Research Article

Shell Extract of Seed from *Canarium odontophyllum Miq.* (dabai) Fruit as Potential Source of Antibacterial Agent

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ABSTRACT

The objective of this study was to evaluate the antimicrobial potential of acetone, ethyl acetate and methanol shell extracts of seed from *C. odontophyllum* fruit at 12.5 mg/ml to 100 mg/ml against two Gram-positive bacteria; *Staphylococcus aureus* ATCC 25923, clinical strain of *Bacillus cereus* and seven clinical strains of Gram-negative organisms; *Salmonella typhimurium, Proteus mirabilis, Shigellasonnei, Escherichia coli, Yersinia enterocolitica, Proteus* spp. and *Acinetobacter baumannii*. The highest yield (1.76 %) was obtained using methanol as extraction solvent despite showing no antibacterial activity. The extraction yield of acetone was 0.59 % whereas the lowest yield (0.29 %) was produced by ethyl acetate. From the antibacterial screening assay, the growth of *Bacillus cereus* was inhibited by ethyl acetate extract with inhibition zone ranging from 9.6 ± 0.1 mm to 14.6 ± 0.1 mm whereas the acetone extract was capable of inhibiting *P.mirabilis* and *A. baumannii* at respectively, 9.6 ± 0.0 mm to 14.0 ± 0.0 mm and7.0 ± 0.1 mm to 13.0 ± 0.1 mm. The MIC and MBC values of ethyl acetate and acetone extract were the same against *Bacillus cereus* and *A.baumannii* at 6.25 mg/ml and 1.563 mg/ml, respectively. As for *Proteus mirabilis*, the MBC value of acetone extract was eight times higher (6.25 mg/ml) than its MIC value at 0.781 mg/ml. Phytochemical screening showed that ethyl acetate extract indicated the presence of flavonoid and tannin whereas acetone extract contained terpenoid and tannin. In conclusion, acetone shell extract of seed from *C. odontophyllum* fruit showed promising antibacterial and thus, have great potential as an alternative phytotherapeutic treatment against infections associated with *A. baumannii* and *P. mirabilis*.

Keywords: *Canariumodontophyllum*, extraction yield, Antimicrobial, Disc Diffusion, MIC, MBC.

INTRODUCTION

Pathogenic microorganisms such as bacteria, fungi, virus can cause various types of diseases that lead to morbidity and mortality. Bacterial infection caused serious problem to human and animal health around the world because pathogenic bacteria has become more resistance towards existing antibiotics with adaptation to their environment[1]. Examples of such bacteria are Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant *Staphylococcus aureus*, penicillin-resistant *pneumococci*, Vancomycin-resistant *enterococci*, multi-drug resistant tuberculosis, *Acinetobacter baumanni, Stenotrophomonas maltophilia*, and *Burkholderiacepacia*[2]. As resistance towards antibiotic arises, it becomes more difficult to cure bacterial infection. It will increase burden to the patient due to ineffective therapy, longer duration of hospital stay and therefore, higher cost of medical expenses. As such, there is a demand for alternative treatment especially from phytotherapeutic sources. *Canariumodontophyllum* (dabai) which belongs to family *Burceraceae,* is locally known as 'dabai' and 'Sibu olive' in Malaysia. It is a seasonal fruit between October until December and *C. odontophyllum* trees have separate sexes. *C. odontophyllum* fruit is white in colour when immature and turn blue black when ripe. The shape is oblong and has thin, edible skin (exocarp). The fleshy mesocarpinside the fruit is yellow or white in colour. It has three faceted diamond shaped seed[3]. The weight of this fruit is 10-13g whereas 3-4cm (long). It also contains single seed with hard and thick endocarp (2.5 -3.5 cm long and 2.2-3.0 cm in diameter). The whole fruit is soaked in warm water 3-5 minutes to soften the pulp[4] before being eaten.

