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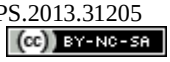
ISSN 2231-3354 

In vitro activity of methanolic extract from *Lagerstroemia speciosa* (Linn. ex. Murray) bark against pathogenic bacteria

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ABSTRACT

Crude methanolic extract of *Lagerstroemia speciosa* barks (Linn. ex. Murray) was subjected to antimicrobial screening including six Gram-positive and eight Gram-negative bacteria. The extract demonstrated significance antibacterial activities on 5 tested bacteria with the inhibition zone ranging from 10-15 mm. The minimum inhibitory concentration (MIC) of the extract was assessed by microdilution method. MIC value of *Bacillus spizizenii* ATCC6633, *Bacillus cereus* and *Streptococcus* coagulase-negative (SCN) was 0.25 mg/ml whilst *Bacillus licheniformis* and *Acinetobacter anitratus* showed MIC value of 0.50 mg/ml. However, only *B. spizizenii* ATCC6633 and *B. licheniformis* showed the minimum bactericidal concentration (MBC) value of 2.00 mg/ml and 1.00 mg/ml, respectively. The morphological changes of *B.spizizenii* ATCC6633 and *A. anitratus* with the treatment of the extract were observed under Scanning Electron Microscope (SEM). The results suggested that the *L. speciosa* methanolic extract had caused membrane structural degenerations of the cells and finally leading to cell-lysis.

INTRODUCTION

The increased of drug-resistant microorganisms has resulted in the evolution of new critical infectious diseases to the world. Infectious disease is one of the causes that jeopardize morbidity and mortality rate globally (Osman *et al*., 2010). In addition, the emergence of antimicrobial resistant microorganisms has also made an impact in global economy. In 1992, the cost for handling aggressive hospital infection caused by *Staphyloccus aureus* was about 122 million US dollar in United State alone and approximately 4.5 billion US dollar for overall nosocomial infection worldwide (Cassell and Mekalanos, 2001). Therefore, to cut down of antimicrobial agent usage has been suggested to bring down total antimicrobial resistant microorganisms. However, it is hard to be implemented and no positive feedback in return (Sandiumenge *et al*., 2006). Thus, the demand on biodiversity of natural resources to search for therapeutic drugs have been increased for the past few decades based on the rich source of bioactive compound. Scientist work on antimicrobial compounds from plants has been widely explored. As reported in Pubmed, there are about 115 medicinal plants that have the potential of anti-infection properties been documented and published in between year 1966 to 1994 and approximately 307 and approximately 307 in between 1995 to 2004 (Ríos and Recio, 2005).

Lagerstroemia speciosa is a plant species mainly distributed in tropical countries like Malaysia. Physically, this plant is a flowering tree that can grow to 18 m in height, with a 9 to 12 m extent. The leaves are dark- green, large, oblong and leathery with the average size of 5 to 10 cm wide by 12 to 30 cm long. It also has flowers that are pink to purple in colour (Gilman and Watson, 1998).

From the researches done, it was found that the corosolic acid derived from the plant has a remarkable ability of lowering blood cholesterol level and as the glucose transport activator for the antidiabetic activity (Sivakumar *et al*., 2009; Takagi *et al*., 2010) and. The antidiabetic property was also reported to be due to the rich contents of tannins and ellagic acid derivatives such as ellagitannins, lagerstroemin, flosin B and reginin A and terpenoids (Hou *et al*., 2009). Such findings show this plant have high potential to become the candidate drug for handling diabetic problems. As for other biological property, the seeds of *L. speciosa* contain caprylic, lauric, mystric,

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palmatic, steric, nonanedioic acid, 12-acetyloxy-9-octadecenoic acid, and 16-methyl-heptadecandic acids with antibacterial activity (Sinhababu *et al*., 1994; Sinhababu *et al*., 1999). Various parts of the plant have also been studied for the antimicrobial property; leaves (Cheeptham and Towers, 2002; Ambujakhshi *et al*., 2009), seeds (Sinhababu *et al*., 1994), and fruits (Brahma *et al*., 2012). However, there is still very little attention given on the *L. speciosa* barks especially regarding to its antimicrobial activity. One study has reported that *L. speciosa* bark contains similar constituents to its leaves which were ellagic acids, beta-sitosterols, and colosolic acids (Faruk *et al*., 2002). Thus, this communication was aim to evaluate the potency of *L. speciosa* barks methanolic extract as an antimicrobial agent. Furthermore, we also described the effect of the extract on the morphologies of bacterial cells after being exposed to it.

