

**THE ANTIMICROBIAL ACTIVITIES OF FIG (*FICUS CARICA*  
L.) LEAVES EXTRACT AGAINST *STAPHYLOCOCCUS*  
*AUREUS* AND *ESCHERECHIA COLI***

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**ABSTRACT**

Increasing risk of antibiotic resistance of pathogenic bacteria has led to the exploration of alternative antibiotics derived from leaves of medicinal plants such as the fig (*Ficus carica* L.). The aim of this study was to determine the antimicrobial activity of the methanolic extract of fig leaves grown under Malaysian tropical environment against pathogenic bacteria linked to antibiotic resistance namely the *Staphylococcus aureus* and *Escherechia coli*. The methanolic extraction was performed by using soxhlet apparatus. The disc diffusion method was used to measure inhibition zone diameter on the Mueller-Hinton agar plate. *Staphylococcus aureus* displayed the highest diameter of inhibition zone against the extract at concentration of 900 mg/ml whilst *Escherechia coli* displayed the highest diameter of inhibition zone against both the 100% crude extract and 700 mg/ml, respectively. This study therefore highlighted the potential of developing alternative antibiotics derived from the methalonic extract of locally grown fig plant.

**KEYWORDS:** *Ficus carica*, antimicrobial agent, *Staphylococcus aureus*, *Escherechia coli*

## INTRODUCTION

*Staphylococcus aureus* is a gram positive bacterium which can cause a wide range of diseases due to infection towards numerous parts of the body. It could cause from minor skin infections such as pimples and scalded skin syndrome, to brain infections or meningitis and may even lead to lung infection or pneumonia. *Escherichia coli* is one of the normal floras in our intestine. Overgrow of this species can cause *E. coli* infection resulting in bloody diarrhea, urinary tract infection and up to kidney failure or severe anemia. Among the practiced treatment for these infections is by providing antibiotics such as ciprofloxacin, gentamicin and penicillin. Antibiotics are basic forms of modern medicine which have a lot of benefit to healthcare especially in increasing life expectancy and reducing childhood mortality. They are also vital in invasive surgery such as chemotherapy. Unfortunately, there are evidences that these bacteria are resistant to antibiotics including penicillin and this condition raises a major problem in clinical practice. Several infections caused by multi-drug resistant bacteria are increasing worldwide (Blair *et al.*, 2015) and those infections have become untreatable. According to Wellington *et al.* (2013), for the past ten years, Gram-negative bacteria that are resistant to multidrug have become a big obstacle for infection control. Therefore, *S. aureus* and *E. coli* had been used in this antimicrobial study because they are among the major pathogens concerned by clinical practitioners due to possessing antibiotic resistant criteria.

In Malaysia, the case of antibiotic resistant pattern among children in Malaysian tertiary hospitals was studied. It was proven that there is a high antibiotic resistance of *E. coli* to several antibiotics especially ampicillin (Mohd Nor *et al.*, 2015). Thus, to overcome this problem, several studies had been conducted to find ways to inhibit the growth of *S. aureus* and *E. coli* by utilizing alternatives for antibiotics. Thus, this leads to investigation on the potential use of medicinal plant including the leaves of the fig. Unlike synthetic drugs, antimicrobial property which is originated from traditional plants has no side effects and it has high potential in curing infectious disease (Blesson *et al.*, 2015).

Medicinal plant has its own benefit to human health that needs to be further studied and explored. The fig is one of the medicinal plants that have its own contributions and potentials. According to Ahmad *et al.*, (2013), the fig is one of the oldest cultivated fruits in the world originated from the Mediterranean region. Adeshina *et al.*, (2010) stated that high antimicrobial activity of *Ficus spp* is based on the abundance of flavonoid compound in the leaves. Flavonoid and phenol contents in the fig leaves portray antibacterial activity against *Methicilin-Resistant Staphylococcus Aureus* (MRSA) (Lee and Jeong, 2010). Hence, several studies have proven that the leaves of this medicinal plant which consist of flavonoid and phenols could contribute to antimicrobial activity against *Staphylococcus aureus* and *Escherechia coli*. In addition, this medicinal plant has also lot of bioactive compounds such as vitamins and flavonoids (Joseph and Raj, 2011).

In the case of fig cultivation in Malaysia, this plant was only been introduced to our country about ten years ago. Although this plant is relatively new, there are efforts made by

several growers, entrepreneurs and research entities on establishing the fig plantations in Malaysia (pers. comm.). In fact, there are several commercial products derived from locally grown fig trees such as herbal tea and soap in the market. However, limited scientific study was reported in relation with our locally grown fig trees. Therefore, this study was conducted to explore the antimicrobial activities of the fig leaves grown under Malaysian tropical environment against pathogenic bacteria linked to antibiotic resistance namely the *S. aureus* and *E. coli*.

