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Artikel Asli/*Original Articles*

Stem Bark of *Canarium odontophyllum* Miq. (Dabai) as Potential Source of Antimicrobial Agent

(Potensi Kulit Dahan *Canarium odontophyllum* Miq. (Dabai) sebagai Sumber Agen Antimikrob)

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ABSTRACT

The objective of this study was to evaluate the antimicrobial potential of methanol, acetone and distilled water stem bark extracts from Canarium odontophyllum against Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 6633, Escherichia coli ATCC 25932, Pseudomonas aeruginosa ATCC 27853, Acinetobacter baumannii strain sensitive, Candida albicans ATCC 64677, Candida glabrata ATCC 90028, Aspergillus niger and Fusarium solani M2781. The extracts from C. odontophyllum stem bark from 3.125 mg/ml to 25 mg/ml were screened against the tested microorganisms using disc diffusion method. The Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts against susceptible organisms were determined using microbroth dilution method and streak-plate technique, respectively. From the antibacterial screening assay, the growth of S. aureus, B. cereus and A. baumannii were inhibited by methanol extract whereas the acetone extract was capable of inhibiting all the tested microorganisms except E.coli, F. solani and A. niger. The lowest MIC value for methanol extract was against A. baumannii (0.195 mg/ml) whereas its MBC value was twice its MIC value (0.391 mg/ml), indicating that methanol extract was bacteriostatic against A. baumannii. While for acetone extract, S. aureus showed bactericidal effect with equal MIC and MBC values at 0.195 mg/ml. In conclusion, stem bark of C. odontophyllum has the potential to be the source of antibacterial agent and can be exploited as an alternative phytoantimicrobial.

Keywords: Canarium odontophyllum; antimicrobial; stem bark extract; MIC; MBC

ABSTRAK

Objektif kajian ini adalah untuk menilai potensi antimikrob bagi ekstrak metanol, aseton dan akeus daripada kulit dahan Canarium odontophyllum ke atas Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 6633, Escherichia coli ATCC 25932, Pseudomonas aeruginosa ATCC 27853, Acinetobacter baumannii jenis sensitif, Candida albicans ATCC 64677, Candida glabrata ATCC 90028, Aspergillus niger dan Fusarium solani M2781. Ekstrak daripada dahan C. odontophyllum pada kepekatan 3.125 mg/ml sehingga 25 mg/ml telah disaringkan ke atas sembilan mikroorganisma kajian menggunakan kaedah resapan cakera. Kepekatan perencatan minima (MIC) dan kepekatan bakterisidal minima (MBC) ekstrak terhadap organisma rentan telah ditentukan menggunakan kaedah pencairan mikrokaldu dan teknik coretan plat, masing-masing. Daripada ujian saringan antibakteria, pertumbuhan S. aureus, B. cereus dan A. baumannii direncat oleh ekstrak metanol manakala ekstrak aseton mampu merencat kesemua mikroorganisma kajian kecuali E.coli, F. solani dan A. niger. Nilai MIC yang terendah bagi ekstrak metanol ialah terhadap A. baumannii (0.195 mg/ml) manakala nilai MBC adalah dua kali nilai MIC (0.391 mg/ml), bererti ekstrak metanol bersifat bakteriostatik ke atas A. baumannii. Ekstrak aseton pula, menunjukkan kesan bakterisidal terhadap S. aureus dengan nilai MIC dan MBC yang sama iaitu 0.195 mg/ml. Kesimpulannya, dahan C. odontophyllum berpotensi untuk dijadikan sumber agen antimikrob dan boleh dieksploit sebagai alternatif agen fitoantimikrob.

Kata kunci: Canarium odontophyllum; antimicrob; ekstrak kulit dahan; MIC; MBC

INTRODUCTION

Bacteria, fungi, protozoa, virus and parasite are categorized as pathogenic microorganisms and have the ability to react with the host organisms that can lead to infectious diseases. These microorganisms are capable of producing certain pathogenic factor and will create an ecosystem in humans (Stefanoic et al. 2010). Adaptation of these microorganisms to the environment contributes to the increase in antibiotic resistance (Lister et al. 2009).

