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Phytoconstituent Screening and Antibacterial Activity of the Leaf Extracts from *Canarium odontophyllum* Miq.

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Abstract

Canarium odontophyllum **is one of the underutilized fruits among the local community in Sarawak, Malaysia. The leaf extracts from** ***C. odontophyllum*** **(6.25 mg/ml to 50 mg/m) were screened against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Extraction of the *C. odontophyllum* leaves using methanol produced the highest yield (5.46%) followed by water (4.0%) and acetone (2.83%) whereas hexane extracted the lowest yield (0.98%). Out of the four bacterial species tested, only *S. aureus* was found to be susceptible towards the *C. odontophyllum* leaves extract. Screening result using agar well diffusion method showed that the antimicrobial activity of the acetone and methanol extracts from *C. odontophyllum* leaves was concentration-dependent towards the growth inhibition of *S. aureus*. The MIC values for both MeOH and acetone extracts were 0.391 mg/ml. The MBC value of MeOH extract was twice its MIC value whereas the MBC and MIC values of acetone extract against *S. aureus* were the same. Phytochemical analysis showed that acetone, MeOH and water extracts contain flavonoid, tannin, terpenoid and phenol. These findings conclude that the leaves of *C. odontophyllum* may contain therapeutically-useful compounds against *S. aureus*, which are mostly concentrated in the acetone extract. As such, *C. odontophyllum* have the potential to be developed as an alternative treatment against *Staphylococcus aureus*-associated skin and soft tissue infections.**

Keywords

Leaves, *Canarium odontophyllum*, Flavonoid, Saponin, Antibacterial, Agar Well Diffusion, *Staphyloccocus aureus*, MIC, MBC

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1. Introduction

Infectious diseases are transmissible diseases that comprise clinically evident illnesses which result from infection of pathogenic biological agents in an individual host organism. Infectious diseases represent a leading cause of morbidity and mortality worldwide, accounting for more than 26% of all death with developing countries carrying the major burden [1]. Pathogenic biological agents include some viruses, bacteria, fungi, protozoa and multicellular parasites that have the ability to interact with human being by creating a community and cause an infection which reflected in possession of certain pathogenic factors [2].

Nosocomial infection is known to be a major cause of death and increased morbidity among hospitalized patients. It was reported that the most frequent nosocomial infections are infection of surgical wound, urinary tract and lower respiratory tract infection commonly associated with *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [3]. Besides nosocomial infection, foodborne diseases especially diarrheal diseases, are also an important cause of morbidity and mortality that occur worldwide caused by bacteria such as *Bacillus cereus*. According to the World Health Report [4], 1.8 million childhood death annually due to acute diarrheal illness which is also a very common illness in developed countries.

Antimicrobial agents or antibiotics are used to prevent, control and reduce the occurrence of the infection. Antibiotics are semi-synthetic or synthetic substances produced by the natural metabolic processes of some microorganisms that can inhibit or destroy other microorganism. The greatest number of antibiotics is derived from bacteria from the genera *Streptomyces* and *Bacillus* and molds from genera *Penicillium* and *Cephalosporium* [5] whereas only 7% of antimicrobial metabolites are plant-derived compounds [6]. Currently, the phenomenon of antibiotic resistance is the greatest challenge on the treatment of bacterial infection. The number of bacteria that are resistant toward these antibacterial agents has increased each year where 70% of the bacteria that cause infections in hospitals are resistant to at least one of the most commonly antibiotic agents [7] [8]. As such, new sources of antimicrobial agents need to be discovered and it has become a worldwide challenge. Many scientists from academic institutions and pharmaceutical companies have made an effort to find and discover novel, safe and effective biologically active compound. One of the approaches is by testing the compound derived from the plant origin.

Plants are found to be an enormous source for variety of bioactive compounds with diverse molecular structure and function. These molecules are primarily derived from the secondary metabolism of plants and were used to protect it against predation by microorganisms, insects and herbivorous [9]. Antibacterial secondary metabolites are usually classified in three large molecule families which are phenolic, terpene and alkaloid [10]. The use of plant as traditional medicine has been discovered for thousands of years and was passed down from generation to generation all around the world. It was estimated that about 80% of the population in most Asian and African countries used traditional medicine as their part of standard healthcare [11]. Nowadays, physicians have been prescribing many drugs that are either directly isolated from plant or are artificially modified version of natural product. The use of these types of medicine as a safe remedy for diseases of both microbial and non-microbial origin has been supported by World Health Organization [12].

