MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 2(10) pp. 229-237, October, 2014
Available online http://www.meritresearchjournals.org/er/index.htm

Original Research Article

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

Lailuma Momand[1], Robaiza Zakaria[3], Maryam Mikail[1], Tara Jalal[1], Muhammad Ibrahim[2,4] and Ridhwan Abdul Wahab[1,2*]

[1]Department of Biomedical Science Kulliyyah of Allied Health Science IIUM

[2]Integrated Center for Research Animal Care and Use (ICRACU) IIUM

[3]School of Medical Science University Sains Malaysia

[4]Department of Nutrition Science Kulliyyah of Allied Health Science IIUM

***Corresponding Author Email ridhwan@iium.edu.my**
Tel +6095704281; Fax +6095716744

Abstract

The research application 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. Thus, a systematic investigation was undertaken to screen for antibacterial activity from *Baccaurea angulata*. Plant that belongs to the family is used as food as well as treatment of infectious diseases such as diarrhoea, skin infections and gonorrhoea. The anti-microbial activity of the *B. angulata* fruit extracts have revealed different antimicrobial properties, that vary between three parts (whole fruit, fruit skin, and berry), three solvents (methanol, ethanol and aqueous), using agar well diffusion, and microdilution methods and different pathogens (*Streptococcus pneumoniae, Staphylococcus epidermidis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa).* The highest observed antimicrobial activity was in ethanol extract of fruit skin using agar well diffusion against *S. pneumoniae.* Among tested Gram negative bacteria, *K. pneumoniae* was the most susceptible bacterium which showed the highest bacteriostatic and bactericidal activity using microdilution method. All parts of fruit extracts poses the antimicrobial activities against all human pathogenic microorganisms used in this study.

Keywords: Antimicrobial activity, *Baccaurea angulata*, Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC)

INTRODUCTION

Pathogenic bacteria increase the incidence of resistant strains worldwide and thus become an important cause of mortality and morbidity around the world (Kraiczy and Würzner, 2006).

Nosocomial and community-acquired infections are both caused by emerging resistant strains to antimicrobials (Oskay et al., 2010). The lowest concentration of antibiotic that inhibits obvious growth of bacteria after overnight incubation is considered as MIC (Andrews, 2004). 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 (Levison, 2004).

In contrast, bactericidal 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 that could be dangerous for human (Puttaswamy et al., 2011). Therefore, treatment of

infectious diseases is becoming more difficult due to emerging resistance and low susceptibility of strains to antibiotics (Wendakoon et al., 2012).

Medicinal plants contain compounds that are capable of providing health benefits by initiating certain physiological actions in the body (Oskay et al., 2010). 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 (Dubey et al., 2012). Different parts of medicinal plants are used as raw drugs and demonstrated numerous medicinal properties (Mahesh and Satish, 2008). Consumption of different fruit species showed an important role due to their health and immunity promoting effects as well as vegetable, nuts and grains in a well-balanced diet, which could decline the risk of inflammatory, metabolic and neurodegenerative diseases (Zia-Ul-Haq et al., 2014). Euphorbiaceae is the fourth major family of the angiosperms, containing more than 300 genera and 7,500 species. Plants that belong to the family are used as food and in the treatment of infectious diseases such as diarrhea, dysentery, and skin infections (Uduak and Kola, 2010). Medicinal and nutritious fruits are abundantly available in Malaysia. The plenty availability of medicinal plant in this geographic area is due to their prolong exposure to sunlight through the year (Ibrahim et al., 2013).

B. angulata is a species that belongs to the family Euphorbiaceae (Lim, 2012) 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 (Jauhari et al., 2013). Antioxidant constituents of *B. angulata* fruit and their nutritional value due to dietary fiber were found by some previous studies and consumption of aqueous juice of the whole fruit in daily meal also recommends (Ibrahim et al., 2013). Hypocholesterolemic effect of this fruit for the treatment and prevention of atherosclerosis and heart disease have recently been found (Mikail et al., 2014).

