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Antimicrobial Effect of *Baccaurea angulata* Fruit Extracts against Human Pathogenic Microorganisms

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Abstract

The application and research for drugs and food supplements derived from plants extracts have increased in recent years. Plants extract and their constituents are recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies. The potential of higher plants as a source for new drugs is largely unexplored. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of medicinal plants have not been adequately evaluated. And an increasing number of pathogens are left off treatment, due to emergent resistance strains. Thus, a systematic investigation was undertaken to screen for antibacterial activity from *Baccaurea angulata* (BA). The *Baccaurea angulata* belongs to the family of Euphorbiaceae. Plant that belongs to the family is used as food as well as treatment of infectious diseases such as diarrhea, skin infections and gonorrhoea. The anti-microbial activity of the BA fruit extracts havee revealed different antimicrobial properties, that are various between three parts (whole fruit, fruit skin, and berry), three solvents (methanol, ethanol and aqueous), different methods (agar well diffusion, and microdilution method) and differently listed pathogens (*Streptococcus pneumonia*, Staphylococcus epidermidis, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*). The highest observed antimicrobial activity was in ethanol extract of fruit skin using agar well diffusion against *S. pneumonia*. Among tested Gram negative bacteria *K. pneumoniae* was the most susceptible bacterium which showed the highest bactericidal and bacteriostatic activity using microdilution method.

Keywords: Antimicrobial activity; *Baccaurea angulate*; Fruit extract; MIC; MBC; Bacterial resistance; Phytochemicals

Introduction

Pathogenic bacteria increase the incidence of resistant strains worldwide, and thus become an important cause of mortality and morbidity around the world [1,2].

Nosocomial and community-acquired infections are both caused by emerging resistant strains to antimicrobials [2]. The lowest concentration of antibiotic that inhibits obvious growth of bacteria after overnight incubation is considered as MIC [3]. The lowest inhibitory concentration could be either mainly bacteriostatic or bactericidal. Bacteriostatic effect of antimicrobial needs the help of human defense system. If human defense system is not satisfactory or has already been impaired due to infection such as meningitis and endocarditis, the existing infection will relapse after stopping bacteriostatic dose. This circumstance requires bactericidal dose [4].

In contrast, bacteriostatic dose is not preferable for the treatment of streptococcal infections and *C. gangrene* as the cidal dose causes the release of internal toxin from dying cells which could be dangerous for human [5]. Therefore, treatment of infectious diseases is becoming more difficult due to emerging resistance and low susceptibility of strains to antibiotics [6].

Medicinal plants contain compounds that are capable of providing health benefits by initiating certain physiological actions in the body [2]. According to an estimation made by the World Health Organization (WHO), around 80% of people in developing countries prefer medicinal plants than drugs for their simple health problems [7]. Different parts of medicinal plants are used as raw drugs, and demonstrated numerous medicinal properties [8]. Euphorbiaceae is the fourth major family of the angiosperms, containing more than 300 genera and almost 7,500 species. Plants that belong to Euphorbiaceae family are used as food and in the treatment of infectious diseases such as diarrhoea, dysentery, and skin infections [9]. *B. angulatais* a species that belongs to the family Euphorbiaceae [10], native to Borneo island of Malaysia. The soft whitish part of this fruit (berry) is edible, while the red part (skin) is sour, and usually cooked by the rural communities [11]. The antimicrobial activity of genus of Euphorbia, which belongs to the family Euphorbiaceae, has been carried out against many bacteria. Ethanol, acetone, and aqueous extracts of *Euphorbia fruticosa* and methanol extracts of *Euphorbia macroclada* demonstrated antimicrobial effect against

S. aureus [12]. The antimicrobial activity of *B. angulata* fruit extracts is not previously tested. Therefore, the aims of this study are to: investigate the bacteriostatic and bactericidal effects of *B. angulate* (whole fruit, fruit skin, and berry) fruit extracts on a number of human pathogenic Gram-positive bacteria, and Gram-negative bacteria, evaluate different methods for screening antimicrobial property of *B. angulate* using, agar well diffusion, and micro dilution methods, and compare bacteriostatic and bactericidal effects of different extracts from different parts of the fruit using different solvents.