Previous study[5] on the exocarp of *C. odontophyllum* reported its antioxidant potential because of the high content of total phenolic, flavonoid and anthocyanin. Research on the defatted *C. odontophyllum* showed that methanol and aqueous extract have high total phenolic and Trolox equivalent antioxidant capacity (TEAC) compared with the ethyl acetate, ethanol and acetone extracts[6]. As far *Canarium* spp. is concerned, it was shown that essential oil from the resin *Canariumschweinfurthii* exerted bactericidal effect[7] and the ethanol extract of leaves and barks from *Canariumpatentinervium* accumulated substantial amounts of antimicrobial constituents[8]. However, our previous investigation[9] on the pulp extract of *C. odontophyllum* showed that it was not active against the bacteria. To date, there is still no antimicrobial study done on the shell from seed of *C. odontophyllum* fruit and this is the first report on the antibacterial activity of the shell extract from the seed of this underutilized fruit.

MATERIALS AND METHODS

Plant Material

The fresh fruit of *C. odontophyllum* was purchased from a local market in Miri, Sarawak, Malaysia and deposited in Herbarium Unit in UKM Bangi for authentication with

voucher specimen number UKMB 40052. The fleshy pulp was manually separated from the seed of *C. odontophyllum* fruit as shown in Figure 1 and the latter was chopped into half before the kernel seed was discarded using a toothpick. The shell from seed of *C. odontophyllum* fruit was dried in the oven for several days and was grinded into powder using an electrical blender.

Figure 1: The seed of *C. odontophyllum* fruit showing (A) shell and (B) kernel

Preparation of Extracts

The crude extracts were prepared in ethyl acetate, acetone and methanol as extraction solvents in the ratio 1:5. In the preparation of acetone extract, 224g of dry powdered shell from the seed of *C. odontophyllum* fruit was mixed with 1120ml acetone and the mixture was shaken using magnetic stirrer at a speed of 100 rpm before being left to dissolve overnight for 24 hours at room temperature. Then the mixture was filtered, the resulting filtrate was collected whereas the remaining residue with mixed with 1120ml fresh acetone. The second filtrate obtained was combined with the first filtrate and was then concentrated under low pressure using a rotary evaporator. The crude extract obtained was dried in a fume chamber and weighed to determine the percentage yield of the extract. The whole process was repeated using the remaining dried residue for the preparation of the crude ethyl acetate and methanol extracts. Each of the extract was dissolved into its respective extraction solvent by passing through a 0.45µm membrane pore filter and the final concentration of each of the extracts were prepared at 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml. The screening for phytochemicals was based on the standard protocol[10] to detect the presence of phytoconstituents only in the shell extracts of seed from *C. odontophyllum* fruit that showed antimicrobial activity.

Preparation of Microorganism Strains

The microorganisms used in this study were two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), clinical strain of *Bacillus cereus* and seven clinical strains of Gram-negative bacteria such as *Salmonella typhimurium, Proteus mirabilis, Shigellasonnei, Escherichia coli, Yersinia enterocolitica, Proteus* spp. and *Acinetobacterbaumannii*. All the bacterial strains were grown and maintained on Mueller Hinton agar (MHA) and nutrient agar (NA) slant. Preparation of bacterial inoculum began with subculturing the bacteria on Mueller-Hinton agar plates and incubated overnight at a temperature of 37°C. Then, three to five bacterial colonies on agar plates were cultured and transferred into Mueller-Hinton broth using a sterile wire loop at a temperature of 37°C for 24 hours and the turbidity of the bacterial suspension was adjusted between 0.08 to 0.10 using a spectrophotometer at wavelength of 625nm.

Screening of Antimicrobial Activity

The bacterial inoculum was inoculated evenly on the surface of Mueller-Hinton agar plate by streaking thrice with a sterile cotton swab. Then sterile disc at extract concentration of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, negative control disc and positive control disc were placed onto the agar surfaces that have been inoculated with the tested bacteria. The test was performed in triplicates and the plates were incubated overnight at a temperature of 37°C. The size of the diameter zone of inhibition was observed and measured. Next, the Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined on the tested bacteria that showed inhibitory effect towards the extract.

RESULTS AND DISCUSSION

The result of the percentage yield of the shell extract from seed of *C. odontophyllum* fruit are presented in Table 1.