Antimicrobial agent or antibiotic is a drug that consists of synthetic and semi-synthetic substances which is used to prevent, reduce and control the incidence of infections. Misuse of antibiotic is the main reason of

antibiotic resistance and this phenomenon is the ultimate problem when treating any infections (CDC 2013; Raka et al. 2009).

Canarium odontophyllum Miq. is classified under family *Burseraceae* and it can be found in the tropical rainforest of Sarawak. *C.odontophyllum* is commonly known as 'Dabai' or 'Sibu Olive' by local community in Sarawak (Ding & Diana 2013). The fruit is seasonal and can only be obtained during the months of October until December. The colour of the ripe fruit is blue-black and white when it is immature. The fruit is oblong in shape with a single three-angled seed. The fruit can be consumed after the fresh fruit is soaked in warm water for a few minutes to soften the pulp (Chew et al. 2012).

Previous study revealed that the peel of *C. odontophyllum* contains high level of antioxidant compounds such as phenolic and flavonoid (Prasad et al. 2010). Recently, research on pulp, seed and leave of *C. odontophyllum* were found to have the potential as antiyeast agent (Basri et al. 2014a), antibacterial agent (Basri et al. 2014b) and anti-MRSA agent (Basri et al. 2014c). With the other *Canarium* species, hexane extract from stem bark of *Canarium patentinervum* Miq. was found to have antimicrobial activity against Gram-positive and Gram-negative bacteria (Mogana et al. 2011). Unfortunately, the antimicrobial activity of stem bark extracts from *C. odontophyllum* against bacteria, yeast and filamentous fungi has not been done. To date, antimicrobial work on the *Canarium odontophyllum* was mainly focussed on the leaves of this plant and hence, this is the first report on the potential of its stem bark extracts as a natural source for antimicrobial agent.

MATERIALS AND METHODS

PLANT MATERIAL

Stem bark of *Canarium odontophyllum* Miq. was obtained from Kuching, Sarawak, Malaysia. All plant parts were identified and authenticated by Mr. Sani Misran and deposited in the Herbarium of the Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia with a voucher specimen number of UKMB 40052.

PREPARATION OF EXTRACT SOLUTION

The methanol and acetone extracts were dissolved with 10% DMSO whereas the aqueous extract was dissolved in sterile distilled water to final concentrations of 3.125, 6.25, 12.5 and 25 mg/ml. All the extracts were sterilized by passing through a 0.45 µm membrane filter.

PREPARATION OF MICROORGANISM STRAINS

The microorganisms used in this study were two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 6633), three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumanii* sensitive strain), two yeast strains (*Candida albicans* ATCC 90028 and *Candida glabrata* ATCC 64677) and two filamentous fungi (*Fusarium solani* M2781 and *Aspergillus niger*). All the bacterial strains were grown and maintained on Mueller Hinton agar (MHA) and nutrient agar (NA) slant whereas the fungus were grown on Potato Dextrose agar (PDA). Preparation of inoculum began with subculturing the bacteria on MHA plates and the fungus on PDA. The plates were then incubated for 24 hours for bacterial and yeast strains whereas three to four days for filamentous fungus. The inoculum size of all microorganisms was standardized using spectrophotometer by adjusting the optical density of the bacterial and yeast suspension, respectively, at turbidity corresponding to an absorbance (A) reading at 0.08 to 6.25 and 530 nm. As for the filamentous fungi, the inoculum size was standardized to absorbance (A) reading within the range of 0.09 to 0.13 at 530 nm.