Antimicrobial activity of *B. angulata* fruit extracts is not previously tested. Therefore, the aims of this study are to: investigate bacteriostatic and bactericidal effects of *B. angulata* (whole fruit, fruit skin, and berry) fruit extracts on human pathogenic Gram-positive bacteria, and Gram-negative bacteria, screen antimicrobial property of *B. angulata* using different method namely agar well diffusion, and microdilution, and compare bacteriostatic and bactericidal effects of different extracts from different parts of the fruit using different solvents.

MATERIALS AND METHODS

Plant Material

B. angulata 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^{o}C at the Department of Nutrition Kulliyyah of Allied Health Science IIUM, Kuantan.

Extraction Method

The three parts of the *B. angulata* fruit (whole fruit, fruit skin and berry) were freeze-dried and reduced to a coarse powder. All 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 include 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).

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. Then, the collected microorganisms were cultivated in non-selective media (screened human donor blood) and cultured overnight at 37^{o}C. The aforementioned pathogenic microorganisms were identified by standard laboratory methods including Gram staining, catalase test, optochin test and biochemical tests such as indole, TSI, urea and motility tests.

Antimicrobial Assays

The antimicrobial activity of each extract of *B. angulata* fruit with different concentrations was tested *in vitro*. Antimicrobial assay was performed using agar well diffusion and microdilution methods. Inoculum was prepared from overnight cultures of different microorganisms (0.5 McFarland). A broad spectrum antibiotic (diluted Ciprobay-200 mg infusion 10/100 µL/mL dH_2O) was used as a positive control and DMSO was used as a negative control throughout the study.

Agar well diffusion method

Agar well diffusion method was carried out 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 sterilised bent glass rod spreader. Next inoculated 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 sterilised tips. Then 100 µL of organic extracts from each part of the fruit (whole fruit, fruit skin and berry) with different concentrations (1,000.0, 500.0, 250.0, 125.0, 62.0, 31.0 µg/mL) were put into each hole. Likewise, the same volume of negative and positive controls was introduced into two wells. All plates were incubated at 37ºC with upside down position (Parekh and Chanda, 2006; Donkor et al., 2012). 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 incubation at 37ºC. All experiments were carried out three times.

Microdilution Method

Microdilution method was carried out on low susceptible microorganisms (*S. epidermids* and *K. pneumoniae*) according to Klančnik (2010). 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 to broth added to each well of the first row. Then the plant extracts were serially diluted to create a concentration of 500.0, 250.0, 125.0, 62.0, 31.0, 15.6, 7.5 and 3.75 µg/mL. Later on, 50 µL of adjusted inoculum to 0.5 McFarmland was introduced into each well. Negative control wells were prepared from Mualler Hintun broth (MHB) and bacterial suspension only, while positive control wells were prepared from MHB, diluted broad spectrum antibiotic (Ciprobay-200) and the adjusted inoculum. The plate was then shaken up for 1 min before being transfer to the incubator for 24 h at 37ºC. The MBC of extract was determined by sub culturing the content of each 96 well on MHA and followed by further incubation for 24 h at 37ºC. 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 the 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 the plates in a dark place. 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.

Statistical Analysis

The experiments were carried out in triplicate, and one way ANOVA 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 (Mahesh and Satish, 2008).

The medicinal properties of *B. angulata* are mostly deposited in the skin of the fruit. In this study, the antimicrobial activity of the extract against aforementioned bacteria carried out using agar well diffusion and microdilution 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 at the highest (1,000 µg/mL) concentration with the ethanol extract of fruit skin (37±1.0 mm), methanol extract of fruit skin (33±1.0 mm) and ethanol extract of fruit berry (30±1.0 mm) against *S. pneumoniae*. The potential effect of the skin extract of *B. angulata* may be due to flavonoid in the fruit skin (Jauhari et al., 2013).

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 (31.0 µg/mL). The highest antimicrobial effect was found in DB extract of the fruit (14.3±0.5 mm) at the concentration of 31.0 µg/mL against *S. pneumoniae* (Table 3). This antimicrobial activity of aqueous extract of the fruit may be due to the fact that fruit components dissolve in aqueous solution (Ördögh et al., 2010). In another study, the antimicrobial effect of apricot juice against different Gram-positive bacteria, as well as Gram negative bacteria was stated the large zone of inhibition (12 mm) by aqueous extract of apricot against *P. mirabilis* (Yiğit et al., 2009).

