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Activity of *Cassia fistula* L. Barks fractions as antibacterial agent

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Abstract

Cassia fistula L. has been used as alternative medicine indicated to have various efficacies. Some studies proved that some parts of this plant have antibacterial and antifungal activity, but activity from the barks of this plant has not been styudied yet. In this study, the sample were extracted by Ethanol, continued by fractination by n-hexane, ethyl acetate and water. Phytochemical screening were performed by methods explained by Fansworth. Antibacterial study of the extract were conducted by diffusion agar method against Escherichia coli and Staphylococcus aureus. The result showed that Minimum Inhibitory Concentration of ethyl acetate fraction was at 0,625 % agaianst S. aureus while water fraction was more than 10%. MIC against E. coli of water fraction was more than 10% and ethyl acetate fraction was at 1,25%. Antibacterial activity study was perfomed by difusion method and was compared to that of amoxicillin as marketed oral antibiotic. The results showed that ethyl acetate fraction showed strongest activity ainst both S. aureus and E. Coli. The study concluded that pontential antimicrobial properties of ethyl acetate fraction of Cassia fistula ethyl illustrates the promising activity in exploring new antibacterial agent.

Keywords: Cassia fistula, antimicrobe, fractions

INTRODUCTION

Certain plant species belonging to the genus Cassia (Leguminosae) have been used for medicinal purposes (Perry, 1980; Veerachari, and Bopaiah, 2011). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Cassia is a native plant in southeast Asia, Africa, Northern Australia and Latin America (Parsons & Cuthbertson, 1992). It was found that this plant contains flavonoids, alkaloids, cardiac glycosides, tannins (Mossa, et al., 1991).This plant has been described to have activity against skin diseases, liver troubles, tuberculoses glands and its use into the treatment of hematemesis, pruritus, leucoderma, and diabetes has been suggested. *Cassia fistula* is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections. The leaves are laxative, antiperiodic, depurative, anti-inflammatory, and are useful in skin diseases, boils, carbuncles, ulcers, intermittent fever, gouty arthritis, and rheumatalgia. *Cassia fistula* are known to have important source of secondary metabolites, notably phenolic compounds. Indian people are using the leaves to treat inflammation; *Cassia fistula* plant organs are known to be an important source of secondary metabolites. It exhibited significant antimicrobial activity and showed properties in the treatment of some diseases as broad-spectrum antimicrobial agents. The root is prescribed as a tonic, astringent, febrifuge and strong purgative (Gupta et al., 2010: Gupta et al, 2008: Kirtikar, 2006: Nadkarni, 2009; Chopra et al., 2006; The Wealth of India, First Supplement Series, 2007; Agarwal et al., 2005). The leaves extract reduced mutagenecity in E. coli. Extract of the root bark with alcohol can be used for backwart fever. The leaves are laxative and used externally as emollient, a poultice is used for chilblains, in insect bites, swelling, rheumatism and facial paralysis (Gupta et al., 2010: Ayurvedic Pharmacopoeia of India, 2001; Nadkarni, 2009).

Many reports have shown that some of the Cassia species have acquired antimicrobial substances and antioxidant activity (Zhenbae et al., 2007). *Cassia alata, C. fistula* and *C. tora* are recommended for primary healthcare in Thailand to treat ringworm and skin diseases (Farnsworth & Bunyaprapatsara, 1992). There are reports showed that seeds possess antiinflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal activity (Mascolo et al, 1998; Markouk et al, 2000). The flower of the plant was reported to possess wound healing activity (Dewan, et al, 2000; Rasik, et al, 1999).