SCREENING OF ANTIMICROBIAL ACTIVITY

The three extracts from the stem bark of *Canarium odontophyllum* were used for antibacterial and antifungal screening test using disc diffusion method. Mueller Hinton agar was uniformly seeded with test bacteria by spreading the inoculum on the surface of the agar plate with a sterile swab. On the other hand, MHA incorporated with glucose and methylene blue was used as the seed medium for yeast whereas PDA was used for filamentous fungus. The sterile disc with the extract concentration of 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml, positive control disc and negative control disc were placed onto the agar surface that have been seeded with the tested microorganisms. For positive control, gentamicin disc (10 µg) was used for bacteria whereas amphotericin B (20 µg) was used for yeast and filamentous fungi. Extraction solvents served as negative control. The bacteria and yeast plates were incubated at 37°C for 24 hours whereas the filamentous fungi plates were incubated at 30°C for 36 hours. The test was done in triplicates to ensure the reliability of the extract assayed in order to calculate the mean value. The antimicrobial activity was performed by measuring the diameter of the zone of inhibition in mm.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The MIC value of each extract was identified by using two fold-serial microdilution method in 96-well microtiter plate. Firstly, 50 µl of the test extract at concentration 25 mg/ml was added to 50 µl sterile broth media. Subsequently, 50 µl of the diluted microorganism suspension with the final inoculum of 10^5 microorganism/ml was added to the 96-well plate. The microdilution was done at a final extract concentration ranging from 0.012 mg/ml to 6.25 mg/ml. Each extract was tested in triplicate. The extracts in broth

were used as negative control to ensure the sterility of the medium while the bacterial suspensions served as positive control to make sure there was growth of the bacteria in the broth. The lowest concentration to inhibit the growth of microorganisms was identified as MIC value which showed no changes in colour after an indicator was added. The MBC value was determined by subculturing the well that showed no colour changes on a sterile agar plate. The lowest concentration that showed no obvious growth on agar plate was recorded as the MBC value.

RESULTS

SCREENING OF ANTIMICROBIAL ACTIVITY

The antibacterial activity of methanol and acetone from stem bark of *Canarium odontophyllum* against nine tested microorganisms was tabulated (Table 1 & 2). The methanol stem bark extract was capable of inhibiting the growth of *S. aureus* ATCC 25923, *B. cereus* ATCC 6633 and *A. baumannii* sensitive strain whereas *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *C. albican* ATCC 90028, *C. glabrata* ATCC 64677, *F. solani* M2781 and *A. niger* were not susceptible towards the methanol stem bark extract. Among the three susceptible bacterial strains, *S.aureus* displayed the largest inhibition zone of 8.00 ± 0.57 mm to 12.00 ± 0.57 mm. On other hand, the acetone extract did not show any antimicrobial activity for *E.coli*, *F. solani* and *A. niger*. Out of the six susceptible microorganisms, *S. aureus* exhibited the biggest inhibition zone of 11.70 ± 0.57 mm to 18.00 ± 0.57 mm followed by *C. glabrata* from 10.00 ± 0.00 mm to 15.00 ± 0.57 mm. Among all the nine tested microorganisms, *S. aureus* was found to be the most susceptible towards both methanol and acetone extracts. Aqueous extract was not capable of inhibiting the growth of all the microorganisms tested.

TABLE 1. Mean diameter of inhibition zones of methanol stem bark extract from *Canarium odontophyllum* against nine tested microorganisms

Microorganisms	Positive control (10 mg/ml)	Concentration of methanol extract (mg/mL)			
		25.0	12.5	6.25	3.125
S. aureus ATCC 25923	27.00 ± 0.00	12.00 ± 0.57	8.00 ± 0.57	-	-
B. cereus ATCC 6633	22.00 ± 0.00	8.00 ± 0.50	7.50 ± 0.00	-	-
E. coli ATCC 25853	23.00 ± 0.00	-	-	-	-
P. aeruginosa ATCC 27853	20.00 ± 0.00	-	-	-	-
A. baumannii sensitive strain	35.00 ± 0.00	10.00 ± 0.00	8.67 ± 0.57	7.30 ± 0.57	7.00 ± 0.00
C. albicans ATCC 64677	17.00 ± 0.00	-	-	-	-
C. glabrata ATCC 90028	20.00 ± 0.00	-	-	-	-
Fusarium solani M2781	12.00 ± 0.00	-	-	-	-
Aspergillus niger	19.00 ± 0.00	-	-	-	-

