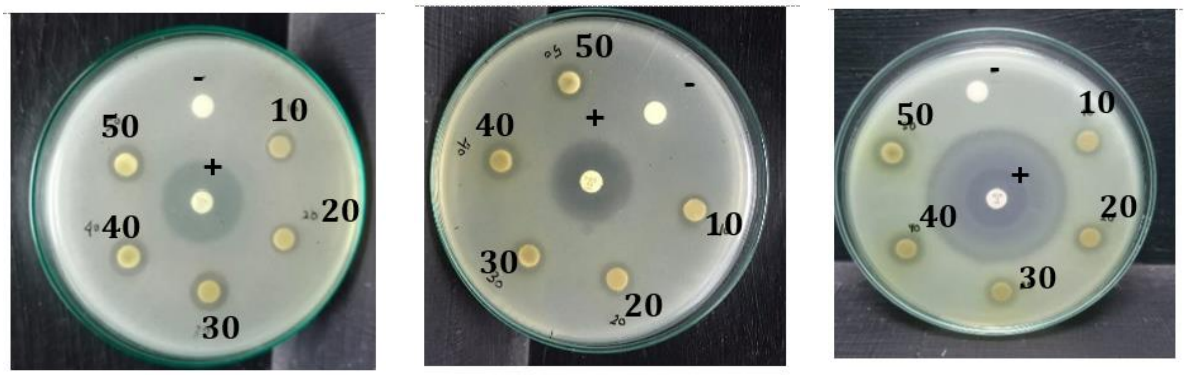
A B C

Note: 10 = 10% concentration is equal by 1mg/disk
20 = 20% concentration is equal by 2mg/disk
30 = 30% concentration is equal by 3mg/disk
40 = 40% concentration is equal by 4mg/disk
50 = 50% concentration is equal by 5mg/disk
(-) = negative control
(+) = positive control

Figure 3. The results of antibacterial activity test of arum manis mango bud ethyl acetate extract against S. aureus (A), E. coli (B) and P. aeruginosa (C) bacteria

Arum manis mango bud ethyl acetate extracts has antibacterial activity against *S. aureus, E. coli, P. aeruginosa* and their resistant species at all of concentration such as 1-5mg/disk. Result of antibacterial activity this extract show at **Table 2, Figure 3** and **Figure 4**. The zone of inhibition

aeruginosa multidrugs resistant growth with clear zone of inhibition (the irradical zone) but the positive control (Tigecycline 15µg/disc) shown the radical zone with diameter of inhibition zone at 19.2 mm. It is caused the



A **B** **C**

Note: 10 = 10% concentration is equal by 1mg/disk
20 = 20% concentration is equal by 2mg/disk
30 = 30% concentration is equal by 3mg/disk
40 = 40% concentration is equal by 4mg/disk
50 = 50% concentration is equal by 5mg/disk
(-) = negative control
(+) = positive control

Figure 4. The results of antibacterial activity test of arum manis mango bud ethyl acetate extract against resistant spesies *MRSA* (A), *E. coli* (B) and *P. aeruginosa* (C)

intrinsic factor on *P. aeruginosa* multidrug resistant.

Previous research had proven that arum manis mango peel (*Mangifera indica* L.) ethanolic extracts had antibacterial activity against methicillin resistant *S. aureus* (MRSA) and it had flavonoids, tannins, triterpenoids, alkaloids and saponins (Wulandari & Sulistyarini, 2018). Arum manis mango bud hydroalcoholic extracts had antibacterial activity against *S. aureus* and MRSA (Wulandari, 2023) . 70% ethanolic extract could to extract gallic acid, quercetin, protocatechuic acid, mangiferin and others on mango leaves (Kumar et al., 2021). Epicatechin- 3-O-β-glucopyranoside, 5-hydroxy-3-(4-hydroxyl phenyl) pyrano chromene-4 (8H)-one, 6-(phydroxybenzyl) taxifolin-7-O-β-D-glucoside, quercetin-3-O-α-glucopyranosyl-(1-2)-β- D-glucopyranoside, and epicatechin(2-(3,4dihydroxyphenyl)-3,4-dihydro-2H-chromene- 3,5,7-triol were major flavonoids compound on mango leaves extract that had antifungal activity (Kanwal et al., 2010). Flavonoid total content on arum manis mango peel ethanolic extracts was 3.27% (Noviyanty et al., 2022). Mangiferin, a xanthone C-glycosyl

Table 2. The diameter of zone of inhibition from arum manis mango bud ethyl acetate extract, Ø paperdisk = 6mm

Bacteria	The average of diameter of zone (mm)						
	1mg/disk	2mg/disk	3mg/disk	4mg/disk	5mg/disk	Positive control	Negative control
S. aureus	8.74±0.20	9.78±0.22	10.75±0.16	11.36±0.35	11.83±0.05	23.76±0.11	-
MRSA	7.61±0.23	8.84±0.08	9.87±0.08	10.46±0.1	10.82±0.24	22.50±0.20	-
E. coli	7.43 ± 0.25	8.2 ± 0.2	8.80 ± 0.1	9.54 ± 0.12	10.6 ± 0.15	21.1 ± 0.31	-
E. coli multidrug resistant	6.76 ± 0.05	7.58 ± 0.17	8.24 ± 0.15	8.62 ± 0.16	9.31 ± 0.12	20.3 ± 0.44	-
P. aeruginosa	8.50 ± 0.10	9.48 ± 0.28	10.42 ± 0.29	11.45 ± 0.27	12.20 ± 0.26	33.80 ± 0.20	-
P. aeruginosa multidrug resistant	7.29 ± 0.07	8.20 ± 0.1	9.35 ± 0.05	10.10 ± 0.1	11.43 ± 0.13	19.2 ± 0.1	-

compounds, extracted on mango leaves that has antimicrobial activity by iron chelating activity, membrane disruption and damage to genetic materials (Kumar et al., 2021).

CONCLUSIONS

The arum manis mango bud ethyl acetate had known antibacterial activity *against S. aureus, E. coli, P. aeruginosa* and their resistant spesies at all of concentration such as 1-5mg/disk. Suggestion for future experiment is determination of flavonoids, alkaloids and mangiferin content on arum manis mango bud ethyl acetate extracts.

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AUTHORS' CONTRIBUTIONS

Controlling the implementation of research, review and editing draft manuscript : DAKM; do research, data analyze and writing original manuscript : MCN, DNS, AUH.

CONFLICT OF INTERESTS

All authors declare no conflict of interest.

ETHICAL CONSIDERATION

Plagiarism checking has been carried out

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