In the current investigation study on antimicrobial activity of *Cassia fistula* barks fractions against pathogenic bacteria was carried out in order to explore new sources of antimicrobial agents. Hence, the aim of study was to investigate antibacterial *Cassia fistula* L. barks fraction agains *E. Coli* and *S. aureus* as candidate of oral antimicrobial agent

MATERIAL AND METHODS

1. Plant materials

The barks were collected from the Manoko herbal plantation, Lembang, Bandung Indonesia

2. Preparation of extract and fractions

The dried powder of sample was extracted using ethanol as extraction solvents, at ambient temperature. The extracts were evaporated under vacuum using rotary evaporator at 60°C. For antibacterial assays, extracts were dissolved in DMSO and diluted with water, in order to obtain a final concentration of 100 mg/mL. The method of fractionation can be summarized as follows; 20 g of *C. fistula* extract was taken in a separating funnel and dissolved in 50 ml of distilled water. Hexane was added and then shaken vigorously. The hexan layer was then collected by filtration and dried by using the rotary evaporator. To the left over layer was added by Ethyl acetate and shaked. Ethyl acetate layer was separated and dried to get Ethyl acetate fraction. The left over fraction 50 ml of ethanol was added and shaken to get the methanol soluble substance and Methanol fraction is prepared by drying the filtered solution. The remaining layer or filtrate was collected and evaporated to get the residual fraction or the aqueous fraction (Rout, et al, 2015).

3. Phytochemical screening:

The screening were carried out on the extract using standard procedures to identify the constituents as described by Harborne [1998] and Edeoga [2005].

4. Determination of Minimum Inhibitory Concentration

Determination of MIC were performed by using scratch method. The samples were mixed with liquid nutrient agar in a sterile petri dish using a certain ratio. Petri dish were shaken until the mixture becomes homogeneous, allowed to solidify at room temperature, then streaking the bacterial suspension test using a wire loop. All petri dishes that were scratched with test bacteria were incubated at 37 ° C for 18-24 hours.

5. Antibacterial activity test of fractions

The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibiotic activity. Bacterial suspensions were put into

20 µL petri dish, then 20 ml of agar medium was added and shacked gently in order to make bacterial suspension and the media homogeneously solidified. Then the media were perforated and the extract were injected into the hole at various concentrations. After it was incubated for 18-24 hours at 37 °C, diameter of inhibition formed were observed.

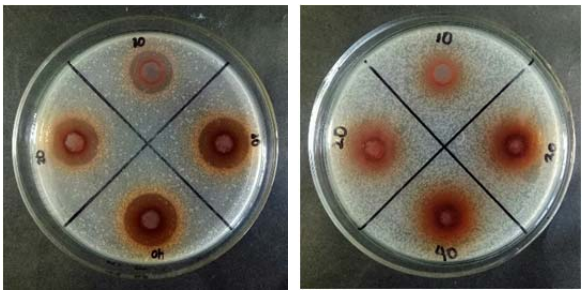
RESULTS AND DISCUSSION

Phytochemical screening of fractions

It was found that ethanolic extracts of *Cassia fistula* L. barks contained tannins, flavonoids, polyphenols, saponins, triterpenoids, and anthraquinones.

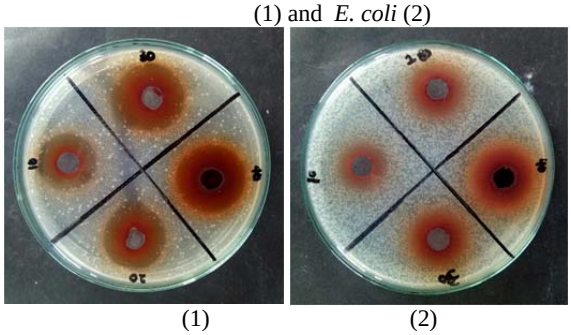
Antibacterial activity

The antimicrobial activity of *Cassia fistula* barks fractions were studied against Gram-negative *Escherichia coli* and *Staphylococcus aureus* as gram positive in different concentrations (10, 20, 30 and 40% b/v). Antibacterial activity of fractions were evaluated as Minimum Inhibition Concentration (MIC) value and zone of inhibition of bacterial growth. The results of the (MIC) determination against *Escherichia coli* and *Staphylococcus aureus* showed in Table 1 - 2 and Figure 1 - 2. The results showed that the fraction with strong activity were ethyl acetate followed by water fractions. Hexane fraction showed no activity (Figure 3). With regard to the results of phytochemical screening, the antibacterial activity could be due to the presence of flavonoids, polyphenols or anthraquinones.



