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Preliminary Screening for Antimicrobial Activity of the Pulp of *Canarium odontophyllum* Miq. (Dabai) Fruit

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Abstract: *Canarium odontophyllum* Miq. or locally known as '*C. odontophyllum*' in Sarawak, Malaysia, is one of the underutilized fruits consumed for their antioxidant properties by the local community in Borneo. The aim of the present study was to evaluate the antimicrobial activity of crude extracts from the pulp of *C. odontophyllum* against pathogenic microorganisms. The air dried flesh pulp of *C. odontophyllum* was extracted using four solvents with different polarity against two Gram-negative bacteria; *Pseudomonas aeruginosa* and *Escherichia coli*, two Gram-positive bacteria; Methicillin-susceptible *Staphylococcus aureus, (*MSSA) and Methicillin-Resistant *Staphylococcus aur*eus (MRSA), three yeast species; *Candida albicans* ATCC 90028*, Candida glabrata* ATCC 64677 and *Candida krusei* ATCC 6258 and two filamentous fungus; *Aspergillus fumigatus* ATCC 204305 and *Aspergillus niger* ATCC 6275. The hexane, acetone, methanol and aqueous extracts of C. *odontophyllum* pulp at 25, 50, 75 and 100 mg/ml were screened for antimicrobial activity using agar well diffusion method. The yield of the *C. odontophyllum* was the highest when methanol was used as the extraction solvent (12.46 %) followed by acetone (8.72%) and hexane (6.22%). The lowest yield was recorded for the aqueous extract of *C. odontophyllum* (2.78%). These findings showed that all the crude extracts of *C. odontophyllum* were not active against any of the bacteria tested. Out of all the fungus studied, only *C. glabrata* was susceptible towards the *C. odontophyllum* pulp extract. The acetone extract displayed moderate antimicrobial activity against *C. glabrata* with inhibitory zone of 8.0 ± 0.00 mm at all tested concentrations whereas *C. glabrata* was susceptible towards hexane extract only at 100 mg/ml compared to the positive control (Gentamicin at 10 µg/ml) with inhibitory zone of 18.0 ± 1.41mm. The present study indicated that *C. odontophyllum* is a good source of obtaining an alternative phytotherapeutic agent which can be developed as antiyeast agent.

Key words: Canarium Odontophyllum · *Burseraceae* · Extraction Yield · Antimicrobial Agar · Well Diffusion

INTRODUCTION

Nosocomial infections are the major cause of death and increased morbidity in hospitalized patients [1, 2]. Infections of surgical wounds, urinary tract infections and lower respiratory tract infections are among the major nosocomial infections [3]. The regular use of antimicrobial for therapeutic or prophylactic indication promotes the development of resistance. In addition to nosocomial infections, fungal diseases in human are also becoming more resistance to currently available drugs. Therefore it is important that novel antimicrobial agents be identified and developed. Attention has been focused on the search for new antimicrobial agents from natural plants as alternative phytotherapeutic agent to combat bacterial and fungal infectious diseases [4].

Canarium odontophyllum Miq. belongs to *Burseraceae* family and mainly found in tropical rain forest of Sarawak, Malaysia [5]. The fruits are highly seasonal and only available during the months of October-December [6]. The fruit is known as "*dabai*" among local community and has been dubbed

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Fig. 1: Photograph showing the fleshy pulp from *Canarium odontophyllum*

"Sibu olive" because its physical appearance, smooth texture and rich flavor are similar to olive fruits. The skin of the fruit turns dark purple black colour when fully ripe (Fig. 1). The flesh is bright yellow in color with a single three-angled seed [7]. They are sometimes viewed by outsiders as nutritionally inferior fruit. In fact, these fruits are classified as underutilized or underexploited fruits due to lack of promotion, minimal planting area with their economic potential not being fully explored [8].

C. odontophyllum fruit is a very nutritious with high content of lipid, carbohydrates, proteins and minerals such as potassium, phosphorus, calcium and magnesium [9]. In addition to this, *C. odontophyllum* fruit has the potential to be developed as healthy cooking oil as it contain a lot of unsaturated fatty acids [10]. Previous study has reported that *C. odontophyllum* fruit, especially the skin, contain high content of antioxidant compounds such as phenolic compound, flavonoids and anthocyanins thus, it can serve as a major source of natural antioxidants [11]. Extracts of peel, pulp and kernel of *C. odontophyllum* have consistently shown antioxidant capacities [12] as well as antiatherosclerotic effect [13]. However, the current usage of *C. odontophyllum* fruit is still limited to human consumption and as such, there is an urge for scientific evidence to realize the full potential of *C. odontophyllum* fruits.

