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Original Article

THE POTENTIAL OF CANARIUM ODONTOPHYLLUM MIQ. (DABAI) AS ANTI-METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AGENT

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ABSTRACT

Objective: The present study evaluates the antimicrobial potential of *C. Odontophyllum* leaves against Methicillin-resistant *Staphylococcus aureus, Candida albicans, Candida glabrata, Candida krusei, Candida tropicalis*, *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus flavus*.

Materials: The extracts from *C. odontophyllum* leaf were prepared using acetone, methanol and distilled waterprior to screening at concentrations from12.5 mg/ml to 100 mg/ml against the test 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.

Results: Water produced the highest yield of extract (5.03%) followed by methanol (2.65%) and acetone (1.79%). Out of all the microbes tested, only MRSA was found to be susceptible towards acetone and methanol extracts of *C. odontophyllum* leaves which showed concentration-dependent growth inhibitory effect against MRSA. Despite the highest extractive potential of water, no antimicrobial activity was observed by the aqueous extract from the screening assay. The MIC values for methanol and acetone extracts were respectively,6.25 mg/ml and 3.125 mg/ml. The MBC value of methanol extract was twice its MIC value which was 12.5 mg/ml whereas the MIC and MBC values of acetone extract against MRSA were the same (3.125 mg/ml).

Conclusion: *C. odontophyllum* leaves have the potential to be developed as an alternative phytotherapeutic agent against MRSA infection.

Keywords: *Canarium odontophyllum*, Antimicrobial, Disc diffusion, MRSA, MIC, MBC.

INTRODUCTION

Canarium odontophyllum Miq. is a species in the family of Burseraceae and commonly known as "dabai". It is one of the popular yet underutilized fruit of Sarawak, Malaysia [1]. The fruit is highly seasonal and only available during the months of October-December[2]. The fruit has been dubbed as "Sibu olive" because its physical appearance, smooth texture and rich flavor are similar to olive fruit. The fruits of *C. odontophyllum* are oval in shape and very nutritious with high content of lipid, carbohydrate, protein and mineral such as potassium, phosphorus, calcium, and magnesium [3]. The fruits are rich in beneficial nutrients which contain high levels of total phenolics, flavonoid and anthocyanin associated with antioxidant property [4]. Extract from peel, pulp and kernel of *C. odontophyllum* have consistently showed antioxidant capacity [5], antifungal activity [6] as well as antiatherosclerotic effect [7]. However, the current research on *C. odontophyllum* is still limited to its fruit and as such, there is an urge for scientific evidence to realize the full biomedical potential of the leaves from *C. odontophyllum*. However, based on previous studies on the other *Canarium* species such as *Canarium album* [8], *Canarium patentinervium* [9] and *Canarium schweinfurthii* [10], the leaves from *C. Odontophyllum* contain bioactive compounds such as terpenoid, tannin, saponin, flavonoid and phenolic compounds that can produce antimicrobial property. Unfortunately to date, no pharmacological activity study has been done on the leaves of *C. odontophyllum* and this is the first report that investigated the potential of the leaves from *C. odontophyllum* as a potential source of anti-MRSA and anti fungal agents.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Canarium odontophyllum* were obtained from Sarawak, Malaysia and were authenticated at the Unit Herbarium, Universiti Kebangsaan Malaysia, Malaysia with voucher specimen no. UKMB 40052. The leaves were dried in the oven and grinded into powdered form using electric grinder. All the extracts used in this study were prepared from the powdered *C. odontophyllum* leaves based on our previous literature [11]and the percentage extraction yield was recorded. The extracts were dissolved in their respective solvent to a final concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml for disc diffusion method and 100mg/ml for broth microdilution method.

Microorganisms

The microorganisms used in this study were Methicillin-resistant *Staphylococcus aureus* ATCC 33591, four yeast strains (*Candida albicans* ATCC 90028, *Candida glabrata* ATCC 64677, *Candida krusei* ATCC 6258, and *Candida tropicalis* MM 13001532) and three filamentous fungi (*Aspergillus fumigatus* ATCC 204305, *Aspergillus niger* MM 895 and *Aspergillus* flavus MM 1938). These standard bacterial and fungal strains were obtained from Novel Antibiotic Laboratory, Universiti Kebangsaan Malaysia. The bacterial strains were grown and maintained on nutrient agar slants while fungal strains were grown and maintained on Sabouraud dextrose agar. Inoculum size of each microorganism for disc diffusion assay was standardized using spectrophotometer by adjusting the optical density of the bacterial and yeast suspension, respectively, at turbidity corresponding to an absorbance reading of 0.08 at 625 and 520 nm. As for the filamentous fungi, the inoculum size was equivalent to absorbance reading adjusted to 0.08 at 530 nm.