Canarium odontophyllum Miq. belonging to Burseraceae family is a tree that can be found in tropical rainforest of Sarawak, Malaysia. It bears fruit pulp known as “dabai” which is blue-black in colour when ripe and is famous among the people in Sarawak. This fruit is rich in mineral, protein, carbohydrate and fat with high level of total phenolic, flavonoid and anthocyanin which is related to its antioxidant activity [13] [14]. In our recent report, it was shown that the pulp extract from *C. odontophyllum* did not possess antibacterial activity but exhibited antifungal effect against *Candida glabrata* [15]. Unfortunately, no research has been done on the leaves of *C. odontophyllum* and this is the preliminary report on the antimicrobial activity from the leaves of *C. odontophyllum*.

Therefore, the present study aimed at evaluating the antimicrobial potential of crude extracts from the leaves of *C. odontophyllum* against two Gram-positive bacteria and two Gram-negative bacteria.

2. Materials and Methods

2.1. Plant Material

Fresh leaves of *Canarium odontophyllum* were obtained from Sarawak, Malaysia and was deposited at the Herbarium Universiti Kebangsaan Malaysia in Bangi, Malaysia with voucher specimen no. UKMB 40052. The

leaves were dried in the oven and grinded into powdered form using electric grinder. The preparation of extracts from *C. odontophyllum* leaves was adopted from Basri and Fan [16].

2.2. Preparation of Organic Extracts

The powdered *C. odontophyllum* leaves were sequentially extracted by three organic solvents based on the different order of polarity. The hexane extract was prepared by immersing 100 g of the dried material in 500 ml hexane and shaked at 100 rpm for 24 h at 50˚C. The mixture was then filtered through Whatman No. 1 filter paper. The residue was further extracted twice by adding 300 ml of fresh solvent each time, after then all the filtrate were combined together. The remaining residue were air-dried and further extracted with acetone, followed by methanol by similar procedure carried out for hexane extraction. The solvent from the combined filtrate was evaporated using rotary evaporator until it formed a pellet. Finally the pellet was pounded to dryness under hot air-dryer to remove the remaining solvent. The final yield of each extract was weighed and stored at 4˚C until further use.

2.3. Preparation of Aqueous Extract

In the preparation of aqueous extract, 100 g of the powdered leaves were macerated in 500 ml distilled water and shaked at 100 rpm for 24 hr at room temperature. Then the mixture was centrifuged at 3000 rpm for 5 minutes. The supernatant was then filtered and the whole process were repeated using the remaining residue with 300 ml distilled water. The filtrates were combined and freeze-dried at −50˚C under vacuum for 24 h to produce a fine crystal-like crude aqueous extract. The extract was weighted and stored in air-tight jar at 4˚C until further use.

2.4. Preparation of Extract Solution

The extracts were dissolved in their respective solvent to a final concentration of 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml and 50 mg/ml for agar-well diffusion method and 100 mg/ml for broth microdilution technique.

2.5. Microorganisms

The bacterial species used in this study were two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 6633) and two Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). All the bacterial strains were grown and maintained on nutrient agar slants. The inoculum size of each test strain was 10^8 CFU/ml for agar well diffusion assay which was standardized by adjusting the optical density of the bacterial suspension to a turbidity corresponding to spectrophotometric absorbance of 0.08 (OD_{620} = 0.08) at 620 nm.