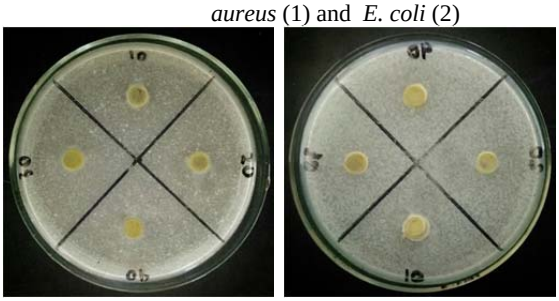
(1) (2)

Figure 1. Microbial activity of water fraction againts *S. aureus* (1) and *E. coli* (2)



(1) (2)

Figure 2. Microbial activity of ethyl acetate fraction againts *S. aureus* (1) and *E. coli* (2)



(1) (2)

Figure 3. Microbial activity of n-hexane fraction againts *S. aureus* (1) and *E. coli* (2)

Table 1. Microbial activity of water fraction

Microbes	Concentration (%)	Diameter (mm)			Means (mm)
		I	II	III	
E. coli	10	13,17	12,17	13,67	13,00
	20	16,67	15,00	14,33	15,33
	30	17,33	16,83	16,67	16,94
	40	18,17	17,83	17,83	17,94
S. aureus	10	15,17	16,50	17,33	16,33
	20	17,83	19,33	19,17	18,78
	30	19,33	19,50	18,83	19,22
	40	19,67	21,67	21,67	21,00

Tabel 2. Microbial activity of ethyl acetate fraction

Microbes	Concentration (%)	Diameter (mm)			Mean (mm)
		I	II	III	
E. coli	10	16,50	14,00	15,17	15,22
	20	18,00	18,07	18,00	18,02
	30	19,17	19,67	19,17	19,33
	40	22,83	21,67	23,00	22,50
S. aureus	10	21,00	20,33	22,67	21,33
	20	24,67	24,67	24,50	24,61
	30	26,00	24,67	25,33	25,33
	40	27,67	26,00	28,17	27,62