## MATERIALS AND METHODS

### Materials

The fig leaves were collected from fig garden in Janda Baik, Pahang. *S. aureus* and *E. coli* bacteria were used and they were obtained from Microbiology Laboratory of Kuliyyah of Allied Health Sciences (KAHS) International Islamic University Malaysia (IIUM) Kuantan campus, Pahang. Mueller-Hinton agar was purchased from Merck, Germany. Dimethyl Sulphoxide (DMSO) was from Gibco®, USA. Methanol and absolute ethanol were purchased from Fisher chemical, UK and Honeywell, USA respectively. Penicillin sensitivity disc was obtained from Oxoid Ltd, Basingstoke, England. Cellulose extraction thimbles was from FAVORIT, and normal saline was from HmbG, Saksen- Anhalt, Germany.

### Extraction of *Ficus carica* leaves

The leaves were washed with distilled water (Blesson, 2015) and they were shade dried in a dark room at the Research Laboratory of KAHS, IIUM Kuantan for approximately fifteen days based on previous study by Al Askari et al. (2012). After two weeks, the dried *Ficus carica* leaves were grinded into powder by using industrial grade blender. The powdered leaves were then put into a thimble and the powdered leaves were extracted for 8 hours with 400 ml of methanol solvent by using soxhlet apparatus (Sharma and Sharma, 2010). Further drying process to remove methanol was performed using a rotary evaporator.

### Bacterial characterization

*S. aureus* and *E. coli* were obtained from the Microbiology Laboratory of Biomedical Science Department, KAHS. Prior to culturing these strains for further experiments, bacterial characterization using gram staining was performed. There were three different stains that had been used in the Gram stain which are crystal violet, Gram's iodine, and safranin.

### Culturing *S. aureus* and *E. coli*

The bacteria were sub-cultured before proceeding to disc diffusion method. A single colony of *S. aureus* was cultured on the Mueller Hinton agar. The agar plate was incubated at 37°C for 24 hours. Then, the same method was applied to culture *E. coli* bacteria. Aseptic

technique was performed throughout the process which was done in laminar flow at Research Laboratory of KAHS.

### **Preparation of 10% Dimethyl Sulphoxide (DMSO)**

A 10% v/v DMSO solution was used in this study as negative control and as diluting solvent to prepare different concentrations of the sample extract. DMSO 10% v/v solution was prepared by diluting 10 ml of undiluted DMSO which was obtained from Microbiology Laboratory of KAHS with 90 ml sterile distilled water. Final concentration of DMSO 10% v/v solution was stored in a dark place at room temperature until further use.

### **Preparation of different concentrations of sample extract**

The stock solution was prepared by diluting 1.0 g of crude extract with 1ml of 10% DMSO and it was stored at 4°C. The methanol extract of the fig leaves were prepared in different concentrations of 500mg/ml, 600mg/ml, 700mg/ml, 800mg/ml, 900mg/ml and undiluted of crude extract itself. In order to prepare 500mg/ml, 150µl of the stock solution had been diluted with 150µl of DMSO 10% v/v to obtain 300µl total volume. This study started the concentration with 500 mg/ml according to a previous study by Sharma and Sharma, (2010).

### **Disc diffusion method (Kirby Bauer Assay)**

Then, each eight agar plates were inoculated with the bacterial strain of *S. aureus* and *E. coli* with side to side motion by using sterile swabs. Before inoculating, the bacterial strains were suspended in normal saline and it was adjusted to 0.5 McFarland standard turbidity (Matuschek *et al.*, 2013). 40 µl of each concentration of methanol extract was dripped on the blank discs. DMSO 10% v/v had been used as diluting solvent (Sharma and Sharma, 2010) and it was set as negative control whereas penicillin was set as positive control. Then, all control discs and blank discs with each concentration of methanol extract were placed on the inoculated agar plate. Each concentration of methanol extract was placed in three quadrants of the agar plate to measure the average of three readings of diameter of inhibition zone (Ahmad *et al.*, 2013).

### **Data Analysis**

For antibacterial study, the diameter of inhibition zone for each bacterium was recorded. Then, the mean and standard deviation were calculated by using Microsoft Excel. Then, data obtained were analyzed with Kruskal Wallis Test by using SPSS 12.0.1 software to compare between treatments of each bacterium with different concentration and also to compare the concentrations of the sample extract with the positive control group.