MATERIALS AND METHODS

Sample collection and extraction

The sample of *Lagerstroemia speciosa* bark was collected from a healthy tree located in front of the School of Biological Sciences, Universiti Sains Malaysia. The barks were gently scrapped off from the tree trunk using a sharp clean knife. The cleaned samples were chopped into smaller pieces and dried in an oven at 45 ºC until a constant weight was a chieved. Dried barks of *L. speciosa* were then ground into powder form. Forty gram of the powdered sample was soaked in 800 ml of 100% methanol (ratio 1:20 w/v) for 3 days at room temperature (30±2 ºC). The mixture was stirred from time to time to ensure the homogeneity of the soaking process. The 3 days old mixture was filtered twice, first with a Muslin cloth followed by Whatman No.1 filter paper (Whatman). The filtrate was evaporated to dryness using a rotary evaporator (Heidolph, Korea) at 50 ºC and 150 rpm. The concentrated extract was allowed to dry in a fume hood for several days until dark brown paste was obtained, and kept in a fridge at 4 ºC until further use.

Test microorganisms

A total of 14 bacteria strains (ATCC and clinical isolates) which were provided by the Industrial Biotechnology Research Laboratory (IBRL) and the Phytochemistry Laboratory of School of Biological Sciences, Universiti Sains Malaysia. The bacterial strains consisted of six Gram-positive (Methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus* coagulase negative (SCN), *Bacillus spizizenii* ATCC 6633, *Bacillus cereus* ATCC 10876, *Bacillus subtilis*, and *Bacillus licheniformis* ATCC 12759) and eight Gram- negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter anitratus*, *Pseudomonas aeruginosa* ATCC 27583, *Citrobacter freundii*, *Shigella boydii*, *Pseudomonas stutzeri* ATCC 17588 and *Yersinia* sp.). All the bacterial strains were maintained on nutrient agar (NA)(Merck, Germany) slants and incubated for 24 hours at 37 ºC. The cultures were kept in a fridge 4 ºC until further use.

Preliminary antimicrobial screening

In this study, the standard Kirby Bauer disc diffusion method was followed (Bauer *et al*., 1966) with slight modifications. The bacterial suspension of each strain was prepared by inoculating one loopful of 24-hour old bacterial colonies into 10.0 ml sterile distilled water. The bacterial suspension was thoroughly mixed and adjusted to match the turbidity of 0.5 McFarland standard solution (approx. 1.0 x 10^8 cells/ml). An aliquot of 1.0 ml of the bacterial suspension was transferred on NA plate and spread uniformly on the medium agar using a sterile bend glass- rod. 20.0 µl of 50 mg/ml and 100 mg/ml of *L. speciosa* methanol extract was pipette onto the 6.0 mm discs. Chloramphenicol (30 µg/ml) and methanol (99.5 %) were used as the positive and negative controls, respectively. The dried discs which contained the extract were placed onto the bacterial- seeded plates and incubated at 37º C for 24 hours. The experiments were performed in triplicate and the means of the diameter of the inhibition zones were measured.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

All the bacterial strains that were positively inhibited by the methanol extracts of *L. speciosa* barks during the preliminary screening were preceded with the MIC microdilution assay.

The extract stock was prepared by a two-fold dilution of 7.80- 4000.00 µg/ml. The microtiter wells were filled with 5.0 µl inoculums (5 x 10^5 cells/ml), 95.0 µl nutrient broth (NB)(Merck, Germany) and 100.0 µl of the extract. The final concentrations of the extract were in between 3.90- 2000.00 µg/ml. The mixture of each well in a microtiter plates were mixed thoroughly and incubated at 37º C for 24 hours. After the incubation period, 40.0 µl of 0.6 mg/ml colourless p-iodonitetrazolium violet (INT) salt (Sigma, Germany) was added into each well and the plate was re-incubated for another 30 minutes at 37 ºC. Any changes of purple color within the wells indicated the presence of living bacteria. The well of the lowest concentration of the extract showing no colour change was identified as the MIC value. To determine the MBC value in this study, the streak plate method was performed. A loopful of inoculum from each of the colourless well were streaked onto NA and incubated for 24 hours at 37 ºC. The MBC value was determined when there was no visible growth on the agar plates after incubation. The test was done in triplicates.