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Materials and Methods

Plant material

B. angulate fruits were purchased from Bau, Sarawak, Malaysia in 2013. The fruits were wrapped in papers and placed in a box, before being transferred via airmail. The fruit was identified by Mr. Kamaruddin Salleh (Forest Research Institute of Malaysia). The collected fruits were later stored at -30°C at the Department of Nutrition Kulliyyah of Allied Health Science IIUM, Kuantan.

Extraction method

The three parts of the *B. angulate* fruit (whole fruit, fruit skin and berry) were freeze dried, and reduced to a coarse powder. All the three parts of the fruit were then extracted using three solvents (methanol, ethanol and water) in a shaker incubator for 24 h. Next, the extracts were centrifuged for 15 min at 4,000 rpm and filtered (whatman No. 55 filter paper) before the solvents were evaporated using rotary evaporator. The obtained different extracts including methanol extract of whole fruit (MW), methanol extract of fruit skin (MS), methanol extract of fruit berry (MB), ethanol extract of whole fruit (EW), ethanol extract of fruit skin (ES), ethanol extract of fruit berry (EB), aqueous extract of whole fruit (DW), aqueous extract of fruit skin (DS), aqueous extract of fruit berry (DB) were used against listed pathogens.

Tested microorganisms

Microorganisms used in this study were *S. pneumoniae*, *S. epidermidis*, *K. pneumoniae*, and *P. aeruginosa*. All patients' isolates used in this study were obtained from diagnostic microbiology lab USM Kelantan. Then, the collected microorganisms were cultivated in non-selective media (screened human donor blood), then cultured overnight at 37°C.All these microorganisms were identified by standard laboratory methods including Gram staining, catalase test, optochin test and biochemical tests (Indole, TSI, urea and motility tests) for Gram negative isolates.

Antimicrobial assays

The antimicrobial activity of each extract of *B. angulate* fruit with different concentration was tested in vitro against the listed isolates. Inoculum was prepared from overnight cultures of different cultured microorganisms (0.5 McFarland). A broad spectrum antibiotic (Ciproboy-200 infusion 100 µL/mL dH_2O) was used as a positive control and DMSO was used as a negative control throughout the study.

Shortly, after inoculum preparation (15-20 min) 100 µL of the adjusted inoculum (0.5 McFarland) was transferred to the surface of Müller-Hinton agar (MHA) plate and spread uniformly using a sterilized bent glass rod spreader, Nextinoculated medium (MHA) was left for 15 min at room temperature to allow the agar to absorb the inoculum. Then, seven holes with 6 mm diameter were made using sterilized tips. Then, 100 µL of organic extract from each part of the fruit (whole fruit, fruit skin and berry) with different concentrations (1,000, 500, 250, 125, 62.0, 31.0 mg/mL) was introduced into each hole. Same volume of DMSO and diluted ciprobay-200 infusions were also loaded as a negative and positive control. All plates were incubated at 37°C with upside down position [13,14]. The results were obtained by measuring the zone diameter using calipers. MIC was defined as the lowest concentration that shows a zone of inhibition around the well after 24 h or 48 h incubation at 37°C. All experiments were carried out three times.

Microdilution Method

Microdilution method was carried out on low susceptible microorganisms (*S. epidermidis* and *K. pneumoniae*) according to Klančnik [15]. Antimicrobial activity of plant extracts was determined using sterile 96-well plate. In this method, all wells were filled with 120 µL of broth. Subsequently fruit extracts (whole fruit, fruit skin and berry) with the same volume was added to the first wells of first row containing nutrient broth. Then the plant extract was serially diluted to create a concentration of 500, 250, 125, 62.0, 31.0, 15.6, 7.5 and 3.75 mg/mL. Later on, 50 µL of adjusted inoculum to 0.5 McFarland was introduced into each well. Negative control wells were prepared from Mueller-Hinton broth (MHB) and bacterial suspension only, while positive control wells were prepared from MHB and diluted broad spectrum antibiotic (ciproboy-200). The plate was then shaken for 1 min prior to transfer it to incubator for 24 h at 37°C. The final dilution of the plant extracts that maintained its inhibitory effect resulting in 85% growth of isolates was recorded as MIC value of the extract. The MBC of extract was determined by sub culturing each well on MHA and further incubation for 24 h at 37°C. The highest diluted well that yielded no growth or 95% growth on MHA was taken as MBC. Then the respiratory activity of cultured microorganisms into 96 well plate was determined after introducing 10 µL of TTC (2-p-iodophenyl-3-pnitrophenyl-5-phenyl tetrazolium chloride, 20 mg/mL dH_2O) into each well, and growth of bacteria was then observed after 30 min incubation of plate in a dark place.