Tabel 3. MIC of fractions against *S. aureus*

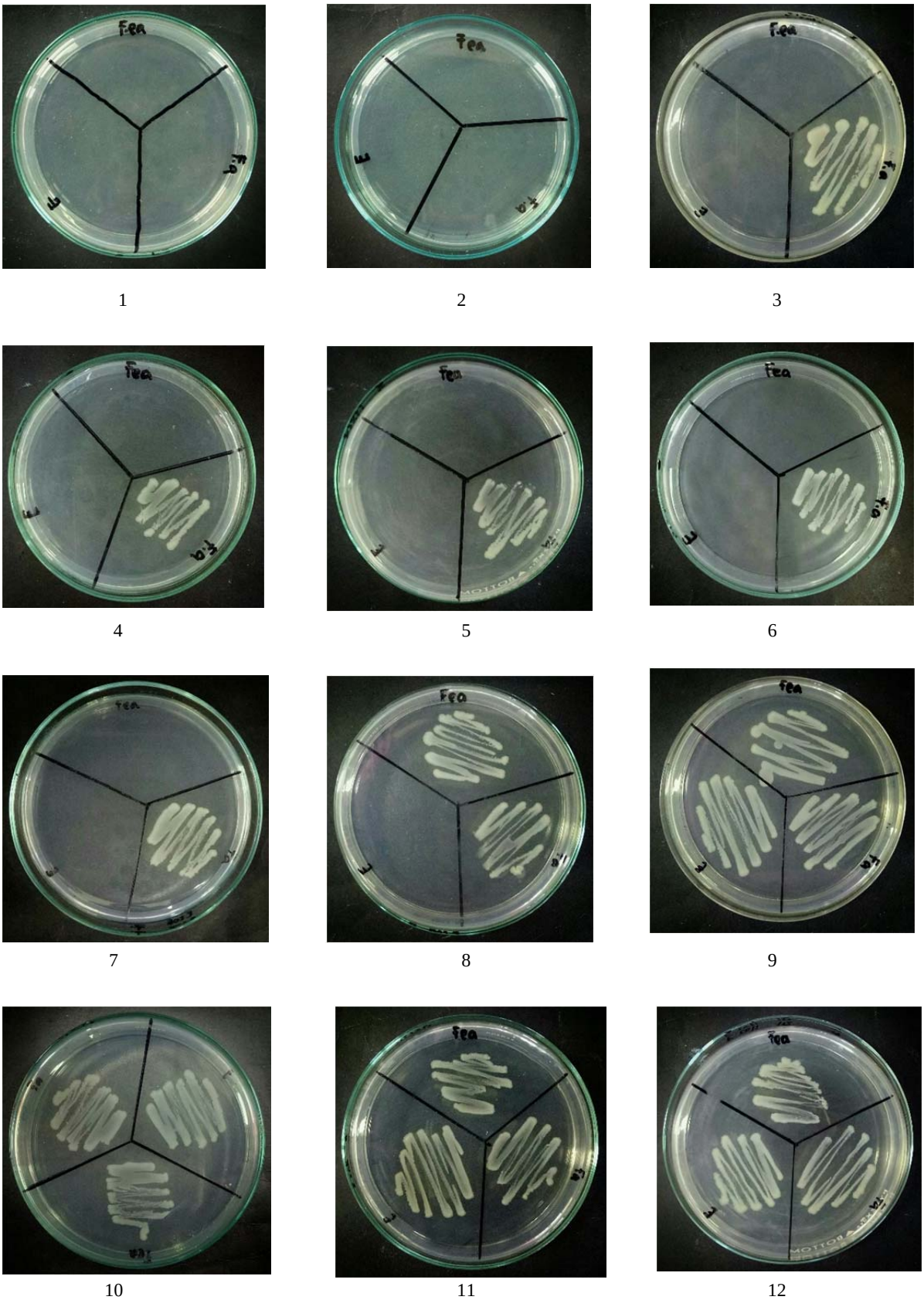
Well	Concetration (% b/v)	Extract	Water fraction	Ethyl acetate fraction
1	Media	-	-	-
2	Media + sample	-	-	-
3	10	-	V	-
4	5	-	V	-
5	2.5	-	V	-
6	1.25	-	V	-
7	0.625	-	V	-
8	0.3125	-	V	V
9	0.15625	V	V	V
10	0.078125	V	V	V
11	DMSO + *S. aureus*	V	V	V
12	*S. aureus*	V	V	V

Tabel 4. MIC of fractions against *E. coli*

Well	Concentration (% b/v)	Extract	Water fraction	Ethyl acetate fraction
1	Media	-	-	-
2	Media + sample	-	-	-
3	10	-	V	-
4	5	-	V	-
5	2.5	-	V	-
6	1.25	-	V	-
7	0.625	-	V	V
8	0.3125	V	V	V
9	0.15625	V	V	V
10	0.078125	V	V	V
11	DMSO + microbes	V	V	V
12	microbes	V	V	V

Minimum Inhibition Concentration (MIC) value of fractions of *Cassia fistula* barks were also studied against *Escherichia coli* and *Staphylococcus aureus* (Figure 4 -5, Table 3 - 4). The resuts showed that MIC against S. aureus of extract was 0,3125 %, while water fraction was more than 10% and ethyl acetate fraction was at 0,625 %. The result were also shown in Figure 4.

Minimum Inhibition Concentration (MIC) value of ethanolic fractions of Cassia fistula barks were also studied against