Scanning electron microscope (SEM) observation

The destructive changes of Gram-positive and Gram-negative bacteria (*B. spizizenii* ATCC 6633 and *A. anitratus*, respectively) were observed under the SEM. These two bacterial strains were selected based on the significant results they produced in the preliminary screening, MIC and MBC. The method for this assay was carried out according to Nor Afifah *et al*., (2012).

The bacterial suspension of each bacteria was prepared according to the method in the preliminary screening (1.0 x 10^8 cells/ml) and diluted to the size of 2.6 x 10^7 cells/ml. The extract stocks were prepared at 13.0 mg/ml and 26.0 mg/ml for *B.*

spizizenii ATCC 6633 and *A. anitratus*, respectively. An aliquot of 1.0 ml of the bacterial suspension and 1.0 ml of the extract were pipetted into an Erlenmeyer flask containing 25.0 ml of NB. The flasks were incubated separately for 12, 24 and 36 hours at 37ºC. For the control set, extract was replaced with 95 % methanol.

After the incubation, the mixture was transferred into 15 ml Falcon tubes and centrifuged at 3000 rpm (ALC CENTRIFUGETTE 4206) for 15 minutes. The pellets were then collected into a 2.0 ml Eppendorf tube and suspended in Mc Dowell-Trump fixative solution, and allowed to settle down for at least 4 hours. These procedures were applied for all the 12, 24 and 36 hours of treatment for both *B. spizizenii* ATCC 6633 and *A. anitratus* samples.

The samples were processed according to the method by Glauert (1980), Nation (1983) and Dykstra (1992). The fixed pellets were centrifuged and their supernatants were discarded. The pellets were then resuspended in 0.1 M phosphate buffer solution and re-centrifuged twice. After the buffer wash process completed, the pellets were then subjected to the post fixation step. The pellet samples were re-suspended in 1% Osmium tetraoxide in phosphate buffer for an hour, followed by centrifugation and the supernatant was discarded. The pellets were then re-suspended in distilled water for post-fixation wash and repeated once. Then, the pellets were subjected to dehydration process with 50 %, 75 %, 95 %, and 100 % (twice) of ethanol consecutively. This was followed by suspending the pellets in hexamethyldisilazane (HMDS) for 10 minutes. The suspension was centrifuged and supernatant was discarded after each dehydration process. The tubes with the processed samples were then allowed to be air-dried in a desiccator. The dried samples were then mounted on a SEM specimen holder and coated with gold prior to viewing under the SEM (Leo Supra 50vp Field Emission SEM equipped Oxford INCA 400 emerge disperse x-ray microanalysis system) with different magnifications and resolutions to observe any changes in the bacterial cell morphology.

RESULTS AND DISCUSSION

L. speciosa bark was selected to be investigated for its antimicrobial activity based on its ethnopharmacology history. Initially, the tree bark of *L. speciosa* is used to treat diarrhea and stomach ache (Rahman *et al.*, 2011); in which it is suspected to comprise of bioactive compounds that exert antimicrobial activity. However, there is still lack of information on the antimicrobial activity of the *L. speciosa* bark as compared to the leaves (Cheeptham and Towers, 2002; Ambujakhshi *et al*., 2009), seeds (Sinhababu *et al*., 1994), and fruits (Brahma *et al*., 2012). Based on these literal evidences, it can be postulated that there is high possibility that the bark of *L. speciosa* also contains wide range of antimicrobial compounds that can be used to treat chronic infectious diseases.

The processing part of the plant material was done cautiously in order to avoid the lost of desired bioactive compounds. A firm force was applied while peeling the tree bark as to prevent injury to the phloem. The phloem itself might contain bioactive compound and the distortion of the system might unintentionally eliminate those compounds (Mani *et al*., 1998). The temperature of 45º C that were applied during the drying process efficiently aided the water content to evaporate in a shorter time and thus prevent the growth of any fungi and bacteria that could alter the phytochemicals of the plant.