Statistical analysis

The experiments were carried out in triplicate, and the statistical analysis for comparisons was done by GraphPad® Prism V.6.04. The results were expressed as mean ±SD. A difference was considered statistically significant if $P<0.05$.

Result and Discussion

Different parts of medicinal plants have various medicinal properties. Parts such as roots, stem, leaves, flowers, and fruit are used traditionally as raw drug [8]. The medicinal properties of *B. angulate* are mostly deposited in the skin of the fruit. In this study, the antimicrobial activity of the extract against listed bacteria carried out using agar well diffusion and micro dilution methods. In terms of diameter of the zone of inhibition, all extracts showed active inhibitory effect against the tested bacteria. *S. pneumoniae* was highly susceptible among all the four isolates. Result showed maximum inhibition with the ethanol extract of fruit skin (37 ± 1 mm), followed by methanol extract of fruit skin (33 ± 1 mm) and ethanol extract of fruit berry (30 ± 1 mm) against *S. pneumoniae* at the concentration of 1,000 mg/mL. The potential effect of the skin extract of BA may be due toflavonoid in the fruit skin [11]. Whole extract of the fruit, using methanol, ethanol and aqueous was less effective than observed antimicrobial activity in the fruit skin at the minimum concentration (31 mg/mL), this was found to be highest in DB extract of the fruit (14.3 ± 0.5 mm) at the concentration of 31 mg/mL against *S. pneumoniae* (Table 1). This antimicrobial activity of aqueous

C	DW	DS	DB
1,000	14.0 ± 1.0^{a}	12.0 ± 1.0^{a}	14.0 ± 1.0^{a}
500	21.0 ± 1.0^{ab}	22.3 ± 0.5^{ab}	21.0 ± 1.0^{ab}
250	14.5 ± 0.5^{bfh}	22.0 ± 1.0^{bfh}	19.3 ± 0.5^{bfh}
125	14.0 ± 1.0^{cfhi}	18.0 ± 1.0^{cfhi}	16.3 ± 0.7^{cfhi}
62	13.0 ± 1.0^{dfhij}	15.6 ± 0.5^{dfhij}	16.0 ± 0.0^{dfhij}
31	9.6 ± 0.5^{eghij}	12.3 ± 0.5^{eghij}	14.3 ± 0.5^{eghij}

C: concentration (mg/mL), DW: aqueous extract of whole fruit, DS: aqueous extract of fruit skins, DB: aqueous extract of fruit berries. Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different. ($p < 0.05$).

Table 1: Mean and SD of aqueous extracts of against *S. pneumonae* on agar well diffusion method (mm).

extract of the fruit may be due to dissolving fruit components in aqueous solution [16]. In another study, the antimicrobial effect of apricot juice against different Gram-positive bacteria, as well Gram negative bacteria was stated that the large zone of inhibition by aqueous extract of apricot observed against *P. mirabilis* with a 12 mm inhibition zone [17]. Antimicrobial activity of EW against *S. epidermidis* showed the same inhibition (28 ± 1 mm) zone to MW against *S. pneumonia* at the highest concentration (1,000 mg/mL) on agar well diffusion (Tables 2 and 3). MS was highly active (15 ± 1.0 mm) against *P. aeruginosa* at the highest concentration (1,000 mg/mL) (Tables 4-10), while the minimum zone (6.8 ± 1.0 mm) was shown by aqueous extract of whole fruit on agar well diffusion method (Figure 1). The activities of aqueous extract of fruit skin and fruit berry were not observed at the concentration of less than 500 mg/mL. This low activity of extracts at the lowest concentration may be due to less permeability in the outer membrane of *P. aeroginosa*, which is one of the important causes of resistance of this bacterium to antibiotics. This permeability is 10–100 folds lower in *P. aeruginosa* than some other bacteria such as *E. coli*. Other than that, in this bacterium, antibiotic is transported to the outer membrane of bacterial cell through efflux pump, which is located in membrane of *P. aeruginosa*, thus causing intrinsic resistance [18] (Figure 2). In this study, the minimum concentration in microdilution was absorbed at the concentration of 7.8 mg/mL in MW, MS, ES, and DS on microdilution method against *S. epidermidis* and *K. pneumoniae*. Similarly, a study on ethanol, methanol, and aqueous extracts of *Dasmodium gangeticum*, *Nelumbo nucifera*, Canabis, as well as white sesame, and black sesame was carried out using agar well diffusion method. The study shows maximum inhibitory zone in aqueous extract