## RESULTS

### Extraction yield

The yield of leaves extract was calculated with the formula given as (weight of the crude extract (g)/ weight of the powder (g)) × 100% which is (10.859 g/23.094 g) × 100%. The percentage yield for *Ficus carica* leaves was 47%.

### Diameter of inhibition zone

The antibacterial activity of sample extract was determined by measuring the diameter of inhibition zone using the disc diffusion method. Sample extracts of 500 mg/ml, 600 mg/ml, 700 mg/ml, 800 mg/ml, 900 mg/ml and undiluted crude extract were tested against *S. aureus* and *E. coli*. Then, the diameter of zone inhibitions was measured, the mean of triplicate was recorded and the outcomes were tabulated in Table 1. Based on the Table 1, both *S. aureus* and *E. coli* displayed inhibition zone against all concentrations of extract. For *S. aureus* bacteria, it displayed the highest diameter of inhibition zone against 900 mg/ml of extract concentration whereas *E. coli* had the highest diameter of inhibition zone against 700 mg/ml of extract concentration and undiluted crude extract. Figure 1 (A & B) showed the greatest antibacterial activity of sample extract against all tested bacteria and Figure 1 (C & D) indicated the antibacterial activity of positive control (Gentamicin) against *S. aureus* and *E. coli*. No zone of inhibition was observed against negative control, which is DMSO 10% v/v.

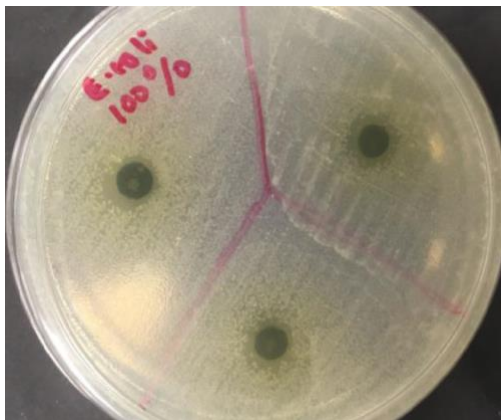
**Table 1** Diameter of *S. aureus* and *E. coli*. inhibition zone using disc diffusion method

	Concentration (mg/ml)	N	Median (iqr*)	p-value	
<i>Staphylococcus aureus</i>	500	3		0.040	
	600	3	0.90		
	700	3	0.80		
	800	3	0.70		
	900	3	0.80		
	100% crude extract	3	1.00		0.80
	Positive control group (Gentamicin)		3		1.80

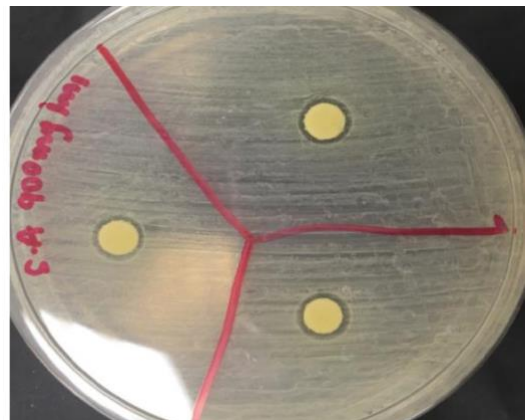
<i>Escherichia coli</i>	500	3	0.80	0.012
	600	3	0.80	
	700	3	1.00	
	800	3	0.80	
	900	3	0.90	
	100% crude extract	3	1.00	
	Positive	3	2.60	
control group (Gentamicin)				

Table 1. Comparing diameter of inhibition zone for *S. aureus* and *E. coli* between different concentrations of methanolic extract of *Ficus carica* leaves and with controls. The result showed that there is significant difference between at least one pair of the different concentration or with controls as the p-value is less than 0.05. From post-hoc test, the significant difference is between all concentrations with positive control.