According to Durling *et al*., (2007), the size of samples, extraction time, types and ratio of extractant, as well as temperature applied are the main factors that affect the recovery percentage of bioactive compound from plants. For this experiment, the bark samples were ground to powder form to increase the effectiveness of the extraction. Other factors that largely determine the type of bioactive compounds to be extracted is depend on the extraction procedures and the type of solvent used (Verpoorte, 1998). Different solvents will exert different types of attraction force and strength towards different types of compounds. This attraction force has enabled the “attracted” compounds to be extracted out from the tissues into the solvent. The selection of methanol as the extraction solvent is based on the ability of this solvent to extract wide range of hydrophilic compounds and the bioactive compound being targeted for. In addition, methanol is efficient in degrading the plant cell walls thus caused the polyphenols to be released from the cells.

The methanol extract of *L. speciosa* bark showed moderate results in its antimicrobial activity towards the test bacteria (Table 1). Four Gram-negative and one Gram-positive bacteria were effectively inhibited by the methanolic extract. The 2 mg/disc sets showed wider inhibition zones as compared to the 1 mg/disc. The inhibition zones of all affected bacteria were in the range of 7 mm to 16 mm (data not shown).

Table 2 shows the MIC values of the susceptible bacteria. The lowest MIC value of 0.25 mg/ml by *B. cereus* ATCC 10876, *B. spizizenii* ATCC 6633 and SCN. On the other hand, *B. licheniformis* ATCC 12759 and *A. anitratus* exhibited a higher MIC value which was 0.50 mg/ml. As for the MBC test, only *B. spizizenii* ATCC 6633 and *B. licheniformis* ATCC 12759 were completely killed by the methanol extract of *L. speciosa* at 2.0 mg/ml and 1.0 mg/ml, respectively. The MBC for the three other bacterial species (*B. cereus* ATCC 10876, *A. anitratus*, and SCN) revealed a negative result for the MBC test.

Based on the data obtained the methanol extract did significantly showed its antibacterial activity by inhibiting some of the tested Gram-positive and Gram-negative bacteria. The results revealed that the antimicrobial compound of the extract did possess a broad spectrum of antibacterial potential, and likely to be more efficient against Gram-positive bacteria as compared to the Gram-negative bacteria.

The antimicrobial property of this species was also proven by the study done by Meléndez and Capriles (2006), who showed that the methanolic extract of *L. speciosa* leaves was able to inhibit the *B. subtilis* and *S. aureus*. The variation in the types of bacteria that were inhibited by our study and Meléndez and Capriles (2006) might reveal that different parts of plant organ of

the same species contain different content of phytochemicals. This phytochemical content will determine the bioactivity of the plant. The MIC and MBC results had quantitatively demonstrated the antimicrobial activity of the *L. speciosa* towards the susceptible bacteria. From the results, the MIC values that varied between *B. spizizenii*, *B. cereus*, *B. licheniformis*, SCN and *A. anitratus* indicating that their susceptibility towards the extract is diverse. This discrepancy may lie in the differences in the way of the bioactive compounds acted on different types of strains. According to Rajabi *et al*., (2005), the MBC value that equals or more than eightfold of its MIC value will have higher tendency as bacteriostatic agent. This indicate that the methanolic extract of *L. speciosa* behave more as a bacteriostatic agent than a bactericidal agent (against *B. cereus*, SCN and *A. anitratus*).

Figures 1 represent the morphological changes of the non- treated and treated *B. spizizenii* ATCC 6633. Figure 1A shows the SEM micrographs of bacterial cells without the methanolic extract treatment. The figure revealed the normal rod shape cell structure without any shrinkage or cavity formation as the surface was smooth and regular. Figure 2B shows the morphology of the cell after 12 hours of treatment with the extract. The damage was still subtle as only a few of the bacterial cell surfaces were slightly furrowed. However, after the 24 hours of exposure the bacterial cells started to show multiple defects. Figure 2C revealed that cells' cavities were formed and some of them were also severely shrunk. After 36 hours of exposure, the bacterial cells were seemed to be totally deformed. The cells were collapsed, clumping together and hence lost their original rod shape as compared to the control cells in Figure 1A.