C	MW	MS	MB
1,000	28.0 ± 1.0^{a}	33.6 ± 1.0^{a}	28.0 ± 0.5^{a}
500	25.6 ± 0.5^{af}	19.3 ± 0.5^{ab}	11.6 ± 0.5^{ab}
250	21.0 ± 1.0^{bfh}	18.3 ± 0.5^{bfh}	11.3 ± 0.5^{bfh}
125	14.0 ± 1.0^{cfhi}	15 ± 1.0^{cfhi}	11.0 ± 1.0^{cfhi}
62	12.3 ± 0.5^{dfhij}	14.0 ± 1.0^{dfhij}	10.0 ± 1.0^{dfhij}
31	11.0 ± 1.0^{eghij}	13.0 ± 1.0^{eghij}	9.3 ± 0.5^{eghij}

C: concentration (mg/mL), MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries. Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different.(p <0.05).

Table 2: Mean and SD of methanol extracts against *S. pneumonae* on agar well diffusion method (mm).

C	EW	ES	EB
1,000	28.6 ± 0.5^{a}	14 ± 1.0^{a}	7.6 ± 0.5^{a}
500	12.8 ± 0.7^{ac}	11.8 ± 0.5^{ad}	9.3 ± 0.5^{ac}
250	14 ± 1.0^{acd}	11.3 ± 0.5^{adf}	9 ± 0.5^{ace}
125	11 ± 1.0^{bcd}	10 ± 1.0^{bdfh}	7.8 ± 0.2^{bde}
62	NIZ	9 ± 1.0^{cegh}	NIZ
31	NIZ	NIZ	NIZ

C: concentration (mg/mL), EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries, NIZ: no inhibition zone Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different.(p <0.05).

Table 3: Mean and SD of ethanol extracts against *S. epidermidis* on agar well diffusion method (mm).

C	EW	ES	EB
1,000	26.0 ± 1.0^{a}	37.0 ± 1.0^{a}	30.0. ± 1.0^{a}
500	18.5 ± 0.7^{ab}	21.6 ± 1.5^{ab}	12.0 ± 0.2^{ab}
250	18.0 ± 1.0^{bfh}	19.6 ± 0.5^{bfh}	10.3 ± 0.5^{bfh}
125	15.3 ± 0.5^{cfhi}	16.6 ± 0.5^{efhi}	9.0 ± 1.0^{cfhi}
62	13.3 ± 0.5^{dfhij}	10.3 ± 0.5^{dfhij}	8.3 ± 0.5^{dfhij}
31	11.0 ± 1.0^{eghij}	10.0 ± 1.0^{eghij}	7.3 ± 0.5^{eghij}

C: concentration (mg/mL), EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries, Values in each column with same superscript have no significant difference, and values with different superscripts in each column are significantly different.(p <0.05).

Table 4: Mean and SD of ethanol extracts against *S. pneumonae* on agar well diffusion method (mm).

C	MW	MS	MB
1,000	14 ± 1.0^{a}	18 ± 1.0^{a}	13 ± 1.0^{a}
500	1 8 ± 0.7^{ae}	18.5 ± 0.5^{ac}	10.5 ± 0.5^{ac}
250	18 ± 0.2^{aei}	18 ± 0.7^{aci}	10.3 ± 0.3^{aci}
125	12 ± 0.2^{bfil}	14.6 ± 0.5^{bfil}	10 ± 0.2^{bfil}
62	7 ± 0.0^{cgjm}	14.5 ± 1.3^{cgim}	8.8 ± 0.4^{cgim}
31	7 ± 0.7^{dhkn}	10 ± 1.0^{dhkn}	6.7 ± 0.3^{dhkn}

C: concentration (mg/mL), MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries, Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different. (p <0.05).

Table 5: Mean and SD of methanol extracts against *S. epidermidis* on agar well diffusion method (mm).

