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# ANTIBACTERIAL ACTIVITY OF PAWPAP (*Carica papaya*) AGAINST MULTIDRUG-RESISTANT *Staphylococcus aureus* AND MULTIDRUG-RESISTANT *Escherichia coli*

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This study was designed to evaluate in vitro antibacterial activity of several part of pawpaw (*Carica papaya*) against multidrug-resistant bacteria. Leaves, stems, and roots were extracted with ethanol and tested for in vitro antibacterial activity using agar dilution method against multidrug-resistant *Staphylococcus aureus* and multidrug-resistant *Escherichia coli*. Stems extracts show highest antibacterial activity against the test bacteria with minimum inhibitory concentration (MIC) value of 30 mg/mL for both bacteria. Leaves and roots extract have antibacterial activity against multidrug-resistant *Staphylococcus aureus* with MIC values of 70 and 50 mg/mL, respectively. The MIC values of leaves and roots extract on multidrug-resistant *Escherichia coli* were 80 and 70 mg/mL, respectively. Several phytochemicals in the extracts are responsible for the antibacterial activity.

Key words: antibacterial, *Carica papaya*, *Escherichia coli*, *Staphylococcus aureus*, multidrug-resistant

## INTRODUCTION

One of public health problems that threaten the effectiveness of infectious diseases treatment is antimicrobial resistance. Microbes have been reported resistant to almost every antibiotic discovered (Taiwo, 2011). Multidrug-resistant microbes are considered a substantial threat to public health because of their significant impact on morbidity and mortality (Spellberg et al., 2007). Microbial resistance to antibiotic impacts on every field of medicine and successful therapy using antibiotic is much more difficult to achieve. Choices of antibiotic to treat infection is becoming limited and almost no new antibiotic is invented (Gould, 2009). Not only physicians interest in botanical medicine but also public in general who seem to prefer products containing “natural extracts” than those based on “synthetic” substances (Gomez-Flores et al., 2008).

Pawpaw (*Carica papaya*) that belongs to caricaceae has many medicinal properties to treat various diseases. Every part of pawpaw tree has a medicinal use (Srivastava et al., 2010). The latex, leaves, and fruits of pawpaw are used medicinally and for various other purposes. Pawpaw contains a broad range of natural products such as sterols, flavonoids, saponins, lectins, fats and oils, glycosides, alkaloids, proteins, enzymes, minerals, vitamins, polysaccharide, etc (Krishna et al., 2008). Numerous study reported the antimicrobial activity of pawpaw. However, no research has been carried out to test antibacterial activity of pawpaw against multidrug-resistant bacteria. The present work investigates the antibacterial activity of ethanol extract of pawpaw against multidrug-resistant *Staphylococcus aureus* and multidrug-resistant *Escherichia coli*.

## MATERIAL AND METHODS

### Plant materials

Leaves, stems, dan roots of pawpaw were obtained from Tawangmangu, Karanganyar, Central Java and authenticated at Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT) Tawangmangu. Plant materials were washed under running water to remove dirt and sliced into thin pieces except leaves. These materials were dried and then blended into coarse powder.

## **Extraction**

Fifty grams of powdered plant materials were extracted with 500 mL of 96% ethanol using Soxhlet extractor. At the end of extraction, ethanol was evaporated using rotary evaporator to obtain ethanol extract of leaves, stems, and roots of pawpaw.

## **Antibiotic sensitivity test**

*Staphylococcus aureus* and *Escherichia coli* were tested for their sensitivity toward several antibiotics such as chloramphenicol, tetracycline, ampicillin, and erythromycin. The sensitivity test was carried out using disc diffusion method.

## **Antibacterial activity test**

Agar dilution method was used to test antibacterial activity of the ethanol extracts. Extracts that are suspended in 0.5% CMC Na was mixed with warm sterile Mueller Hinton media and allowed to solidify at room temperature. Fifty microliter of  $10^6$  CFU/mL of *Staphylococcus aureus* and *Escherichia coli* were streak on the surface of media. Later, cultures were incubated at 37°C for 24 hours and after which the presence or absence of bacterial growth were examined. Minimum inhibitory concentration (MIC) was determined as the lowest concentration of extract that prevented bacterial growth.