After explosion of methanolic crude extract in the cultivations medium, the *A. anitratus* cells exhibited changes in their morphology (Fig. 2). Figure 2A which acts as the control shows healthy bacteria cells with rod-shaped structure and uninterrupted surface that stay intact. Figures 2B-2D show the morphological changes of *A. anitratus* after being exposed to the methanolic extract at different durations of time. Figure 2B shows the bacterial cells after 12 hours of exposure to the extract. It shows crumples, shrunken cells and cavity formations on the bacterial cell surfaces. The result of the 24 hours of exposure to the extract (Fig. 3C) shows a very drastic morphological change of the cells compared to the 12 hours exposure. At this point, the cells shrunk, curled up and contracted. Figure 3D shows the same destruction characteristics as the 24 hours exposure but at a higher degree of injury. The cavities and wrinkle formed were very deep due to the shrinkage effect. SEM observations hypothesized that the effects of the extract were exerted on the outer membrane of the wall which then altered the membrane cell structure and also the permeability of the cells. At the early stage (12 hour) both bacteria exerts a slight difference in their morphologies compared to control cells. However, the cells started to show more aberrant morphology over time. It can be summarized that the lethal action of treated cells starts with the shrinkage of the cells and then followed by the accelerated pores or cavity formations and unusual cell morphogenesis. It might have been because of the changes in the membrane cell structure resulted from the breakage of the hydrogen bonds that functions in keeping the rigidity of the membrane (Neu and Gootz, 1996). The same characteristics were observed in other antimicrobial studies of plant extract against pathogenic bacteria (Darah *et al*., 2011; Nor Afifah *et al*., 2012). Hyde *et al*., (2006) suggested that the morphological changes of the antibiotic-treated bacteria occur when the antimicrobial agent attacked the cell membrane. The bioactive compound of the methanolic extract of *L. speciosa* barks that locked on the cell surface structure and had permeabilized the bacterial membranes. As shown by the SEM micrographs where the cells became crumpled and exhibited formation of cavity. These damages may indicate the lost of cellular materials and organelles from the cell cytoplasm (Al-Adham *et al*., 1998). These unstable and altered cells were observed to be completely collapsed. Black (2008) stated that a cell with non- rigid, non- sturdy, and abnormal usually tends to burse when expose to the low osmotic pressure. The interaction between the active compounds and the lipid layer in the cell wall of the bacterial cells also could lead to cell leakage. There were reports on the uptake of antimicrobial compounds presence in the plant extract which affect greatly on the lipid content in the cell wall of microorganisms (Salton, 1964; Straus and Hancock, 2006).

Table. 1: Antimicrobial activity of the methanol extract of *Lagerstroemia speciosa* barks on test bacteria.

MICROORGANISMS	1.0 Mg/Disc	2.0 Mg/Disc	C	M
Gram-positive bacteria				
Bacillus cereus ATCC 10876	+	+	+++	-
Bacillus licheniformis ATCC 12759	+	+	++	-
Bacillus spizizenii ATCC 6633	++	+++	+++	-
Bacillus subtilis	-	-	++	-
Methicillin resistant *Staphylococcus aerues* (MRSA)	-	-	++	-
Streptococcus coagulase negative (SCN)	++	+++	+	-
Gram-negative bacteria				
Acinetobacter anitratus	+	+	+++	-
Citrobacter freundii	-	-	++	-
Escherichia coli	-	-	++	-
Klebsiella pneumoniae ATCC 13383	-	-	+++	-
Pseudomonas aeruginosa ATCC 27583	-	-	++	-
Pseudomonas stutzeri ATCC 17588	-	-	++	-
Shigella bodyii	-	-	+++	-
Yersinia sp.	-	-	+++	-

The inhibition zones were in millimeter (mm) unit and grouped according to the following scale: +++ = ≥ 15mm, ++ = 11 to 14mm, + = ≤ 10mm, - = No inhibition zone, NT = Not tested, C = Chloramphenicol, M = Methanol.

Table. 2: MIC and MBC values of the methanol extract of *L. speciosa* bark on test bacteria.