Antimicrobial activity of EW against *S. epidermidis* showed the same inhibition (28±1.0 mm) zone to MW against *S. pneumoniae* at the highest concentration (1,000.0 µg/mL) on agar well diffusion (Table 1).

MS was highly active (15±1.0 mm) against *P. aeruginosa* at the highest concentration (1,000.0 µg/mL) (Table 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 the fruit skin and fruit berry were not observed at the concentration of less than 500.0 µg/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. aeroginosa* than some other bacteria such as *E. coli*. Other than that, in this bacterium, antibiotic is

Table 1. Mean±SD of methanol extracts in different concentrations (µg/mL) against *S. pneumonae* on agar well diffusion method (mm)

C	MW	MS	MB
1,000.0	28.0±1.0*	33.6±1.0*	28.0±0.5*
500.0	25.6±0.5	19.3±0.5*	11.6±0.5*
250.0	21.0±1.0*	18.3±0.5*	11.3±0.5*
125.0	14.0±1.0*	15±1.0*	11.0±1.0*
62.0	12.3±0.5*	14.0±1.0*	10.0±1.0*
31.0	11.0±1.0*	13.0±1.0*	9.3±0.5*

C: concentration, MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 2. Mean±SD of ethanol extracts in different concentrations (µg/mL) against *S. pneumonae* on agar well diffusion method (mm)

C	EW	ES	EB
1,000.0	26.0±1.0*	37.0±1.0*	30.0.±1.0*
500.0	18.5±0.7*	21.6±1.5*	12.0±0.2*
250.0	18.0±1.0*	19.6±0.5*	10.3±0.5*
125.0	15.3±0.5*	16.6±0.5*	9.0±1.0*
62.0	13.3±0.5*	10.3±0.5*	8.3±0.5*
31.0	11.0±1.0*	10.0±1.0*	7.3±0.5*

C: concentration, EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 3. Mean±SD of aqueous extracts in different concentrations (µg/mL) against *S. pneumonae* on agar well diffusion method (mm)

C	DW	DS	DB
1,000.0	14.0±1.0*	12.0±1.0*	14.0±1.0*
500.0	21.0±1.0*	22.3±0.5*	21.0±1.0*
250.0	14.5±0.5	22.0±1.0*	19.3±0.5*
125.0	14.0±1.0	18.0±1.0*	16.3±0.7*
62.0	13.0±1.0	15.6±0.5	16.0±0.0
31.0	9.6±0.5*	12.3±0.5	14.3±0.5

C: concentration, DW: aqueous extract of whole fruit, DS: aqueous extract of fruit skins, DB: aqueous extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 4. Mean±SD of methanol extracts in different concentrations (µg/mL) against *S. epidermidis* on agar well diffusion method (mm)

C	MW	MS	MB
1,000.0	14±1.0*	18±1.0*	13±1.0*
500.0	18±0.7*	18.5±0.5	10.5±0.5*
250.0	18±0.2*	18±0.7	10.3±0.3*
125.0	12±0.2	14.6±0.5*	10±0.2*
62.0	7.0±0.1*	14.5±1.3*	8.8±0.4*
31.0	7.0±0.7*	10±1.0*	6.7±0.3*

C: Concentration, MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 5. Mean±SD of ethanol extracts in different concentrations (µg/mL) against *S. epidermidis* on agar well diffusion method (mm)

C	EW	ES	EB
1,000.0	28.6±0.5*	14±1.0*	7.6±0.5*
500.0	12.8±0.7*	11.8±0.5	9.3±0.5*
250.0	14±1.0*	11.3±0.5	9.0±0.5*
125.0	11±1.0*	10±1.0*	7.8±0.2*
62.0	NIZ	9.0±1.0*	NIZ
31.0	NIZ	NIZ	NIZ

C: concentration, EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 6. Mean±SD of aqueous extracts in different concentrations (µg/mL) against *S. epidermidis* on agar well diffusion method (mm)

C	DW	DS	DB
1,000.0	18.6±0.5*	18±1.0*	13±1.0*
500.0	16±1.0*	15.3±0.5*	8.0±1.0*
250.0	13.8±0.7*	12.3±0.5*	7.5±0.8*
125.0	NIZ	7.5±0.7*	NIZ
62.0	NIZ	NIZ	NIZ
31.0	NIZ	NIZ	NIZ