Table 1: Result of extraction yield of the shell extract from the seed of *C. odontophyllum* fruit

Solvent	Weight of the original sample (g)	Weight of extract (g)	Percentage of yield (%)
Acetone	224	1.32	0.59
Ethyl Acetate	220	0.64	0.29
Methanol	95	1.67	1.76

The highest yield (1.76 %) was obtained using methanol as extraction solvent followed by acetone at 0.59%, whereas the lowest yield was recorded by ethyl acetate at 0.29%. This is in line with[11] that the yield of extractive value from the stem bark of *Alstoniascholaris* and stem of *Tinosporacordifolia* and *Enicostemahyssofolia* were higher in methanol as compared to acetone as extractive solvent. In another study[12] which compared the extractive capacity of methanol, ethanol, acetone and ethyl acetate from *Solanumscrabrum*, methanol extract gave the highest yield while the lowest yield was obtained from ethyl acetate extract. Being highly polar, the ability of methanol to dissolve greater number of secondary metabolites in plants could probably account for its high extractive potential[13]. However, no antimicrobial activity

was recorded by the methanol extract despite the finding that methanol appeared to be the best extraction solvent to dissolve more polar compounds from the shell of the seed from *C. odontophyllum* fruit. This is supported by[9] that the methanol pulp extract of *C. odontophyllum* showed no antimicrobial activity despite its highest percentage of extraction yield. As such, Table 2 and Table 3 showed only the result of antimicrobial screening assay for ethyl acetate extract and methanol extract respectively, against a total of 9 bacterial species studied. The ethyl acetate shell extract was capable of inhibiting the growth of *S. aureus* ATCC 25923, *Proteus* spp., *P. mirabilis*, *Shigellasonnei*, *Yersinia enterocolitica* and *B. cereus* whereas *Acinetobacterbaumannii*, *Salmonella typhimurium* and *E. coli* were not susceptible towards the ethyl acetate shell extract. Among the six susceptible bacterial strains, *B. cereus* displayed the largest inhibition zone of 14.6 ± 0.1 mm at 100 mg/ml compared to other pathogens within the range of 9.6 ± 0.0 mm to 13.0 ± 0.1mm. As far as acetone extract is concerned (Table 3), only *E. coli* did not show the diameter of inhibition zone whereas *S. typhimurium* was only inhibited at 100 mg/ml. Out of the eight susceptible bacterial species, *P. mirabilis* exhibited the biggest inhibition of 14.0 ± 0.0 mm followed by *A. baumannii* (13.0 ± 0.1 mm) at 100 mg/ml. It can be deduced that acetone extract showed wider spectrum of antimicrobial coverage compared to ethyl acetate shell extract from the seed of *C. odontophyllum* fruit.

The result for comparison of MIC and MBC values of ethyl acetate shell extract from seed of *C. odontophyllum* fruit against six susceptible bacterial strains was presented in Table 4 whereas Table 5 referred to that of acetone extract against eight bacterial species. The broth micro dilution method and the preliminary antimicrobial test correlated well in which the most susceptible bacteria in the screening study showed the lowest MIC value in the microbroth assay. As seen from Table 4, the lowest MIC value of ethyl acetate shell extract was against *B. cereus* at 6.25 mg/ml whereas that of acetone shell extract was 0.781 mg/ml against *P. mirabilis* followed by *A. baumannii* at 1.563 mg/ml (Table 5). Low MIC value indicated that bacteria are more sensitive, as a good indicator that the extract has high efficacy against the bacteria[14].

Table 2: Mean diameter of inhibition zones of ethyl acetate shell extract from the seed of *C. odontophyllum* fruit against nine bacteria species