Bacteria	MIC value (mg/ml)	MBC value (mg/ml)
Acinetobacter anitratus	0.50	-
Bacillus cereus ATCC 10876	0.25	-
Bacillus licheniformis ATCC 12759	0.50	1.00
Bacillus spizizenii ATCC 6633	0.25	2.00
Streptococcus coagulase negative (SCN)	0.25	-

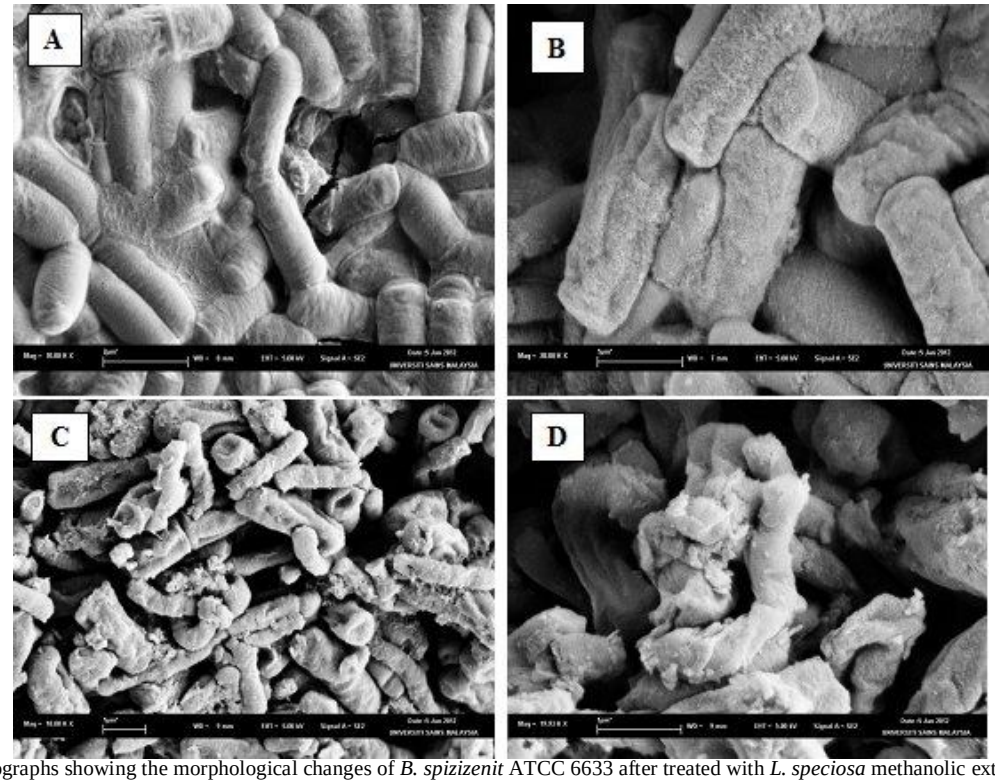

Fig. 1: SEM micrographs showing the morphological changes of *B. spizizenit* ATCC 6633 after treated with *L. speciosa* methanolic extract at 0.25mg/ml. Control cell without extract treatment (A), after 12 hours of treatment (B), after 24 hours of treatment (C), after 36 hours of treatment (D).