The data is presented as a mean of 3 replicates ± SD; (-) = no inhibition zone

TABLE 2. Mean diameter of inhibition zones of acetone stem bark extract from *Canarium odontophyllum* against nine tested microorganisms

Microorganisms	Positive control (10 mg/ml)	Concentration of methanol extract (mg/mL)			
		25.0	12.5	6.25	3.125
S. aureus ATCC 25923	23.00 ± 0.00	18.00 ± 0.57	11.70 ± 0.57	-	-
B. cereus ATCC 6633	23.00 ± 0.00	8.00 ± 0.00	7.00 ± 0.00	-	-
E. coli ATCC 25853	19.00 ± 0.00	-	-	-	-
P. aeruginosa ATCC 27853	19.00 ± 0.00	8.50 ± 0.70	7.00 ± 0.00	-	-
A. baumannii sensitive strain	35.00 ± 0.00	11.67 ± 0.57	9.33 ± 0.57	7.33 ± 0.57	6.50 ± 0.00
C. albicans ATCC 64677	18.00 ± 0.00	10.50 ± 0.70	8.50 ± 0.70	-	-
C. glabrata ATCC 90028	18.00 ± 0.00	15.00 ± 0.57	10.00 ± 0.00	-	-
Fusarium solani M2781	11.00 ± 0.00	-	-	-	-
Aspergillus niger	20.00 ± 0.00	-	-	-	-

The data is presented as a mean of 3 replicates ± SD; (-) = no inhibition zone

DETERMINATION OF MIC AND MBC VALUES

The result for MIC and MBC values of methanol and acetone extract from stem bark of *C. odontophyllum* against susceptible microorganisms was presented in Table 3 and Table 4, respectively. The lowest MIC value for methanol extract was 0.195 mg/ml against *A. baumannii* sensitive strain with MBC value at 0.391 mg/ml. Interestingly, the MBC value for acetone extract against *S. aureus* was the same as its MIC value which was at 0.098 mg/ml. This showed that acetone extract portrayed bactericidal effect against *S. aureus*.

TABLE 3. MIC and MBC values for methanol extract from stem bark of *C. odontophyllum*

Bacteria	MIC (mg/ml)	MBC (mg/ml)
S. aureus	0.391	ND
B. cereus	3.125	ND
A. baumannii sensitive strain	0.195	0.391

ND: not determined

TABLE 4. MIC and MBC values for acetone extract from stem bark of *C. odontophyllum*

Microorganism	MIC (mg/ml)	MBC (mg/ml)
S. aureus	0.195	0.195
B. cereus	1.563	ND
A. baumannii sensitive strain	0.049	0.391
P. aeruginosa	0.098	ND
C. albicans	0.098	ND
C. glabrata	0.098	ND

ND: not determined

DISCUSSION

Acetone, methanol and water were the solvents used in this study with different level of polarity to extract a wide range of active compounds from stem bark of *C. odontophyllum*. In this study, acetone and methanol extracts were found to possess antimicrobial activity against nine tested microorganisms. However, no antimicrobial activity was recorded by aqueous extract from stem bark of *C. odontophyllum* against all nine tested microorganisms. Based from the result, the most polar solvent was incapable of extracting active compounds from the stem bark of *C. odontophyllum* to exhibit antimicrobial activity and is supported by Basri et al. (2014) that the aqueous extract from leaves of *C. odontophyllum* showed no anti-MRSA activity despite its higher percentage of extraction yield. On the other hand, the acetone extract revealed a stronger inhibitory effect against the tested bacteria compared to methanol extract. It also indicates that the acetone stem bark extract from *C. odontophyllum* contains wide-spectrum of antibacterial compounds which makes it a potential good source of antimicrobial substance compared to methanol extract. This is because acetone extract is the best solvent for extraction as it has an ability to dissolve many polar and non-polar components from the plant and has low toxicity to the host organisms (Eloff 1998).