Previous study on other *Canarium* species has revealed that *Canarium schweinfurthii* Engl. has high antimicrobial activity [14]. No past researches have been conducted on the antimicrobial activity of *C. odontophyllum* extract against bacteria, yeast and filamentous fungus. Thus, this is the first paper reporting on the antimicrobial activity of *C. odontophyllum*.

MATERIALS AND METHODS

Plant Materials: Fresh fruit of *C. odontophyllum* was purchased from the local market in Miri, Sarawak, East Malaysia. The fruit was authenticated and the specimens were deposited in the Herbarium Universiti Kebangsaan Malaysia in Bangi, Malaysia with voucher specimen no: UKMB 40052. Prior to analysis, the fruits were taken out of freezer, thawed at room temperature and then washed thoroughly under running tap water. The fleshy pulp and seed was manually separated using a knife. The pulp was then spread on the tray and allowed to dry at room temperature for several days. The dried pulp was ground into powder using an electrical blender.

Preparation of Extracts: The extracts were prepared in using four solvents with different degree of polarity according to Basri and Fan [15]. The dried powder was extracted using methanol, acetone, hexane and distilled water. In the ratio of 1:5, 50 g of the powdered *C. odontophyllum* were soaked each in 250 ml methanol. The mixture was then subjected to agitation using electrical shaker for 24 hours at room temperature. The mixture was then filtered using the Whatman filter paper No. 1 whereby the filtrate obtained was collected. The process was repeated using the remaining residue with 250 methanol. Both filtrates were then mixed and concentrated under reduced pressure using a rotary evaporator. The extracts obtained were finally pounded to dryness under fume hood in order to produce a crude methanol extract. The same procedure was repeated in the preparation of crude acetone and hexane extract.

In the preparation of aqueous extract, the mixture of the powdered gall in distilled water was centrifuged at 3000 rpm for 5 min. The supernatant was then collected and the whole process was repeated using the remaining residue with 250 ml distilled water. The supernatant was combined and freeze-dried at -50°C under vacuum for 12 hours in order to produce a fine crystal-like crude aqueous extract. All the extracts were stored in air-tight jars at 4°C until further use.

The methanol, acetone and hexane extracts were dissolved with 5% DMSO whereas the aqueous extract was dissolved in sterile distilled water to final concentration of 25, 50, 75 and 100 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-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 259220), two Gram-positive bacteria (*Streptococcus aureus* ATCC 25923 and Methicillin-Resistant *Staphylococcus aureus* ATCC 33591), three yeast strains (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 64677 and *Candida krusei* ATCC 6258) and two filamentous fungus (*Aspergillus fumigatus* ATCC 204305 and *Aspergillus niger* ATCC 6275). All the bacterial strains were grown and maintained on Mueller Hinton agar (MHA) slants whereas the fungus were grown on Potato Dextrose agar (PDA) and were incubated for 24 hours for bacterial and yeast strains and three to four days for filamentous fungus.

The inoculum size of all the microorganisms was standardized using spectrophotometer. The optical density of the bacterial and yeast suspension was adjusted to turbidity at absorbance (A) reading within the range of 0.08 at 625 which corresponded to 10^8 cfu/ml whereas for the filamentous fungi, the same inoculum size was equivalent to absorbance (A) reading adjusted within the range of 0.09 to 0.13 at 530 nm.

Screening of Antimicrobial Activity: The extracts from the pulp of *C. odontophyllum* were subjected to antibacterial as well as antifungal screening by agar well diffusion method [16]. MHA plates were inoculated with the tested bacteria by spreading the standardized inoculum on the surface of the agar plate with sterile swab. On the other hand, PDA was used as the seed medium for antiyeast screening whereas Mueller-Hilton incorporated with glucose Methylene Blue agar plate was uniformly seeded with filamentous fungus. Wells of diameter 5 mm were punched onto the inoculated agar plate with sterile Pasteur pipette and filled with 20 µl of each of the extract solutions 25, 50, 75 and 100 mg/ml. Gentamicin disc (10 µ/ml) served as positive control whereas the well containing 5% DMSO alone was used as a negative control for antibacterial assay. As for antifungal screening, amphotericin B disc (20 µg/mL) and distilled water were used as positive and negative control, respectively. All the plates were incubated at 37°C for 24 hours (bacteria and yeast) and 36 hours (filamentous fungi). The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition in mm from observation of clear zones formed surrounding each wells. The bioassay was performed in triplicate in order to calculate the mean value.