Bacteria species	Gentamicin (10µg/ml)	Concentration of ethyl acetate extract (mg/ml)			
		100	50	25	12.5
S. aureus ATCC 25923	22.0 ± 0.0	12.0 ± 0.0	11.0 ± 0.1	10.0 ± 0.0	9.6 ± 0.1
Proteus spp.	20.0 ± 0.0	13.0 ± 0.1	12.0 ± 0.1	10.0 ± 0.0	10.0 ± 0.0
Shigellasonnei	18.0 ± 0.0	10.0 ± 0.0	9.0 ± 0.1	7.6 ± 0.1	7.0 ± 0.1
Yersinia enterocolitica	20.0 ± 0.0	12.0 ± 0.0	12.0 ± 0.0	10.0 ± 0.1	9.6 ± 0.1
B. cereus	25.0 ± 0.0	14.6 ± 0.1	12.0 ± 0.0	10.4 ± 0.1	9.6 ± 0.1
P. mirabilis	22.0 ± 0.0	9.6 ± 0.0	9.0 ± 0.1	7.8 ± 0.1	6.8 ± 0.1
Acinetobacterbaumannii	24.0 ± 0.0	-	-	-	-
E. coli	20.0 ± 0.0	-	-	-	-
Salmonella typhimurium	22.0 ± 0.0	-	-	-	-

- : No inhibition zone; The data is presented as a mean of 3 replicates ± SEM

Table 3: Mean diameter of inhibition zones of acetone shell extract from the seed of *C. odontophyllum* fruit against nine bacteria species

Bacteria species	Gentamicin (10µg/ml)	Concentration of acetone extract (mg/ml)			
		100	50	25	12.5
S. aureus ATCC 25923	22.0 ± 0.0	10.0 ± 0.0	9.6 ± 0.1	8.4 ± 0.1	
Proteus spp.	20.0 ± 0.0		12.0 ± 0.0	10.0 ± 0.0	9.6 ± 0.1
Shigellasonnei	22.4 ± 0.0	12.2 ± 0.1	10.0 ± 0.0	9.6 ± 0.1	8.4 ± 0.1
Yersinia enterocolitica	20.0 ± 0.0	10.6 ± 0.1	10.0 ± 0.0	8.2 ± 0.1	7.6 ± 0.1
B. cereus	22.0 ± 0.0	12.6 ± 0.1	10.4 ± 0.1	10.0 ± 0.0	9.0 ± 0.1
P. mirabilis	22.0 ± 0.0	14.0 ± 0.0	10.4 ± 0.1	10.0 ± 0.0	9.6 ± 0.0
Salmonella typhimurium	22.0 ± 0.0	10.4 ± 0.1	-	-	-
Acinetobacterbaumannii	24.0 ± 0.0	13.0 ± 0.1	11.0 ± 0.1	9.6 ± 0.1	7.0 ± 0.1
E. coli	20.0 ± 0.0	-	-	-	-

- : No inhibition zone; The data is presented as a mean of 3 replicates ± SEM

Table 4: Result of comparison of MIC and MBC values of ethyl acetate shell extract from the seed of *C. odontophyllum* fruit against six bacteria species

	Bacteria species	MIC (mg/ml)	MBC (mg/ml)
Gram-positive bacteria	*S. aureus*ATCC 25923	12.5.5	25
	B. cereus	6.25	6.25
Gram-negative bacteria	*Proteus*spp.	12.5	12.5
	Yersinia enterocolitica	12.5	12.5
	Shigellasonnei	12.5	25
	P. mirabilis	12.5	25

Table 5: Comparison of the MIC and MBC value of acetone shell extract from the seed of *C. odontophyllum* fruit against eight bacteria species

	Bacteria species	MIC (mg/ml)	MBC (mg/ml)
Gram-positive bacteria	*S. aureus*ATCC 25923	3.1255	12.5
	B. cereus	6.25	25
Gram-negative bacteria	*Proteus*spp.	6.25	12.5
	Yersinia enterocolitica	6.25	12.5
	Shigellasonnei	6.25	12.5
	P. mirabilis	0.781	6.25
	Acinetobacterbaumannii	1.563	1.563
	Salmonella typhimurium	3.125	6.25

Table 6: Result of phytochemical screening of shell extracts from the seed of *C. odontophyllum* fruit

Test	Acetone extract	Methanol extract	Ethyl acetate extract
Terpenoid	+	ND	-
Alkaloid	-	ND	-
Resin	-	+	-
Flavonoid	-	ND	+
Tannin	+	+	+