C	DW	DS	DB
1,000	18.6 ± 0.5^{a}	18 ± 1.0^{a}	13 ± 1.0^{a}
500	16 ± 1.0^{ab}	15.3 ± 0.5^{ac}	8 ± 1.0^{ab}
250	13.8 ± 0.7^{ab}	12.3 ± 0.5^{acd}	7.5 ± 0.8^{ab}
125	NIZ	7.5 ± 0.7^{bcd}	NIZ
62	NIZ	NIZ	NIZ
31	NIZ	NIZ	NIZ

C: concentration (mg/mL), DW: aqueous extract of whole fruit, DS: aqueous extract of fruit skins, DB: aqueous extract of fruit berries, NIZ: no inhibition zone Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different.(p <0.05).

Table 6: Mean and SD of aqueous extracts against *S. epidermidis* on agar well diffusion method (mm).

C	MW	MS	MB
1,000	18.3 ± 0.5^{a}	21 ± 1.0^{a}	8.5 ± 0.7
500	1 ± 0.7^{bf}	15 ± 1.0^{bg}	NIZ
250	10.5 ± 0.5^{cf}	10 ± 1.0^{cg}	NIZ
125	10.3 ± 0.5^{df}	9.6 ± 0.5^{dg}	NIZ
62	8 ± 1.0^{ef}	10 ± 1.7^{eg}	NIZ
31	NIZ	7.3 ± 0.5^{fg}	NIZ

C: concentration (mg/mL), MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries, NIZ: no inhibition zone Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different.(p <0.05).

Table 7: Mean and SD of methanol extracts against *K. pneumoniae* on agar well diffusion method (mm).

C	EW	ES	EB
1,000	22 ± 1.0^{a}	19 ± 1.0^{a}	15.3 ± 0.5^{a}
500	12 ± 1.0^{bg}	12 ± 1.0^{bg}	11 ± 1.0^{bg}
250	10.3 ± 0.5^{cg}	10.7 ± 0.3^{cg}	10.3 ± 0.5^{cg}
125	9.6 ± 0.5^{dg}	10.6 ± 0.5^{dg}	9.6 ± 0.5^{dg}
62	7.2 ± 0.3^{eg}	9 ± 1^{eg}	9.3 ± 1.0^{eg}
31	7.2 ± 0.3^{eg}	7.3 ± 0.5^{fg}	9 ± 1.3^{fg}

C: concentration (mg/mL), EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries. Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different.(p <0.05).

Table 8: Mean and SD of ethanol extracts against *K. pneumoniae* on agar well diffusion method (mm).

of these plants [19]. In microdilution method bacterial growth in 96 wells showed antimicrobial activity of different extracts, which were further determined using triphenyltetrazolium chloride ri phenyl. Result of microdilution method is recommended as this is the best alternative test that also combines with ATP measurement. This method is cheap, less time-consuming, and designed to screen many bacteria and plant extract together [12]. Moreover, in this study, the results of different parts of extracts against different bacteria on microdilution method also were different. Aqueous extract of skin at the concentration of 15.6 mg/mL was acted as a bactericidal against *K. pneumoniae* on microdilution method, followed by MS at the concentration of 31 mg/mL against *S. epidermidis*. The extracts from *B. angulata* showed potential effect on Gram-positive bacteria and Gram-negative bacteria tested in this study. Mixed results were found, mostly depending on the methods, parts of fruit, and solvents used. The ES was the most sensitive fruit extract using microdilution method against *S. epidermidis* and *K. pneumoniae*.

C	DW	DS	DB
1,000	21 ± 1.0^{a}	12 ± 1.0	10 ± 1.0^{a}
500	11 ± 0.8^{bg}	NIZ	7 ± 1.0^{bg}
250	10 ± 1.0^{cg}	NIZ	6.8 ± 0.2^{cg}
125	9 ± 1.0^{dg}	NIZ	6.6 ± 0.2^{dg}
62	8 ± 1.0^{eg}	NIZ	NIZ
31	7.3 ± 0.5^{fg}	NIZ	NIZ

C: concentration (mg/mL), DW: aqueous extract of whole fruit, DS: aqueous extract of fruit skins, DB: aqueous extract of fruit berries, NIZ: no inhibition zone Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different. (p <0.05).

Table 9: Mean and SD of aqueous extracts against *K. pneumoniae* on agar well diffusion method (mm).