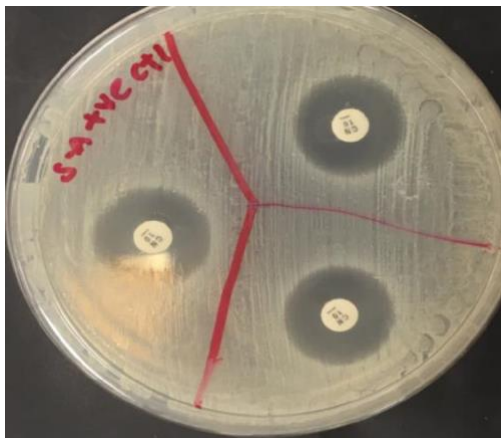
A



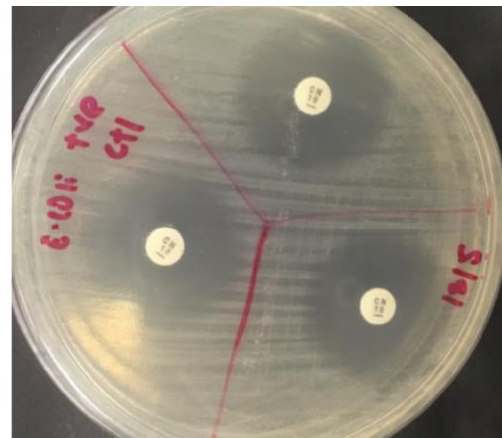
B



C



D



**Figure 1** Antibacterial activity against sample extract

Figure 1. Various concentration of *ficus carica* extracts produced inhibitory zone of bacterial growth. A) *E. coli* displayed highest diameter of inhibition zone against undiluted crude extract itself and B) *S. aureus* growth were inhibited by 900 mg/ml of *ficus carica* methanol extract. C) and D) exhibits zone of inhibition of *S. aureus* and *E. coli* against positive control which is Gentamicin.

**Data analysis**

The results obtained were analyzed with Kruskal Wallis Test by using SPSS 12.0.1 software to compare between the sample extract of each bacterium with different concentrations and to compare different concentrations of the sample extract with the control group.

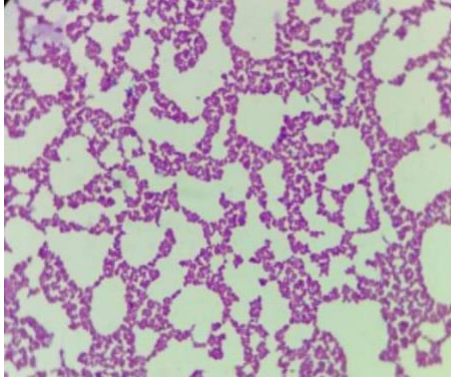
**DISCUSSION**

The extraction yield obtained in this study is high (47.02%) and that is why methanol was used to extract the sample as the resulting yield is depended on the type of solvent used during extraction process. The extraction yield in this study is considered high because previous study done by Setha et al. (2014), the extraction yield obtained was only 3.40%. According to Setha et al. (2014), different solvents like methanol, hexane, chloroform and ethyl acetate were used to extract the *Jatropha curcas* leaves and the findings displayed that the extraction yield of methanol was the highest when compared to others. Among these polar solvent like ethanol, acetone and methanol, extraction using methanol as solvent yield the highest amount of crude extract contain phenolic compound (Syukriah et al., 2014). Methanol is a solvent that dissolves all kinds of compound for instance polar, semi-polar and non-polar. Besides, the use of soxhlet apparatus in the extraction process is one of contributors to the high yield of methanolic extract of the sample. According to Wang and Weller (2006), soxhlet extraction is an efficient method to extract *Quercus infectoria* galls powder because it is able to yield high amount of bioactive compounds including gallic acid and tannic acid. Besides, the method is very simple and soxhlet extraction method can maintain the temperature high enough to obtain the maximum product. In addition, there is no filtration required for crude extracts.

Gram stain was used in the study to identify gram positive bacteria and gram negative bacteria (Peck and Badrick, 2017). It is a process of staining cells whether purple or pink, which relate to the properties of their cell wall. Figure 2 showed the appearance of purple color *S. aureus* that is in cocci shape and arranged in clusters. Usually, the purple color on direct Gram stain displays Gram-positive bacteria whereas pink color shows Gram-negative bacteria. According to Harris et al., (2002) the study has stated that *S. aureus* has diameter of 0.5-1.5µm with cocci shape, which spread into cluster to form grape-like structure. The occurrence of the clusters of Gram-positive cocci on direct Gram stain is defined as *S. species* (Murdoch and Greenlees, 2004). Figure 2 showed the occurrence of pink color *E. coli* which in rod-shaped structure. *E. coli* will stain pink on Gram staining



process (Brown and Hopps, 1973). Gram negative bacteria do not maintain the crystal violet stain and therefore it will stain pink whereas gram positive bacteria retains the crystal violet stain which gives the purple color on direct Gram stain.