RESULTS

Table 1 showed the result of the percentage yield of the crude extract using different solvents from 50 g of dry powdered sample. Out of the four extraction solvents employed, methanol was found to produce the highest yield of extract (12.46%), followed by acetone (8.72%) and hexane (6.22%). The lowest yield was recorded for the aqueous extract at 2.78%. The results obtained indicated that methanol appeared to be the best solvent in the extraction of *C. odontophyllum* whereas water extracted the least yield of the water-soluble components in the fleshy pulp of the fruit.

The data pertaining to the antimicrobial potential of hexane, acetone, methanol and aqueous extracts from *C. odontophyllum* fruit against bacteria, filamentous fungi and yeast are presented in Table 2. All the extracts from *C. odontophyllum* fruit at 25 mg/ml-100 mg/ml, were not capable of inhibiting the growth of all the bacteria tested compared to Gentamicin (10 µg/ml) with diameter zone of inhibition within the range of 18.33 ± 0.58 to 21.67 ± 0.58 mm. *C. odontophyllum* pulp extracts were also not active against filamentous fungi. However, out of the three yeast strains studied, only *C. glabrata* was susceptible towards the *C. odontophyllum* pulp extract. The acetone and hexane extracts from *C. odontophyllum* pulp displayed moderate activity against *C. glabrata*. It was shown that acetone extract from *C. odontophyllum* at concentration of 25 mg/ml-100 mg/ml, displayed inhibitory zone diameter of 8.0 ± 0.00 mm, compared to 18.0 mm ± 1.41 mm by Amphotericin B at 20 µg/mL. On the other hand, the hexane extract from *C. odontophyllum* at 100 mg/ml also weakly inhibit the growth of *C. glabrata* with inhibitory

Table 1: Percentage yield of various solvent extracts from *Canarium odontophyllum*

Solvent	Weight of powder sample (g)	Weight of sample extract (g)	Percentage of yield (%)
Hexane	50	3.1	6.22
Acetone	50	4.36	8.72
Methanol	50	6.23	12.46
Aqueous	50	1.39	2.78

Table 2: Diameter of inhibition zones of extracts from *C. odontophyllum* against tested microorganisms

		Diameter of inhibition zone (mm±SD)				
		Concentrations of extract (mg/ml)				Standard antibiotic
Bacteria	Extracts	25	50	75	100	(10 µg/ml)
	Methanol	-	-	-	-	19.67 ± 0.58
E.coli	Acetone	-	-	-	-	19.67 ± 0.58
	Hexane	-	-	-	-	19.67 ± 0.58
	Aqueous	-	-	-	-	19.67 ± 0.58
	Methanol	-	-	-	-	21.67 ± 0.58
P.aeruginosa	Acetone	-	-	-	-	21.67 ± 0.58
	Hexane	-	-	-	-	21.67 ± 0.58
	Aqueous	-	-	-	-	21.67 ± 0.58
	Methanol	-	-	-	-	18.33 ± 0.58
S.aureus	Acetone	-	-	-	-	18.33 ± 0.58
	Hexane	-	-	-	-	18.33 ± 0.58
	Aqueous	-	-	-	-	18.33 ± 0.58
	Methanol	-	-	-	-	18.67 ± 0.58
MRSA	Acetone	-	-	-	-	18.67 ± 0.58
	Hexane	-	-	-	-	18.67 ± 0.58
	Aqueous	-	-	-	-	18.67 ± 0.58
A.fumigatus	Methanol	-	-	-	-	17.0 ± 1.41
ATCC	Acetone	-	-	-	-	17.0 ± 1.41
204305	Hexane	-	-	-	-	17.0 ± 1.41
	Aqueous	-	-	-	-	17.0 ± 1.41
A.niger	Methanol	-	-	-	-	15.0 ± 1.41
ATCC	Acetone	-	-	-	-	15.0 ± 1.41
6275	Hexane	-	-	-	-	15.0 ± 1.41
	Aqueous	-	-	-	-	15.0 ± 1.41
C.albicans	Methanol	-	-	-	-	21.0 ± 4.24
ATCC	Acetone	-	-	-	-	21.0 ± 4.24
90028	Hexane	-	-	-	-	21.0 ± 4.24
	Aqueous	-	-	-	-	21.0 ± 4.24
C.krusei	Methanol	-	-	-	-	18.0 ± 2.82
ATCC	Acetone	-	-	-	-	18.0 ± 2.82
6258	Hexane	-	-	-	-	18.0 ± 2.82
	Aqueous	-	-	-	-	18.0 ± 2.82
C.glabrata	Methanol	-	-	-	-	18.0 ± 1.41
ATCC	Acetone	8.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.00	18.0 ± 1.41
64677	Hexane	-	-	-	7.0 ± 0.00	18.0 ± 1.41
	Aqueous	-	-	-	-	18.0 ± 1.41

The concentration of Gentamicin and Amphotericin used was 10 µg/ml and 20µg/mL, respectively against bacteria and fungi.