C	MW	MS	MB
1,000	7 ± 1.0^{a}	15 ± 1.0^{a}	10 ± 1.0^{a}
500	11 ± 1.0^{ab}	10.5 ± 0.5^{ab}	10 ± 0.2 ^{ab}
250	10 ± 0.2^{abc}	10.3 ± 0.1^{abc}	9 ± 1.0^{abc}
125	10 ± 1.0^{abcd}	10 ± 1.0^{abcd}	8 ± 1.0^{abcd}
62	9.6 ± 0.5^{abcd}	9.6 ± 0.5^{abcde}	7 ± 0.7^{abcd}
31	NIZ	9 ± 1.0^{gbcde}	NIZ

C: concentration (mg/mL), MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries, NIZ: no inhibition zone Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different.(p <0.05).

Table 10: Mean and SD of methanol extracts against *P. aeruginosa* on agar well diffusion method (mm).

C	EW	ES	EB
1,000	13.3 ± 0.5^{a}	12 ± 1.0^{a}	11 ± 1.0
500	13 ± 1.0^{ab}	10.6 ± 0.5^{ab}	NIZ
250	11 ± 1.0^{ab}	10.3 ± 1.0^{abc}	NIZ
125	9 ± 1.0^{abcd}	10 ± 1.0^{abcd}	NIZ
62	8.3 ± 0.5^{abcde}	9.3 ± 0.5^{abcde}	NIZ
31	7 ± 0.7^{gbcde}	9 ± 1.0^{gbcde}	NIZ

C: concentration (mg/mL), EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries, NIZ: no inhibition zone Values in each column with same superscript have no significant difference, and values with different superscript in each column are significantly different.(p <0.05).

Table 11: Mean and SD of ethanol extracts against *P. aeruginosa* on agar well diffusion method (mm).

Microorganisms	MIC/ MBC	MW	MS	MB	EW	ES	EB	DW	DS	DB
S. epidermidis	MIC	7.8	31.0	500	15.6	15.6	15.6	31.0	250	15.6
	MBC	125	62.5	250	250	125	250	125	500	62
K. pneumoniae	MIC	62.0	62.5	250	62.5	7.8	62.5	31.0	15.6	250
	MBC	125	125	500	250	31.0	250	125	15.6	62

MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries, EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skin, EB: ethanol extract of fruit berry, DW: aqueous extract of whole fruit, DS: aqueous extract of fruit skin and DB: aqueous extract of fruit berry

Table 12: MIC and MBC of extracts against *S. epidermidis* and *K. pneumonia*.

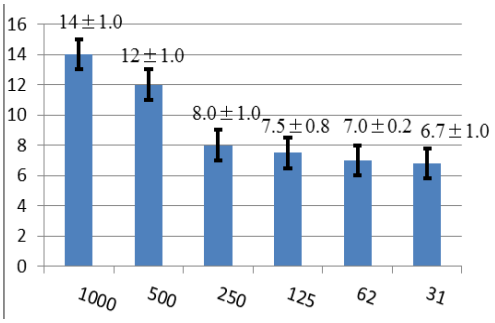

Figure 1: Aqueous extract of whole fruit against *P. aeruginosa* on agar well diffusion method.

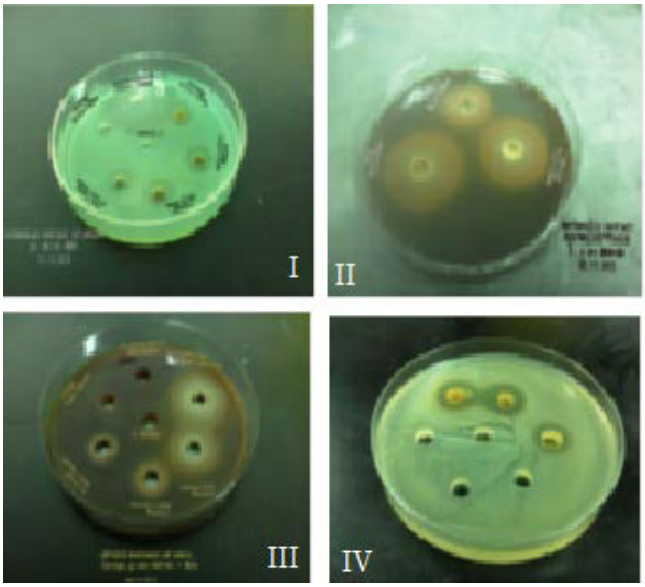

Figure 2: ethanol extract of whole fruit against *P. aeruginosa,* (I), methanol extract of whole fruit, fruit skin and fruit berry against *S. pneumoniae*, (II), aqueous extract of fruit skin against *S. pneumoniae* and ethanol extract of fruit skin against *S. epidermidis* (III).

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