C: concentration, DW: aqueous extract of whole fruit, DS: aqueous extract of fruit skins, DB: aqueous extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 7. Mean ± SD of methanol extracts in different concentrations (µg/mL) against *K. pneumoniae* on agar well diffusion method (mm)

C	MW	MS	MB
1,000.0	18.3±0.5*	21±1.0*	8.5±0.7
500.0	11±0.7*	15±1.0*	NIZ
250.0	10.5±0.5*	10±1.0*	NIZ
125.0	10.3±0.5*	9.6±0.5*	NIZ
62.0	8.0±1.0*	10±1.7*	NIZ
31.0	NIZ	7.3±0.5*	NIZ

C: concentration, MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 8. Mean±SD of ethanol extracts in different concentrations (µg/mL) against, *K. pneumoniae* on agar well diffusion method (mm)

C	EW	ES	EB
1,000.0	22±1.0*	19±1.0*	15.3±0.5*
500.0	12±1.0*	12±1.0*	11±1.0*
250.0	10.3±0.5*	10.7±0.3*	10.3±0.5*
125.0	9.6±0.5*	10.6±0.5*	9.6±0.5*
62.0	7.2±0.3*	9.0±1.0*	9.3±1.0*
31.0	7.2±0.3*	7.3±0.5*	9.0±1.3*

C: concentration, EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 9. Mean±SD of aqueous extracts in different concentrations (µg/mL) against *K. pneumonia*e on agar well diffusion method (mm)

C	DW	DS	DB
1,000.0	21.0±1.0*	12.0±1.0	10.0±1.0*
500.0	11.0±0.8*	NIZ	7.0±1.0*
250.0	10.0±1.0*	NIZ	6.8±0.2*
125.0	9.0±1.0*	NIZ	6.6±0.2*
62.0	8.0±1.0*	NIZ	NIZ
31.0	7.3±0.5*	NIZ	NIZ

C: concentration, DW: aqueous extract of whole fruit, DS: aqueous extract of fruit skins, DB: aqueous extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 10. Mean ± SD of methanol extracts in different concentrations (µg/mL) against *P. aeruginosa* on agar well diffusion method (mm)

C	MW	MS	MB
1,000.0	7.0-±1.0*	15±1.0*	10±1.0*
500.0	11±1.0*	10.5±0.5*	10±0.2
250.0	10±0.2*	10.3±0.1*	9.0±1.0*
125.0	10±1.0*	10±1.0*	8.0±1.0*
62.0	9.6±0.5*	9.6±0.5*	7.0±0.7*
31.0	NIZ	9.0±1.0*	NIZ

C: concentration, MW: methanol extract of whole fruit, MS: methanol extract of fruit skins, MB: methanol extract of fruit berries. *Values in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

Table 11. Mean±SD of ethanol extracts in different concentrations (µg/mL) against *P. aeruginosa* on agar well diffusion method (mm)

C	EW	ES	EB
1,000.0	13.3±0.5*	12±1.0*	11±1.0
500.0	13±1.0	10.6±0.5*	NIZ
250.0	11±1.0*	10.3±1.0*	NIZ
125.0	9.0±1.0*	10±1.0*	NIZ
62.0	8.3±0.5*	9.3±0.5*	NIZ
31.0	7.0±0.7*	9.0±1.0*	NIZ

C: concentration, EW: ethanol extract of whole fruit, ES: ethanol extract of fruit skins, EB: ethanol extract of fruit berries. *Value in each column are significantly different ($p<0.05$) from that of 1000.0 µg/mL