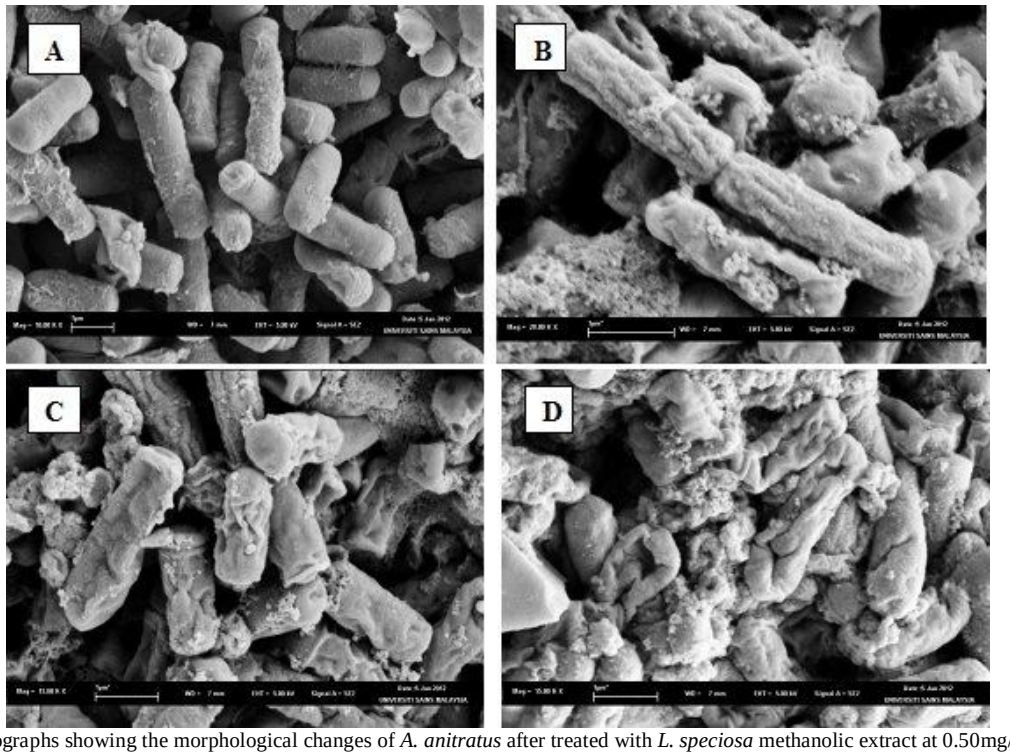

Fig. 2: SEM micrographs showing the morphological changes of *A. anitratus* after treated with *L. speciosa* methanolic extract at 0.50mg/ml. control cells without extract treatment (A), after 12 hours of treatment(b), after 24 hours of treatment(C), after 36 hours of treatment (D).

CONCLUSION

In conclusion, *L.speciosa* barks methanolic extract was found to possess antimicrobial activity. However, this crude extract should be further processed to obtain pure compound that solely exert the antimicrobial property of *L.speciosa* barks methanolic extract which can then be tested for antibacterial activity.

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REFERENCES

Al-Adham ISI, Dinning AJ, Eastwood IM, Austin P, Collier PJ. Cell membrane effects of some common biocides. J Ind Microbiol Biotechnol, 1998; 21: 6-10.

Ambujakshi HR, Surendra V, Haribabu T, Goli D. 2009. Antibacterial activity of leaves of Lagerstroemia speciosa (L) Pers. J Pharma Res, 2009; 2(6): 1028-1032.

Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Technical Bulletin Registry Medica Techno, 1966; 36: 49-52.

Black JG. 2008. Microbiology: Principles and explorations. John Wiley & Sons.

Brahma NS, Singh HB, Singh A, Singh BR, Mishra A, Nautiyal CS. Lagerstroemia speciosa fruit extract modulates quorum sensing-controlled virulence factor production and biofilm formation in Pseudomonas aeruginosa. Microbiol, 2012; 158: 529-538.

Cassell GH, Mekalanos J. Development of antimicrobial agents in the era of new and reemerging infectious diseases and increasing antibiotic resistance. J American Medical Association, 2001; 285: 601-605.

Cheeptham N, Towers GHN. Light-meidated activities of some Thai medicinal plant teas. Fitoterapia, 2002; 73: 651- 662.

Darah I, Lim CL, Nurul Aili Z, Nor Afifah S, Shaida Fariza S. Effects of methanolic extract of a soft sponge sp. on bacterial cells: structural degenaration study. Int J Comprehensive Pharma, 2011; 2: 1- 6.

Durling NE, Catchpole OJ, Grey JB, Webby RF, Mitchell KA, Foo LY, Perry NB. Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using ethanol–water mixtures. Food Chem, 2007; 101: 1417-1424.

Dykstra MJ. 1992. Biological Electron Microscopy: Theory, Techniques and Trouble shooting. Plenum Press, New York.

Faruk MJ, Nahar N, Aziz MA, Mosihuzzaman M, Rashid MA. Two new ellagic acids from Lagerstroemia speciosa Linn. Plant. J Bangladesh Chem Society, 2002; 15: 73-78.