Screening for antibacterial activity

The screening of antimicrobial activity of *C. odontophyllum* leaves were carried out using disc diffusion method [12]. Mueller-Hinton agar plates were uniformly seeded with bacteria whereas Mueller-Hinton agar incorporated with glucose methylene blue and potato dextrose agar were used for screening against yeast and filamentous fungi, respectively. Each test plate comprised of six discs; standard antibiotic disc Vancomycin 30 µg and Amphotericin B 20 µg, respectively for MRSA and fungi as positive control, extraction solvent as negative control and four discs saturated with the extract, each at different concentrations. The plates were then incubated at 30ºCfor 36 hr for fungal strains and 37ºC for 24 hr for MRSA and the diameter of inhibition zones measured. The test was repeated three

times to ensure reliability with each extract assayed in triplicate in order to calculate the mean ± SD value.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC value of the extracts was determined using the two-fold serial microdilution method in 96-well microtiter plate[13]. It will only be carried out on microorganisms that are susceptible towards the extract in the disc diffusion test. The lowest concentration that inhibits the growth of microorganisms was considered as the MIC value. The minimum bactericidal concentration (MBC) was determined by subculture of the well showing no apparent growth in a sterile agar plate. The least concentration showing no visible growth on agar subculture was taken as MBC value.

Statistical Analysis

SPSS version 20.0 and Kruskall-Wallis analysis were used for statistical comparison of the mean ± SD value for inhibition zone obtained from extract and positive control.

RESULTS

In the extraction of *C. odontophyllum* leaves, distilled water produced the highest percentage of extraction yield followed by methanol and acetone. Aqueous extract was obtained from 5.03% of dried powdered leaves while methanol and acetone extracts were respectively, produced from 2.65% and 1.79% of the *C. odontophyllum* leaves.

Inhibitory effect of *C. odontophyllum* leaf extracts against tested microorganisms

Table 1 showed that only MRSA was susceptible towards acetone and methanol leaf extracts from *C. odontophyllum*. Acetone and methanol extracts displayed concentration-dependent inhibition against the growth of MRSA. As shown in Table 2, an increase in the diameter zone of inhibition from 6.67±0.58 mm,7.33± 0.58 mm,9.67 ± 0.58 mm and 14.00 ± 1.00 mm was respectively observed at 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml by the methanol extract. On the other hand, acetone showed a bigger inhibitory zone compared to MeOH extract from 7.33± 0.58 mm,8.33± 0.58 mm, 10.33 ± 0.58 mm and 16.00 ± 1.00 mm as respectively recorded at 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml. However, there was no significantly difference in the size of the inhibition zone between both extracts. Vancomycin (0.03 mg) as the standard antibiotic displayed inhibition zone of 18.00 ± 0.00 mm against MRSA.

Antimicrobial activity of *C. odontophyllum* leaf extracts against MRSA

The MIC and MBC values of the acetone and methanol extracts from *C. odontophyllum* leaves against MRSA was presented in Table 3 and Table 4, respectively. The MIC value of acetone extract was 3.125 mg/ml whereas the MIC value of methanol extract was 6.25 mg/ml. Interestingly, MBC value for acetone extract was the same as its MIC value. As for methanol extract, MBC value was 12.5 mg/ml which was twice its MIC value.

Table 1: **Inhibition zones diameter of extracts (12.5 mg/mL- 100 mg/mL) from *C. odontophyllum* leaves against fungus and MRSA ATCC 33591**

Microorganisms	Diameter of inhibition zone (mm)				
	Extract			Control	
	Aqueous	Methanol	Acetone	Positive	Negative
C. albicans ATCC 90028	-	-	-	26.00 ± 0.00	-
C. glabrata ATCC 64677	-	-	-	24.00 ± 0.00	-
C. krusei ATCC 6258	-	-	-	16.00 ± 0.00	-
C. tropicalis MM 13001532	-	-	-	18.00 ± 0.00	-
A. fumigatus ATCC 204305	-	-	-	25.00 ± 0.00	-
A. niger MM 895	-	-	-	22.00 ± 0.00	-
A. flavus MM 1938	-	-	-	23.00 ± 0.00	-
MRSA ATCC 33591	-	+	+	18.00 ± 0.00	-

(+) Inhibition of bacterial growth, (-) No inhibition of bacterial growth, Positive control comprises Vancomycin (30 µg/disc) for MRSA and Amphotericin B (20 µg/disc) for yeast and filamentous fungus, Negative control comprises respective extraction solvent