## **Thin Layer Chromatography (TLC)**

Chemical compounds in the extracts were separated and detected using TLC method. For separating natural compounds in leaves extracts, cellulose and silica gel GF<sub>254</sub> were used as stationary phase and butanol:acetic acid:water = 4:1:5 (upper phase) was used as mobile phase. Stationary and mobile phase that were used to separate chemical compounds in stems extracts were silica gel GF<sub>254</sub> and chloroform: methanol = 9 : 1, respectively. Compounds in root extracts were separated using silica gel GF<sub>254</sub> as stationary phase and butanol:acetic acid:water = 4:1:5 (upper phase) as mobile phase. In order to identify the chemical compounds, spots on the TLC plates were detected by observing them under UV 254 and 366 nm and sprayed with FeCl<sub>3</sub>, citroborate, methanolic KOH, Dragendorff, and Lieberman-Burchard and exposing to ammonia vapour.

## **Bioautography**

Developed plates were dried to remove the mobile phase completely and placed on inoculated Mueller Hinton agar for 20 minutes. Plates were removed from the culture followed by culture incubation at 37°C overnight. Any clear zone formed was confirmed with TLC result to determine the chemical compounds that has antibacterial activity.

## **RESULTS AND DISCUSSION**

### **Bacterial sensitivity**

As can be seen in Table 1, the results of antibiotic sensitivity test show that *Staphylococcus aureus* resistant to ampicillin, erythromycin, and tetracycline whereas *Escherichia coli* resistant to all antibiotics tested. The bacteria are resistant to more than 2 antibiotics. This results indicated that bacteria, which will be used in antibacterial activity test were multidrug-resistant.

**Table 1. Bacterial sensitivity to several antibiotics**

Antibiotics	Zone inhibition of resistant bacteria (mm)	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
		Zone of inhibition (mm)	Resistant	Zone of inhibition (mm)	Resistant
Ampicillin (10 µg)	≤ 20	12	Resistant	10	Resistant
Erythromycin (15 µg)	≤ 13	0	Resistant	10	Resistant
Chloramphenicol (30 µg)	≤ 11	36	Sensitive	10	Resistant
Tetracyclin (30 µg)	≤ 14	12	Resistant	8	Resistant

**Antibacterial activity**

Results of antibacterial activity of leaves, stems, and roots extracts of pawpaw are shown in Table 2. The results show that all extract has antibacterial activity against multidrug-resistant *Staphylococcus aureus* and multidrug-resistant *Escherichia coli*. Stem extracts have the highest antibacterial activity against the test bacteria compared to roots and leaves extracts. The MIC value of stems extracts against multidrug-resistant *Saphylococcus aureus* is 30 mg/mL followed by roots and leaves extract with MIC values of 50 and 80 mg/mL, respectively. Similar results were observed in antibacterial test against multidrug-resistant *Escherichia coli*. Stems extract has the lowest MIC value that is 30 mg/mL, followed by roots extract with MIC value of 70 mg/mL and leaves extract with MIC value of 80 mg/mL. Stems and leaves extract have the same MIC values against gram positive bacteria (*Saphylococcus aureus*) and gram negative bacteria (*Escherichia coli*). This indicated that there is no difference antibacterial potency toward different gram stain bacteria. The same potency of leaves extracts against *Staphylococcus aureus* and *Escherichia coli* was also reported by Srivastava et al. (2010). The MIC value of roots extracts against gram positive bacteria is higher than that of gram negative bacteria. It means that gram positive bacteria are more susceptible to root extracts.

**Table 2. Antibacterial activity of ethanol extract of pawpaw against multidrug-resistant *Staphylococcus aureus* and multidrug-resistant *Escherichia coli***

Test bacteria	MIC (mg/mL)		
	Leaves extracts	Stems extracts	Roots extracts
Multidrug-resistant <i>Staphylococcus aureus</i>	80	30	50
Multidrug-resistant <i>Escherichia coli</i>	80	30	70

Studies on antibacterial activity of pawpaw have been carried out by several researchers. Rahman et al. (2011) found that ethanol extract of leaves and stems have antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. However, the MIC value of leaves and stems extracts in Rahman et al. (2011) study are lower than MIC value of leaves and stems extracts in this study. The differences in the MIC value may due to differences in bacterial sensitivity to antibiotics. Multidrug-resistant bacteria that were used in this study are resistant to several antibiotics. Hence, it may need higher concentration of extracts to inhibit the bacterial growth. Another research reported that *Staphylococcus aureus* are more susceptible to 70% ethanol and 80% methanol extracts of pawpaw stems than *Escherichia coli* (Khan et al. 2012). Doughari et al. (2007) reported that *Staphylococcus aureus* and *Escherichia coli* have the same susceptibility to methanol extracts of pawpaw roots.