From the disc diffusion assay, the methanol and acetone extracts showed high activity towards *S.aureus* that can be observed from the larger size of the zone of inhibition on agar plates. This is supported by Basri & Nor (2014) who reported the methanol and acetone extracts from the leave of *C. odontophyllum* were capable of inhibiting the growth of *S. aureus*. Other than that, the leave extract from another species *C. schwenfurthii* act as an antibacterial agent against *S. aureus* (Odunkabu & Ganiyu 2012).

In order to determine the antimicrobial activity, it is important to identify and characterize the effect of the extracts against the tested microorganisms by comparing the MBC value with the MIC value of each extract. Current study revealed that the MIC and MBC values of acetone extract against *S. aureus* were the same. This indicated that acetone extract contains bioactive components that give bactericidal action against *S. aureus*. The MBC values were higher than the MIC values of the methanol and acetone extracts against susceptible microorganisms except for the acetone extract against *S. aureus*. Thus, it can be interpreted that they acted against corresponding strains by bacteriostatic activity. The difference of antimicrobial activity shown on the microorganisms tested was due to the different susceptibility of each microorganism to the stem bark extract (Basri et al. 2011).

Therefore, the high amounts of tannin in the methanol and acetone extracts from stem bark of *C. odontophyllum* could be the bioactive compounds responsible for the antimicrobial activity. The antimicrobial activity of extract from galls of *Quercus infectoria* against Gram-positive and Gram-negative bacteria was previously reported to be due to high amounts of tannin (Basri et al. 2005). Previous study on phytochemical screening of methanol and acetone from stem bark of *C. odontophyllum* showed that it contains terpenoid, flavonoid, saponin, tannin and phenolic compound (Basri et al. 2014). In general, tannin is a bioactive compound and can produce antimicrobial activity. Tannin is able to inhibit the growth of bacteria by binding to the cell wall of ruminal bacteria that will cause lack of substrates and lower the production of protease from microbial growth. Besides that, tannin can react and disrupt the mechanism of oxidative phosphorylation that will cause damage to the microorganism and extracellular enzyme (Scalbert 1991). However, Deschamps et al. (1983) found that some bacteria, yeast and fungi are resistant to the tannin compounds.

CONCLUSION

The methanol and acetone extract from stem bark of *Canarium odontophyllum* exhibited respectively, bacteriostatic activity against *Acinetobacter baumannii* and bactericidal effect against *Staphylococcus aureus*. Hence, the stem bark from *Canarium odontophyllum* has the potential as an alternative phytotherapeutic agent against bacterial infections. However, further investigations are needed to determine and identify the bioactive compounds that are responsible for the antimicrobial activity.

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REFERENCES

Basri, D.F., Fudholi, A. & Ruslan, M.H. 2012. Drying characteristics of the Borneo *Canarium odontophyllum* (Dabai) Fruit. *American Journal of Agricultural and Biological Science* 7(3): 347-356.

Basri, D.F., Saidi, N., Mahari, H., Saari, S. & Santhanam, J. 2014. Preliminary screening for antimicrobial activity of the pulp of *Canarium odontophyllum* Miq. (Dabai) fruit. *Global Journal of Pharmacology* 8(2): 213-220.

Basri, D.F. & Nor, N.H.M. 2014. Phytoconstituent screening and antibacterial activity of the leaf extracts from *Canarium odontophyllum* Miq. *American Journal of Plant Sciences* 5: 2878-2888.

Basri, D.F., Ishak, S.F. & Zin, N.M. 2014. Shell extract of seed from *Canarium odontophyllum* Miq. (dabai) fruit as potential source of antibacterial agent. *International Journal of Pharmaceutical Sciences Review and Research* 28(2): 257-261.

Basri, D.F., Zainal, N.H. & Santhanam, J. 2014. The potential of *Canarium odontophyllum* Miq. (dabai) as anti-methicillin resistant *Staphylococcus aureus*. *International Journal of Pharmacy and Pharmaceutical Sciences* 6(9): 290-293.