+: Present of constituent; - : Absent of constituent; ND: Not done

It was compared that the MIC and MBC values of ethyl acetate and acetone extract were the same against *Bacillus cereus* and *A. baumannii* at 6.25 mg/ml and 1.563 mg/ml, respectively. It can therefore be interpreted that they act against the corresponding strains by bactericidal action. On the other hand, the MBC value of the acetone shell extract against *P. mirabilis* was eight times higher than its MIC value. This indicated that although acetone extract showed the most potent antimicrobial activity against *P. mirabilis* but it acts only through bacteriostatic effect. On the contrary, the bactericidal effect against *A. baumannii* occurred at twice the concentration of acetone shell extract from seed of *C. odontophyllum* fruit that inhibited the growth of *P. mirabilis*. Acetone shell extract was active against all the bacteria in this study except for *E. coli*. The microorganism *E. coli*, which is already known to be multi-resistant to drugs, was also resistant to the plant antimicrobials[15]. This is also supported by[16] most strains of *E. coli* are resistant to an extract from *Adhatodavasica.* Acetone is considered to be the best extraction solvent because it can dissolve a wide range of active compounds from plants including both hydrophilic and hydrophobic components and has low toxicity [17]. The interesting finding from this study was that acetone shell extract from seed of *C. odontophyllum* fruit clearly demonstratedsignificantly stronger antibacterial activity ($p < 0.05$) compared to the ethyl acetate shell extract against mostbacteria species tested.

Table 6 showed the result of phytochemical screening of shell extracts from the seed of *C. odontophyllum* fruit. Due to the fact that methanol extract showed no antibacterial activity, limited phytoscreening analysis was done on methanol extract. In fact, tests to detect the presence of terpenoid, alkaloid and flavonoid were not performed due to its absence of antimicrobial activity. What is more important from the present observation is a deduction that acetone produced higher extraction yield from the shell of the seed from *C. odontophyllum* fruit than ethyl acetate therefore, more active constituents which were dissolved by acetone showed wider spectrum of antimicrobial coverage compared to lower amount of phytocomponents extracted by the less polar ethyl acetate. This is likely due to the presence of terpenoid in the acetone shell extract which was not detected in the ethyl acetate shell extract from seed of *C. odontophyllum* fruit. This is supported by a study [18] that acetone has the capacity to extract terpenoid and alkaloid from the seed of *Ferula gummosa* Boiss. Moreover, terpenoid has been known to serve as plant defence against pathogens, insects and herbivores[19, 20]. It was revealed from our preliminary screening result that tannin was present in acetone, methanol and ethyl acetate extracts and out of these three extracts, only methanol extract showed the presence of resin. The antimicrobial activity of acetone and ethyl acetate shell extract against most bacteria studied could also be attributed to tannin. This is in

agreement with[21] that antibacterial activity of the extract from *Quercus infectoria* against oral pathogens was due to high tannin content. Condensed tannins have been identified to bind to the cell walls of ruminal bacteria by inhibiting the growth due to the lack of the necessary substrate for microbial growth and lack of protease production. Tannin inhibits microbial and extracellular enzyme through its direct action on metabolism via inhibition of oxidative phosphorylation[22]. Despite the fact that tannin is established as remarkable antimicrobial, its presence in the methanol shell extract from seed of *C. odontophyllum* fruit however, did not contribute to the inhibitory effect against the growth of all the bacterial species tested. This could be probably due to interference with resin which was only detected in the methanol shell extract as revealed from the phytochemical screening assay. As such, there is a possibility that the antimicrobial effect of tannin in the extract is antagonized by resin.

Based on[23], the high tannin content in the red propolis is associated with the presence of resin from a legume tree. However, the actual mechanism of this possible antagonistic action warrants further investigation.

CONCLUSION

In general, acetone shell extract from the seed of *C. odontopyhllum* fruit showed a antimicrobial activity with bactericidal action against *Acinetobacter baumannii* despite merely capable of inhibiting *P. mirabilis* growth. The phytochemicals which were revealed to account for this antibacterial potential of acetone shell extracts from seed of *C. odontopyhllum* fruit were terpenoid and tannin and hence, could offer a promising source of an alternative phytotherapeutic application against various bacterial infections.

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