### Thin Layer Chromatography

Thin layer chromatography was done to identify chemical compounds in the extracts. Spots on developed plate were observed under visible light, UV light, and sprayed with several spray reagents to identify the type of chemical compounds. The results of chemical compounds identification by TLC are shown in Table 3. Leaves extracts contain flavonoids and alkaloids meanwhile stems extracts contain antraquinones and saponins. Alkaloids, polyphenols, and flavonoids were detected in roots extracts. Yusha'u et al (2009) demonstrated that ethanol extract of pawpaw leaves contain alkaloids, flavonoids, steroids, and tannins. Whereas Ayoola et al (2008) revealed that ethanol extract of pawpaw leaves possessed reducing sugar, antraquinones, terpenoids, flavonoids, saponins, tannins, alkaloids, and cardiac glycosides. In another study, leaves extracts contain tannins, saponins, phlobatannins, flavonoids, terpenoids, steroids, alkaloids, and carbohydrates (Njoku and Obi, 2009). There are discrepancies in phytochemicals in leaves extracts among these studies. Saponin, alkaloid, tannin, and phenol were identified in methanol extract of pawpaw root (Doughari et al, 2007).

**Table 3. Chemical compound in pawpaw extracts**

Extracts	Chemical compounds
Leaf	Flavonoid and alkaloid
Stem	Antraquinone and saponin
Root	Alkaloid, polyphenol, and flavonoid

### Bioautography

Bioautography was carried out to determine chemical compounds that responsible for antibacterial activity. Flavonoids in leaves extracts are responsible for antibacterial activity against both multidrug-resistant *Staphylococcus aureus* and multidrug-resistant *Escherichia coli*. Saponins and anthraquinones in stems extracts inhibit the growth of multidrug-resistant *Staphylococcus aureus* and multidrug-resistant *Escherichia coli*, respectively. Meanwhile, flavonoids and alkaloids in roots extracts have antibacterial properties towards multidrug-resistant *Staphylococcus aureus* and multidrug-resistant *Escherichia coli*, respectively.

**Table 3. The results of bioautography**

Test bacteria	Compound that has antibacterial activity		
	Leaf extracts	Stem extracts	Root extracts
Multidrug-resistant <i>Staphylococcus aureus</i>	Flavonoids	Saponins	Flavonoids
Multidrug-resistant <i>Escherichia coli</i>	Flavonoids	Anthraquinones	Alkaloids

Natural products may have different antibacterial mechanism of action. Flavonoids are phenolic substances that have hydroxyl groups and present as a C<sub>6</sub>-C<sub>3</sub> unit linked to an aromatic ring. Plants synthesize flavonoids as response to bacterial infection. Therefore it is not surprise that the compounds has antibacterial activity in vitro against a wide range of microbes. Their ability to complex with extracellular and soluble protein and to complex with bacterial cell walls may support their antimicrobial activity. Microbial membrane may disrupted by lipophilic flavonoids (Cowan, 1999). Saponins have different mode of action. Rather than simply altering the surface tension of the extracellular medium, membranolytic properties of saponins contribute to antibacterial effects of saponins (Cheeke, 1999). Alkaloids must be able to interfere with important cellular and molecular target in bacteria in order to inhibit bacterial growth. Biomembrane and DNA in bacteria are target of alkaloid action (Fattorusso and Tagliatela-Scafati, 2008).

## CONCLUSION

Ethanol extract of leaves, stems, and roots of pawpaw inhibit the growth of multidrug-resistant gram positive and gram negative bacteria. Several chemical compounds in the extracts are responsible for the antibacterial activity. These compounds can be used as an alternative to overcome multidrug-resistant bacteria.

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