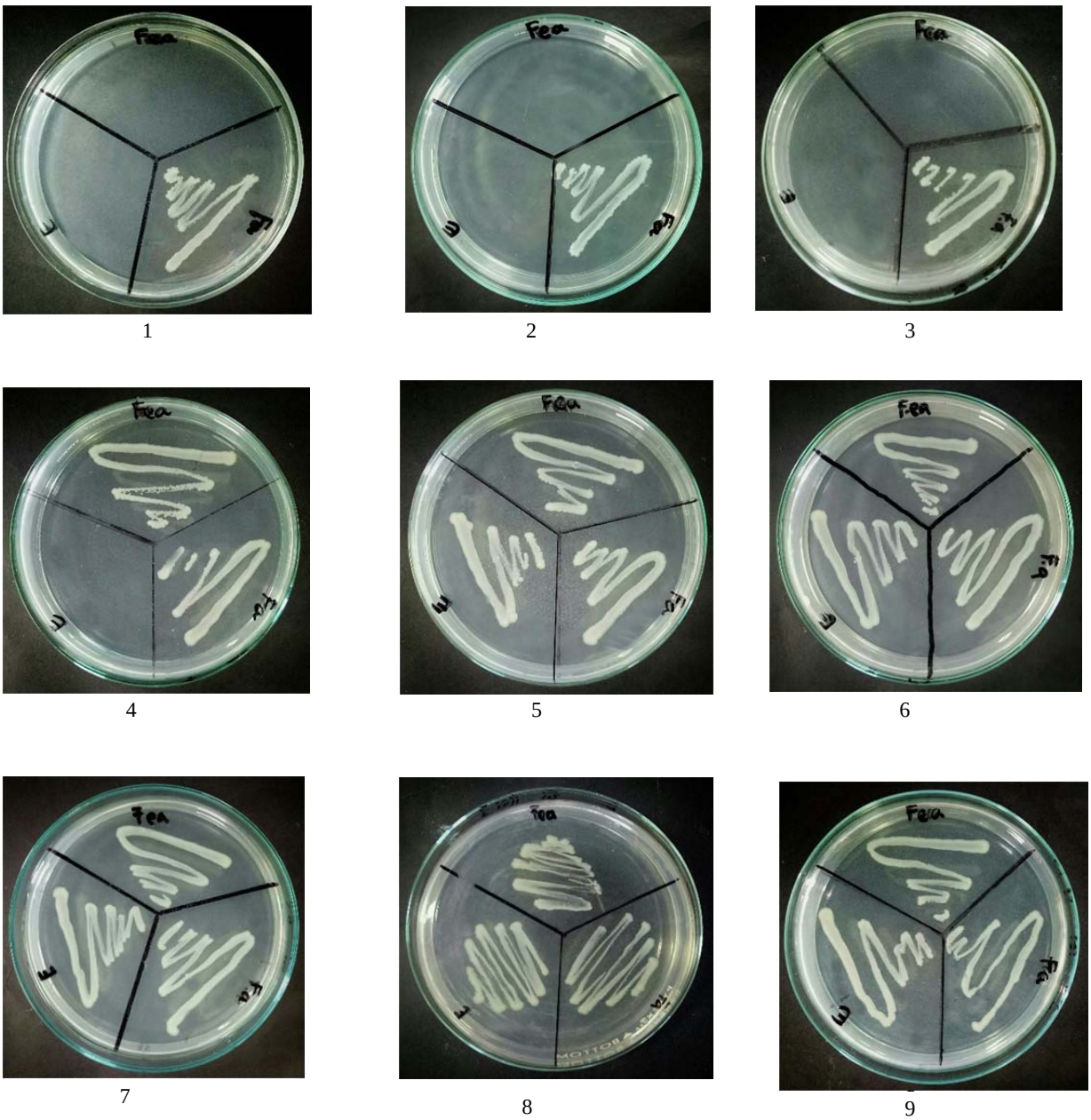
1 2 3

4 5 6

7 8 9

10 11 12

Figure 4. MIC of fractions against *S. aureus* as subculture in MHA media (E = extract, Fea = ethyl acetate fraction, Fa = water fraction)

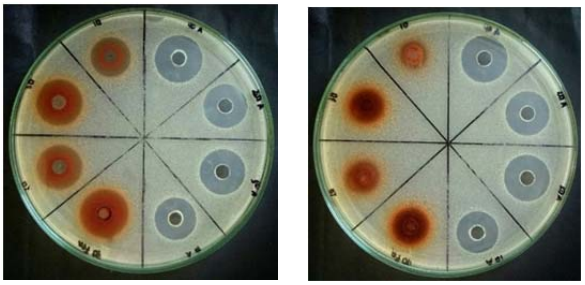


1 2 3

4 5 6

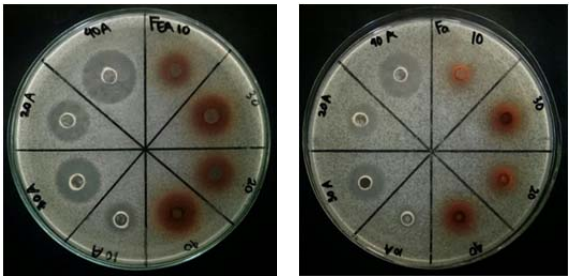
7 8 9

Figure 5. MIC of fractions against *S. aureus* as subculture in MHA media (E = extract, Fea = ethyl acetate fraction, Fa = water fraction)