-: No inhibition zone

zone diameter of 7.0 ± 0.00 mm. This means that hexane extract from *C. odontophyllum* was capable of inhibiting the growth of *C. glabrata* only at the highest concentration tested.

DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop more effective medicines in controlling the growth of microorganism with less toxicity [17]. Throughout the history of mankind, many infectious diseases have been treated with plant extracts. The screening of crude extracts from medicinal plants has shown that some of the screened plants are potentially rich sources of antibacterial and antifungal agents [18]. Antifungal activity of medicinal plants has been reported

from the extract of *Periploca aphylla*, *Ficus sarmentosa* 4 and *Periploca aphylla* against the growth of *Aspergillus flavus, Aspergillus niger, Trichoderma harzianum* and *Fusarium oxysporum* [19].

In the present study, various organic solvents with increasing order of polarity were used for extraction of the fruits of *C. odontophyllum*. The ability of a solvent to dissolve the bioactive ingredients from the extract was then evaluated by calculating the percentage yield of each extract. The yield of the *C. odontophyllum* extract was the highest when methanol was used as the extracting solvent compared to the rest of the solvents. The higher percentage yield of methanol extract indicated that methanol was the best solvent that can dissolved many of the polar active compounds found in the fruits of *C. odontophyllum*. The polarity of methanol and the solubility of plant secondary metabolites in methanol could be the probable reason for the high extractive value of methanol extract [20]. The higher percentage yield obtained using a more polar solvent in comparison to a less polar solvents indicated that the test plant materials were found to possess greater number of polar compounds than non-polar components. This is supported by Azlan *et al.* [11] methanol is the best medium to dissolve most of the phenolic compounds from the defatted *C. odontophyllum*. Low percentage yield of the aqueous extract *C. odontophyllum* indicated that the use of distilled water as solvent may not be able to dissolve the active compound from the plant. It is clear that using organic solvents provided a higher efficiency in extracting compounds for antimicrobial activities compared to water based method. However, greater efficiency in the extraction of solutes is not directly related to greater inhibition [21]. Methanol extract have no ability to extract antimicrobial compound from *C. odontophyllum* since the extract did not exhibit inhibitory effect on the tested bacteria, filamentous fungus and yeast. Interestingly, the lowest extractive yield by distilled water also demonstrated no antimicrobial activity. Previous research suggested that the chemical characteristics of the solvent, the method used during the extraction process and diverse structural and compositional aspects of the natural products result in each material solvent system showing distinct behavior [21].

Results obtained from the screening of antimicrobial activity indicated that acetone and hexane extracts of *C. odontophyllum* did not show any inhibitory effect against all bacteria and filamentous species tested. These extracts were capable of inhibiting the growth of *C. glabrata*. This finding showed that hexane and acetone are useful extractant solvent that have potential to extract active compound from *C. odontophyllum* against *C. glabrata.* Semi polar compound such as acetone are reported to exhibit high antimicrobial activity. Acetone was the best extractant, since it dissolved the active antiyeast compounds from many of the test plants and is low in toxicity to the test organisms [22]. Acetone is a useful extractant because it dissolves wide range of active compounds from plants including both hydrophilic and hydrophobic components [23].

The results indicated that all four extracts of *C. odontophyllum* were not able to inhibit the growth of MRSA, *S. aureus*, *P. aeruginosa* and *E. coli*. The presence of large molecules in the extract itself is one of the reasons why an extract cannot diffuse well through the agar [24]. In addition, the hydrophobic nature of most plant extracts may prevents the uniform diffusion of these substances through the agar medium [25]. As a result, the zone of inhibition was difficult to develop.

The absence of antimicrobial activity may be due to the present of flavonoid sedimentation or the action of flavonoid itself in the extracts. Analysis on phenolic compound in *C. odontophyllum* using HPLC revealed that there are two phenolic acids (ellagic and vanillic acid), five flavonoids (catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, apigenin), three anthocyanidins (cyanidin, pelargonidin and delphinidin), four anthocyanins (malvidin-3,5-di-O-glucoside, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside and peonidin-3-O-glucoside) and ethyl gallate [26].

The occurrence of sedimentation of flavonoids is likely to cause diminished contact between bacterial cells and flavonoid molecules. Consequently, this could be misinterpreted as bacterial growth. Thus, this may lead to false negative reports of antimicrobial activity [27]. Other than that, the mechanism action of active compounds in extract that is not killing the bacterial cells is also one of the reasons why there is the MIC assay showed negative results. Some of active compounds can cause damage to cytoplasmic membrane of bacteria cell and inducing the formation of bacterial aggregates instead of directly killing the bacteria hence, resulting in the reduction of viable counts [28].