Gilman EF, Watson DG. 1998. *Lagerstroemia speciosa* Queens Crape-Myrtle. US Forest Service, Department of Agriculture. Available at http://hort.ifas.ufl.edu/trees/LAGSPEA.pdf . [Accessed on 25 September 2013].

Glauert AM. 1980. Fixation, dehydration and embedding of biological specimens. In: Glauert, A. M. (eds.), Practical Methods in Electron Microscopy. North- Holland, Amsterdam.

Hou W, Li Y, Zhang Q, Wei X, Peng A, Chen L, Wei Y. Triterpene acids isolated from Lagerstroemia speciosa leaves as α-glucosidase inhibitors. Phytotherapy Res, 2009; 23: 614-618.

Hyde AJ, Parisot J, McNichol A, Bonev BB. Nisin-induced changes in *Bacillus* morphology suggest a paradigm of antibiotic action. Proceedings of the National Academy of Sciences of the United States of America, 2006; 103: 19896-19901.

Mani M, Metraux B, Pierre J. Salicylic acid and systemic acquired resistance to pathogen attack. Annals Bot, 1998; 82: 535-540.

Meléndez PA, Capriles VA. Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine, 2006; 13: 272-276.

Nor Afifah S, Darah I, Shaida Fariza S, Nurul Aili Z. Inhibition of Klebsiella pneumoniae ATCC 13883 cells by hexane extract of Halimeda discoidea (Decaisne) and the identification of its potential bioactive compounds. J Microbiol Biotechnol, 2012; 22: 872-81.

Neu HC, Gootz TD. 1996. Antimicrobial Chemotherapy. In: Baron S. (eds.), Medical Microbiology. Galveston (TX), University of Texas Medical Branch at Galveston.

Osman MEH, Abushady AM, Elshobary ME. In vitro screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir bay Alexandria, Egypt. African J Biotechnol, 2010; 9: 7203-7208.

Rahman MDA, Uddin N, Hasanuzzaman M, Rahman AA. Antinociceptive, antidiarrhoeal and cytotoxic activities of Lagerstroemia speciosa (L.) pers. Pharmacologyonline, 2011; 1: 604-612.

Rajabi L, Courreges C, Montoya J, Aguilera RJ, Primm TP. Acetophenones with selective antimycobacterial activity. Letters Appl Microbiol, 2005; 40: 212-217.

Ríos JL, Recio MC. 2005. Medicinal plants and antimicrobial activity. J Ethnopharmacol, 2005; 100: 80-84.

Salton MRJ. 1964. The Bacterial Cell Wall. Elsevier, Armsterdam, Holland.

Sandiumenge A, Diaz E, Rodriguez A, Vidaur L, Canadell L, Olona M, Rue M, Rello J. Impact of diversity of antibiotic use on the development of antimicrobial resistance. J. Antimicrob Chemother, 2006; 57: 1197-1204.

Sinhababu A, Basak B, Laskar S, Chakrabarty D, Sen SK. Effect of different fractions of petroleum ether (60- 80 degrees) extract of the seeds of Lagerstroemia speciosa (Linn. Ex Murray) Pers. on some microorganisms. Hindustan Antibiotics Bulletin, 1994; 36 (1-2): 39- 45.

Sinhababu A, Das S, Laskar S, Thakur S, Sen SK. Characterization and identification of antibacterial components in extracts of seeds from Lagerstroemia speciosa. Adv Food Sci, 1999; 21: 19-22.

Sivakumar G, Vail DR, Nair V, Medina-Bolivar F, Lay JR. Plant-based corosolic acid: Future anti-diabetic drug? Biotechnol J, 2009; 4: 1704-1711.

Straus SK, Hancock RE. Mode of action of the new antibiotic for Gram-positive pathogens daptomycin: comparison with cationic antimicrobial peptides and lipopeptides. Biochimia et Biophysica Acta, 2006; 1758: 1215-1223.

Takagi S, Miura T, Ishihara E, Ishida T, Chinzei Y. 2010. Effect of corosolic acid on dietary hypercholesterolemia and hepatic steatosis in KK-Ay diabetic mice. Biomed Res, 2010; 31: 213-218.

Verpoorte R. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discovery Today, 1998; 3: 232-238.

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