**A.** *Staphylococcus aureus*



**B.** *Escherichia coli*

**Figure 2** Bacterial Characterization using Gram Staining

Figure 2. A) The characterization of *S. aureus* after Gram staining and observing under microscopy. The figure shows purple color staining of the bacteria with cocci shape which are spread into clusters to form grape like structure, B) the characterization of *E. coli* after observing under microscope. The figure shows the bacteria with rod-shaped structure and *E. coli* forms pink color staining.

Concentration of extract (mg/ml)	Diameter of inhibition zone, mm (mean $\pm$ SD )	
	<i>Staphylococcus aureus</i> (n=3)	<i>Escherichia coli</i> (n=3)
500	8.67 $\pm$ 0.58	8.33 $\pm$ 0.58
600	7.67 $\pm$ 0.58	9.00 $\pm$ 0.58
700	7.33 $\pm$ 1.00	10.33 $\pm$ 1.52
800	8.33 $\pm$ 0.58	7.67 $\pm$ 0.58 9.00 $\pm$ 1.00
900	9.00 $\pm$ 0.00	
Undiluted crude extract	8.33 $\pm$ 0.58	10.33 $\pm$ 0.58

**Table 2** Diameter of inhibition zone

Gentamicin	17.67 ± 0.58	25.67 ± 0.58
DMSO 10% v/v	0.00 ± 0.00	0.00 ± 0.00

Table 2 displayed the results for disc diffusion method of methanolic extract against *S. aureus* and *E. coli*. From the results, it was shown that, both *S. aureus* and *E. coli* displayed inhibition zone against all concentrations of extract, which indicates that the methanolic extracts of the fig leaves have antimicrobial properties. According to Ahmad et al. (2013), disc diffusion method determined the antibacterial activity against gram positive and Gram negative bacteria and the result showed that methanolic extracts of *Ficus carica* leaves showed antimicrobial effect against those bacteria. To some extent, this was consistent with another study by Jeong et al. (2009) where methanolic extract of *Ficus carica* leaves exhibited strong antibacterial activity against oral bacteria. It is clearly seen that *Ficus carica* leaves has antimicrobial properties.

There are several factors why the methanolic extract of this sample displays antimicrobial effect against both *S. aureus* and *E. coli*. The phytochemical analysis from previous study has revealed that *Ficus carica* has numerous bioactive compounds such as organic acids, coumarin, phenolic compounds and volatile compounds (Mawa et al., 2013). These bioactive compound present in the leaves of *Ficus carica* are likely to contribute to this antimicrobial effect. According to Lee and Jeong (2010), flavonoid and phenolic compounds are the most significant phytochemicals, which possess antimicrobial properties. This is further supported by Salem et al. (2013) which claimed that the leaves of *Ficus carica* have higher flavanoid content hence contribute to the antibacterial effects rather than other parts of the plant.

Among the mechanisms pertaining to the antimicrobial property is that these compounds interfere with the bacterial cell components and cytoplasmic membrane (Setha et al., 2014). They are also able to inhibit protein synthesis and interfere with the bacterial function of genetic materials. As in Table 2, the inhibition zone of *E. coli* increased steadily after the increase in concentration of the leaves extract. At 700 mg/ml concentration, maximal inhibition zone was observed and this was similarly the same inhibitory zone produced by the use of a 100% crude extract. On the other hand, the inhibitory zone of *S. aureus* showed a moderate increase with the use of 600 mg/ml to 900 mg/ml of extract concentration. Interestingly the undiluted crude extract did not show the highest concentration of the extract that gave rise to the highest diameter of inhibition zone.

A study by Setha et al. (2014) had similarly observed this condition where they reported that when the extract concentration is too high, the penetration of secondary metabolites into the bacterial cell wall could be interfered hence reducing the effectiveness of the extract to produce its antimicrobial effects. There are a lot of other circumstances that may affect the antibacterial activity besides the concentration of the sample extract for instance the species of bacteria, the presence of organic compound and the pH and

temperature of the environment. Therefore, this study proves that *Ficus carica* leaves which was grown in Malaysia shows antimicrobial proper.

## CONCLUSION

From the results, disc diffusion method of sample extract against *S. aureus* and *E. coli* displayed zone of inhibition against all concentrations of methanolic of *Ficus carica* leaves. *S. aureus* showed the highest diameter of inhibition zone which is 0.9 cm for the sample extract at the concentration of 900 mg/ml and *E. coli* has the highest diameter of zone of inhibition which is 1.033 cm for the sample extract at the concentration of 700 mg/ml and undiluted crude extract itself. As a conclusion, this study highlighted the potential of developing alternative antibiotics derived from the methalonic extract of locally grown fig trees against pathogenic bacteria such as *Staphylococcus aureus* and *Escherechia coli*.

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