Basri, D.F., Mohd, M.A.A.R., Meng, C. K., Latif, E.S. & Huyop, F.Z. 2014. Cytotoxic activity of stem bark extracts from *Canarium odontophyllum* Miq. (dabai) against human colorectal carcinoma HCT 116 Cell Line. *American Journal of Plant Sciences* 5: 3925-3933.

Basri, D.F. & Fan, S.H. 2005. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian Journal Pharmacology* 37: 26-29.

Basri, D.F. & Khairon, R. 2012. Pharmacodynamic interaction of *Quercus infectoria* galls extract in combination with vancomycin against MRSA using microdilution checkerboard and time-kill assay. *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 493156, 6 pages, 2012.

CDC, US Centers for Disease Control and Prevention. 2010. *Public Health Image Gallery (PHIL), Department of Health and Human Services*. http://www.cdc.gov/ drugresistance/ about.html [13 September 2014].

Chew, L.Y., Khoo, H.E., Amin, I., Azrina, A. & Lau, C.Y. 2012. Analysis of phenolic compounds of dabai (*Canarium odontophyllum* Miq.) Fruits by high-performance liquid chromatography. *Food Analytical Methods* 5(1): 126-137.

CLSI. 2012. Performance Standard for Antimicrobial Susceptibility Testing; Twenty-Second Information Supplement. *Clinical and Laboratory Standard Institute* 32(3): 1-182.

Deschamps, A.M., Otuk, G. & Lebeault, J. M. 1983. Production of tannase and degradation of chestnut tannins by bacteria. *Journal of Fermentation Technology* 61(1): 55-59.

Ding, P. & Diana, J. 2013. Physico-chemical changes in dabai (*Canarium odontophyllum* Miq.) fruit during modified atmosphere storage. *International Food Research Journal* 20(6): 3033-3040.

Drugbank. 2005. *Gentamicin*. http://www.drugbank.ca/ drugs/ DB00798 [26 May 2015].

Drugbank. 2005. *Amphotericin B*. http://www.drugbank.ca/ drugs/ DB00681 [26 May 2015].

Eloff, J.N. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 60: 1-8.

Lister, P.D., Wolter, D.J. & Hanson, N.D. 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews* 22(4): 582-610.

Mogana, R., Teng-Jin, K. & Wiart, C. 2011. In vitro antimicrobial, antioxidant activities and phytochemical analysis of *Canarium patentinervium* Miq. from Malaysia. *Biotechnology Research International* 2011: 1-5.

Odunkabu, O.A. & Ganiyu, S.K. 2012. Synergism between ethanol leaf extracts of *Canarium Schwenfurthii* and antimicrobial drugs on some pathogenic microbes. *Global Research Journal of Agricultural and Biological Sciences* 3(4): 347-350.

Prasad, K.N., Chew, L.Y., Khoo, H.E., Kong, K.W., Azlan, A. & Ismail, A. 2010. Antioxidant capacities of peel, pulp and seed fractions of *Canarium odontophyllum* Miq. fruit. *Biomed Research International* 2010: 1-7.

Raka, L., Zoutman, D., Mulliqi, G., Krasniqi, S., Dedushaj, I., Raka, N., Ahmeti, S., Shala, M., Vishaj, A. & Elezi, Y. 2006. Prevalence of nosocomial infections in high-risk units in the University Clinical Center of Kosovo. *Infection Control of Hospital Epidemiology* 27: 421-423.

Scalbert, A. 1991. Antimicrobial properties of tannins. *Phytochemistry* 30(12): 3875-3883.

Stefanoic, O., Radojevic, I., Vasic, S. & Comic, L. 2012. Antibacterial activity of naturally occuring compounds from selected plants, in Chapter 1: Antimicrobial Agents, edited by Bobbarala V. Croatia: InTech, Rijeka.

WHO 2013. *Antimicrobial Resistance. Geneva: World Health Organization.* http://www.who.int/mediacentre/ factsheets/ fs194/ [30 September 2014].

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