(a) (b)

Figure 6. Comparison study of (a) ethyl acetate fraction and and (b) water fraction with amoxicilline against *S. aureus* at 10%, 20%, 30%, 40% concentration



(a) (b)

Figure 7. Comparison study of (a) ethyl acetate fraction and and (b) water fraction with amoxicilline against *E. coli* at 10%, 20%, 30%, 40% concentration

Table 5. Comparison study of ethyl acetate fraction with amoxicilline against *E. coli*

Concentration (%)	Diameter (mm)		mean (mm)	
	Fraction	Amoxicilin	Fraction	Amoxicilin
10	16,50	17,00	16,17	17,17
	16,00	17,00		
	16,00	17,50		
20	18,00	23,00	18,17	22,83
	18,50	23,00		
	18,00	22,50		
30	21,00	24,00	20,33	24,33
	20,00	24,50		
	20,00	24,50		
40	23,00	28,50	22,83	28,33
	23,00	28,00		
	22,50	28,50		

Table 6. Comparison study of water fraction with amoxicilline against *E. Coli*

Concentration (%)	Diameter (mm)		mean (mm)	
	Fraction	Amoxicilin	Fraction	Amoxicilin
10	12,00	12,00	11,83	12,17
	12,00	12,50		
	11,50	12,00		
20	13,00	19,00	12,83	19,33
	13,00	19,50		
	12,50	19,50		
30	15,00	23,00	15,17	23,17
	15,50	23,50		
	15,00	23,00		
40	18,00	26,00	17,83	26,17
	17,50	26,50		
	18,00	26,00		

Table 7. Comparison study of ethyl acetate fraction with amoxicilline against *S. aureus*

Concentration (%)	Diameter (mm)		mean (mm)	
	Fraction	Amoxicilin	Fraction	Amoxicilin
10	21,00	23,00	20,67	22,67
	21,00	22,00		
	20,00	23,00		
20	23,50	24,00	23,33	24
	23,00	24,00		
	23,50	24,00		
30	24,00	24,50	24,17	24,67
	24,50	25,00		
	24,00	24,50		
40	26,50	26,50	26,17	26,33
	26,00	26,50		
	26,00	26,00		

Escherichia coli were shown in Figure 5 and Table 4. The resuts showed that MIC against E. coli of extract was 0,625%, while water fraction was more than 10% and ethyl acetate fraction was at 1,25%. The result were also shown in Figure 5.

As comparison with Amoxicilline as standard drugs, the results revealed that antibacterial activity of the extracts against S. aureus were more sensitive compared to that against E. Coli. The antibacterial activities of the extracts increased with increase in concentration of sample either water fraction or ethyl acetate fraction. The result shown in Figu Conclusion

The study concluded that the most active fraction from Cassia fistula L. barks was ethyl acetate, followed by water fraction while n hexane fraction has no activity against S. aureus and E. Coli and illustrates the promising activity in exploring new antibacterial agent fom ethyl acetate fraction.

Table 8. Comparison study of water fraction with amoxicilline against *S. aureus*

Concentration (%)	Diameter (mm)		mean (mm)	
	Fraction	Amoxicilin	Fraction	Amoxicilin
10	14,50	22,00	14,33	22,17
	14,00	22,00		
	14,50	22,50		
20	16,50	24,00	16,67	24,00
	17,00	24,00		
	16,50	24,00		
30	17,50	25,00	17,17	24,67
	17,00	24,50		
	17,00	24,50		
40	19,00	25,00	19,33	25,67
	19,50	25,50		
	19,50	25,50		

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