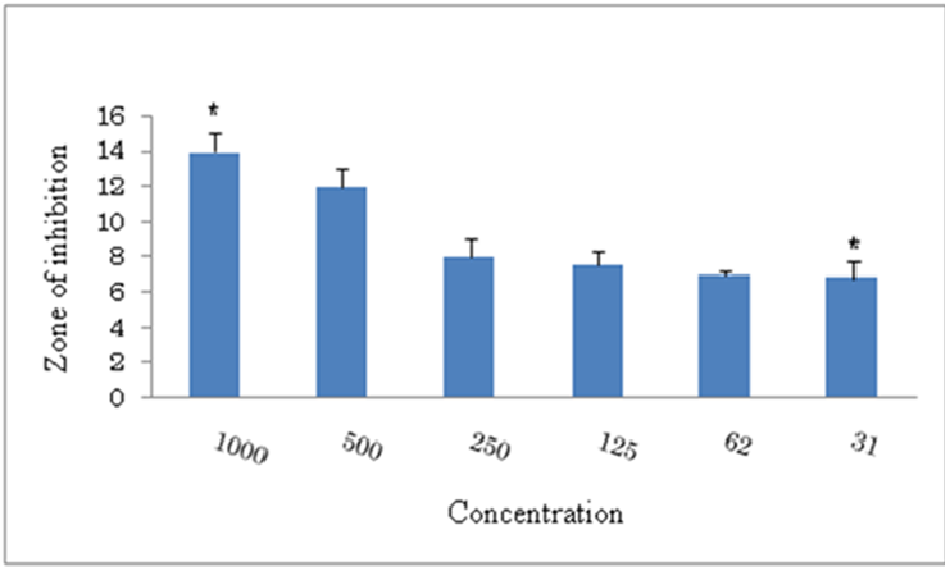

Figure 1. Aqueous extract of whole fruit against *P. aruginosa* on agar well diffusion method. *Values are singnificantly different ($p<0.05$)

Table 12. MIC and MBC of extracts against *S. epidermidis* and *K. pneumoniae* (µg/mL)

Microorganisms	MIC/MBC	MW	MS	MB	EW	ES	EB	DW	DS	DB
S. epidermidis	MIC	7.8	31.0	500.0	15.6	15.6	15.6	31.0	250.0	15.6
	MBC	125.0	62.0	250.0	250.0	125.0	250.0	125.0	500.0	62.0
K. pnumoniae	MIC	62.0	62.0	250.0	62.0	7.8	62.0	31.0	15.6	250
	MBC	125.0	125.0	500.0	250.0	31.0	250.0	125.0	15.6	62.0

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.

transported to the outer membrane of bacterial cell through efflux pump, which is located in the membrane of *P. aeruginosa*, thus causing intrinsic resistance (Hamud-Socoro, 2004).

In this study, the minimum inhibitory concentration using microdilution was observed at the concentration of 7.8 µg/mL of MW against *S. epidermidis* and ES against *K. pneumoniae*. The minimum bactericidal concentration was observed in 15.6 µg/mL of DS against *K. pneumonia*e. 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 of these plants (Singh et al., 2012).

In microdilution method bacterial growth in 96 wells showed antimicrobial activity of different extracts, which were further determined using TTC. Result of microdilution method is recommended as this is the best alternative test that combines with ATP measurement. This method is cheap, less time-consuming, and designed to screen many bacteria and plant extract together (Donkor et al., 2012). Moreover, in this study, the results of different parts of fruit extracts against different bacteria were various. Aqueous extract of skin at the concentration of 15.6 µg/mL was acted as a bactericidal against *K. pneumoniae* this activity is followed by ES at the concentration of 31.0 µg/mL against *S. epidermidis* using microdilution method. All-in-all, the extracts from *B. angulata* showed potential effect on Gram-positive bacteria and Gram-negative bacteria tested in this study.

CONCLUSION

In this study, antimicrobial activity of *B. angulata*, fruit that belongs to the family of Euphrobeacea, were determined. The extracts from the species showed potential effect on tested Gram-positive bacteria as well as Gram-negative bacteria. Various results were found mostly depending on the methods, parts of the fruit, solvents used and the pathogens. Ethanol extract of fruit skin against *S. pneumonia*e on agar well diffusion and methanol extract of whole fruit against *S. epidermidis* and ethanol extract of skin against *K. pneumoniae* showed highest inhibitory activity. The highest bactericidal activity of the fruit extracts was observed in aqueous extract of skin using microdilution method.

ACKNOLEDGEMENT

Authors are acknoledged the whole laboratory staff of Department of Biomedical Science Allid Health Science IIUM Kuantan and laboratory staff of Department of Medical Microbiology and Parasitology school of Medical Science, Health Capmpus University of Sains Malaysia.