Table 2: Mean diameter of inhibition zones of methanol and acetone extracts from *C. odontophyllum* leaves against MRSA ATCC 33591

Final Concentration of extracts in the disc[c]	Mean Zone of Inhibition[a] (mm)[b]	
	Methanol extract	Acetone extract
100	14.00 ± 1.00	16.00 ± 1.00
50	9.67 ± 0.58	10.33 ± 0.58
25	7.33± 0.58	8.33± 0.58
12.5	6.67± 0.58	7.33± 0.58
Vancomycin (30 µg/disc)	18.00 ± 0.00	

[a] – diameter of zone inhibition (mm) including the disc diameter of 6 mm, [b]- mean of three assays ± standard deviation, [c]- sterile disc was saturated with the stated concentration

DISCUSSION

In general, the percentage of extraction yield is higher in polar solvent than solvent with lower polarity. Distilled water resulted in a higher yield compared to acetone and methanol which is in agreement with previous studies that reported the extraction yield from the leaves of *Orthosiphon stamineus* [14] and *Camellia sinensis* [15]. This could well suggest that most of the extracted compounds from *C. odontophyllum* leaves are highly polar. In accordance with this, water is more effective in extracting the solute because it has higher polarity and shorter chain [16].

From the disc diffusion assay, acetone and methanol extracts were able to inhibit the growth of MRSA whereas no anti-MRSA activity was observed by the aqueous extract. It is noteworthy to highlight from this finding that despite showing the lowest extractive yield, acetone was capable of extracting an active antimicrobial phyto component from the leaves of *C. odontophyllum*. In fact, acetone extract showed a stronger inhibitory effect against MRSA compared to methanol extract as can be observed from the larger size of the diameter zone of inhibition on agar plates although this difference was not significant. This means that the total amount of active component extracted by acetone was much higher compared to methanol [17]. In addition, acetone has proved efficient in extracting flavonoid [18,19]and hence,the presence of flavonoid could possibly contribute to the anti-MRSA effect of the*C. Odontophyllum* leaves. This is supported by TLC analysis on the leaves from *Jatropha*

gossypiifolia that suggested flavonoids could be the major compounds in the extract [20]. As far as the flavanone structure is concerned, it is the hydroxyl group at position 5 which is important for their activity against MRSA [21].

A correlation study between antibacterial activity and membrane interference evidenced that flavonoids may demonstrate antibacterial activity by reducing membrane fluidity of bacterial cells [22].

Table 3: **Determination of MIC values of acetone and methanol extracts of *C. odontophyllum* leaves against MRSA ATCC 33591**

Concentration of the extracts	MIC			
	Extract		Control	
	Acetone	Methanol	Positive	Negative
25	-	-	+	-
12.5	-	-	+	-
6.25	-	-	+	-
3.125	-	+	+	-
1.563	+	+	+	-
0.781	+	+	+	-
0.391	+	+	+	-
0.195	+	+	+	-
0.098	+	+	+	-
0.049	+	+	+	-

(-) Absence of growth, clear well (+) Presence of growth, turbid well, Positive control comprises bacterial suspension and Mueller-Hinton broth, Negative control comprises Vancomycin and Mueller-Hinton broth

Table 4: **Determination of MBC values of extract of*C. odontophyllum* leaves against MRSA ATCC 33591**

Concentration of the extracts (mg/ml)	MBC	
	Acetone	Methanol
25	-	-
12.5	-	-
6.25	-	+
3.125	-	+
1.563	+	+
0.781	+	+
0.391	+	+
0.195	+	+
0.098	+	+
0.049	+	+

(-)Absence of growth, (+) Presence of growth

The present study showed that the acetone extract exerted bactericidal effect against MRSA where as methanol extract merely exhibited growth inhibitory action against the bacteria. This difference in the mechanism of antimicrobial activity is probably due to the interaction of variety of phytocomponents working in antagonism that account for the less susceptibility of MRSA towards the methanol extract from *C. odontophyllum* leaves. In addition to flavone, methanol is also capable of extracting other active constituents which are polar in nature such as tannin, saponin, terpenoid and anthocyanin [18].

As such, it is possible that antagonistic relationship among the phytochemicals would affect the efficacy of crude extracts [23]. Therefore, further study to confirm and identify the type of flavonoid in acetone extracts from the leaf of *C. odontophyllum*is strongly recommended to understand its bactericidal mode of anti-MRSA action.

CONCLUSION

The leaf extracts of *C. odontophyllum* have the potential to be developed as an alternative phytotherapeutic agent against MRSA infection. However, *C. odontophyllum* leaf extract specifically the acetone extract should be analyzed further as it may provide a new lead that might be effective against multi-drug resistant *S. aureus*.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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