From the findings of this study, the results obtained from the antimicrobial sensitivity test indicated that all four extracts of *C. odontophyllum* did not have any antimicrobial activity against MRSA, *S. aureus*, *P. aeruginosa* and *E. coli*. This result is comparable to the study by Alam *et al*. (2011) using methanol extract of

Terminalia belerica Roxb. fruits that showed antibacterial activity against *S. aureus, P. aeruginosa* and *E.coli* [29]. Uddin and Rauf (2012) showed that methanol fraction of *Pistacia integerrima* bark was active towards *S. aureus* [30]. These negative results indicated that that the pulp of *C. odontophyllum* possessed no bioactive constituents. The absence of antimicrobial activity may be due to the solvent extractions used in this study was not able to dissolve the active compound with antimicrobial properties from *C. odontophyllum* extracts. Thus, the extracts produced did not contain antimicrobial active compound.

Other than that, the interference of activity in certain active compounds by other compounds that are also present in the same extract may be the other contributing factor to the absence of antimicrobial activity. Alternatively, if the active compound is present in high quantity, there could be other constituents exerting antagonistic effects or positive effects of the bioactive agents. Moreover, there could be numerous compounds present in the extracts that were produced using a single extraction method compared to sequential method [31].

The interesting finding in this study was that the increasing concentration of acetone extract from 50 mg/mL to 100 mg/mL does not affect the inhibitory activity of the growth of *C. glabrata*. The inhibitory activity of the extracts was not concentration-dependent. This finding was in accordance with previous work which had reported that antibacterial effect of alcoholic extracts of some common spices was also not concentration-dependent [32]. Furthermore, hexane extract only showed inhibitory activity at highest concentration which is at 100 mg/mL. In fact, the crude extracts of plant usually have lower antimicrobial activity as compared to pure antibiotic [33]. The aqueous extract did not exhibit antimicrobial activity against all tested organisms. This was also reported by Arunkumar and Muthuselvam (2009), that the aqueous extract of *Aloe vera* leaves did not show any positive results against *S. aureus, P. aeruginosa, E. coli* and *A. niger* [34]. Alcohol is a general solvent and tends to provide a more complete extraction of compounds with a variety of polarities. Thus, aqueous extracts may not contain some of the less polar compounds. It is also possible that climate variation and seasonal discrepancy regarding the harvesting of the plants may account for some of the differences observed. The quantity and quality of active compounds will vary depending on the growing conditions. Besides, storage also can affect the active compound in the extract [35].

Result of screening of antifungal activity of *C. odontophyllum* extracts showed that out of all tested yeast and filamentous fungus, only *C. glabrata* was susceptible to acetone and hexane extract. This finding revealed that plant extract have high potential to resist the growth of *C. glabrata* and was in accordance with previous work which had reported that the aqueous, methanol, ethanol, acetone, ethyl acetate extract of galls of *Quercus infectoria* have the ability to inhibit the growth of *C. glabrata* [36].

Canarium odontophyllum, known as "*dabai*" is classified as an underutilized fruit, popular in Sarawak, Malaysia. There is no previous study that reported about the antimicrobial activity of *C. odontophyllum* fruits. In the present study, Chew *et al.* [37] reported that *C. odontophyllum* fruit were found containing flavonoid (catechin, epicatechin, epicatechin gallate, epigallocatechin gallate and apigenin) and anthocyanidin (cyanidin, pelargonidin and delphinidin). Flavonoids are ubiquitous in photosynthesising cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have been proposed for use against fungal pathogen in human [38]. Present of flavonoid in *C.odontophyllum* implied that flavonoid may be the active compound responsible for the antifungal activity in this study.

CONCLUSION

From the present study, it was concluded that methanol, acetone, hexane and aqueous extracts of *C. odontophyllum* did not exhibit any antimicrobial activity. Thus, *C. odontophyllum* is not effectively used as alternative phyotherapeutic agents to treat nosocomial causing bacteria. However, further evaluation for identification of bioactive compounds present in each extracts of *C. odontophyllum* by gas chromatography-mass spectrometry (GS-MS) analysis should be done.

The results obtained from this study showed hexane and acetone extracts of pulp of *C. odontophyllum* exhibit antifungal effects against *Candida glabrata.* In conclusion, *Canarium odontophyllum* has the potential to be developed as anti-yeast agent. However, further

investigations are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity.

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