REFERENCES

Andrews JM (2004). Determination of minimum inhibitory concentrationJAC; 48:5-16

Donkor AM, Glover RLK, Boateng JK, Gakpo VY (2012). Antibacterial activity of the fruit extract of Physalisangulata and its formulation.JMBS;1:21- 6.

Dubey D, Sahu MC, Rath S, Paty BP, Debata NK, Padhy RN (2012). Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria. Asian Pac J Trop Biomed;2:S846-S54.

Hamud-Socoro AA (2004). Pseudomonas aeruginosa resistance to tetracycline and triclosan.Cantaurus;12:7-9.

Ibrahim D, Hazali N, Jauhari N, Yahya MNA, Ahmed IA, Mikail M A, Ibrahim M(2013).Sub-chronic toxicological evaluation of the Baccaureaangulata(Belimbing Dayak) fruit juice in rats.Ijarnp;6 (4): 23-32.

Jauhari NK, Ibrahim D, Ibrahim M, Yahya MA, Norazmir, Nor Khairul A, ISA MKA, Muhammad NO &Norazlanshah H (2013). Proximate Composition and Antioxidant Activity of Dried Belimbing Dayak (*Baccaureaangulata*) Fruits.Sains Malaysian;42:129-34.

Klančnik A, Piskernik S, Jeršek B, Možina SS (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts.JMicrobiol Methods;81:121-6.

Kraiczy P,Würzner R (2006). Complement escape of human pathogenic bacteria by acquisition of complement regulators.Mol Immunol;43:31-44.

Levison ME (2004). Pharmacodynamics of antimicrobial drugs.Infect Dis Clin North Am;18:451-65

Lim TK(2012).*Edible Medicinal and Non-Medicinal Plants., Fruits* Springer;4.

Mahesh B, Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens.WJAS;4:839-43.

Mikail, MA, Ahmed IA, Ibrahim M, Hazali N, Abdul Rasad, MSB., Abdul GR, Abdul WR, Arief SJ, Isa ML, Draman S, Ishola A, Yahya MNA (2014). Changes in the Markers of Atherosclerosis Following

Administration of Belimbing Dayak (BaccaureaAngulata) Fruit Juice in Experimental Rabbits Fed with Cholesterol Diet. International Journal of Advances in Agricultural & Environmental Engineering (IJAAEE);1(1):2349-1531.

Ördögh L, Galgóczy L, Krisch J, Papp T, Vágvölgyi C (2010). Antioxidant and antimicrobial activities of fruit juices and pomace extracts against acne-inducing bacteria.ActaBiologica Szegediensis;54:45-9.

Oskay M, Oskay D, Kalyoncu F (2010). Activity of some plant extracts against multi-drug resistant human pathogens. IJPR;8:293-300.

Parekh J, Chanda S (2006). In-vitro antimicrobial activities of extracts of Launaeaprocumbensroxb(Labiateae), VitisviniferaL (Vitaceae) and Cyperusrotundus L (Cyperaceae.AJBR;9.

Puttaswamy S, Lee BD, Amighi B, Chakraborty S, Sengupta S (2011).Novel Electrical Method for the Rapid Determination of Minimum Inhibitory Concentration (MIC) and Assay of Bactericidal/Bacteriostatic Activity. JBSBE;*S2-003. doi*.

Singh V, Thakur K, Chauhan PK (2012). Effect of poly herbal.Formulation against *klebsiella pneumonia* causing pneumonia in children's.Asian J Pharm Clin Res;5:69-75.

Uduak AE, Kola KA (2010). Antimicrobial activities of some Euphorbiaceae plants used in the traditional medicine of AkwaIbom State of Nigeria.*Ethnobot Leaflets;*6:2.

Wendakoon C, Calderon P, Gagnon D (2012). Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. IJIMP;1:4

Yiğit D, Yiğit N, Mavi A (2009). Antioxidant and antimicrobial activities of bitter and sweet apricot (*Prunusarmeniaca*) kernels.Braz. J Med Biol Res;42:346-52.

Zia-Ul-Haq M, Riaz M, De Feo V, Jaafar HZE, Moga M (2014). *RubusFruticosus*L Constituents, Biological Activities andHealth Related UsesHeal.ISSN;19:10998-11029.

.