2.6. Screening for Antibacterial Activity

The screening of antibacterial activity of *C. odontophyllum* leaves was carried out using agar-well diffusion method based on [15]. In this assay, Mueller-Hinton agar plates were used for the growth of each bacterial species. Each plate was uniformly seeded with bacteria by dipping in the standardized suspension with sterile swab and streaking it on the surface of the agar plate. The wells of 5 mm in diameter were punched into the inoculated agar media with sterile Pasteur pipette. Each plate for each tested extract comprised six wells to accommodate the extract concentrations at 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml as well as for positive and negative controls. Approximately, 60 µl of the extract solution were dropped into each well, respectively. For positive control, vancomycin (30 µg/ml) was used against *S. aureus*, whereas gentamicin (30 µg/ml) was used for *B. cereus*, *P. aeruginosa* and *E. coli.* The extraction solvent for each extract served as negative control. The plates were pre-incubated for 1 hr at room temperature, allowing the complete diffusion of the samples before incubated at 37˚C for overnight. The antibacterial activity was determined by measuring the diameter of inhibition zone surrounding the well. Each experiment was repeated in triplicate in order to calculate the mean value ± SD value.

2.7. Determination of MIC and MBC Values

Minimum inhibitory concentration (MIC) value of the extracts was determined against the bacterial strains using

the two-fold serial microdilution method carried out performed in 96-well microtiter plate according to Basri and Khairon [17]. This procedure will only be performed on the extract that showed inhibitory growth against any tested bacteria in agar well diffusion screening test. Initially, the tested extract was added into the well containing the Mueller-Hinton broth followed by addition of the standardized bacterial suspension to make up the final concentration ranging from 25 mg/ml to 0.049 mg/ml. Each extract was assayed in triplicate. The extract in the broth was used as negative control to ensure medium sterility while the bacterial suspension served as positive control to control the adequacy of the broth for bacterial growth. To visualize the cell viability, 20 µl of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, 1 mg/ml) and 20 µl of 2,3,5-triphenyltetrazolium chloride (TTC, 2 mg/ml) were added into each well containing respectively, Gram-positive and Gram-negative bacterial strains. 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 the MBC value.

2.8. Phytochemical Analysis

The aqueous, acetone and methanol leaf extracts of *C. odontophyllum* were screened for the presence of phytoconstituents based on [18].

3. Results

Table 1 showed the percentage yield of hexane, acetone, methanol and water of *C. odontophyllum* leaf extracts. In the extraction of *C. odontophyllum* powdered leaves, methanol showed the highest percentage of extraction yield followed by aqueous and acetone extracts, respectively. Methanol extract was produced from 5.46% of dried powdered leaves while aqueous and methanol each produced 4.0% and 2.83%. Hexane appeared to produce the lowest extraction yield of only 0.98% from the dried powdered leaves.

From the result of antibacterial screening assay (**Table 2**), only *Staphylococcus aureus* was susceptible towards acetone and methanol extracts of *C. odontophyllum* leaves. Hexane and aqueous extracts were not capable of inhibiting the growth of all the bacteria tested. **Figure 1** illustrated the diameter zones of inhibition by the extracts against *S. aureus*. It can be seen from **Table 3** that the mean diameter inhibition zone for methanol extract at 6.25 - 50 mg/ml was 7.00 ± 0.00 to 22.33 ± 0.58 mm whereas acetone extract (6.25 - 50 mg/ml) recorded bigger mean inhibition zone from 12.00 ± 0.00 to 24.33 ± 0.15 mm. Both acetone and methanol extracts at 25 mg/ml displayed stronger inhibitory effect against *S. aureus* (20.33 ± 0.58 mm and 18.67 ± 0.58 mm, respectively) compared to the positive control (vancomycin 0.03 mg/ml) with inhibition zone of 15.00 ± 0.00 mm. However, acetone extract exhibited significantly bigger ($p < 0.05$) inhibition zone than the methanol extract at all concentrations studied. The MIC values of the acetone and methanol extracts from *C. odontophyllum* leaves against *S. aureus* were shown in **Table 4**. Interestingly, both extracts were found to exhibit similar MIC values against *S. aureus* (0.391 mg/ml). The MIC values of both extracts correlated to the screening test result. The standard drug used in the study, vancomycin showed MIC value of 0.0075 mg/ml (**Table 5**). This means that the MIC values of acetone and methanol extract were about 50 times less potent than standard antimicrobial agent. The MBC values of the methanol and acetone extracts from *C. odontophyllum* leaves against *S. aureus* were tabulated in **Table 6**. The MBC value for acetone extract was the same as its MIC value which is, 0.391 mg/ml. This showed that the acetone extract have bactericidal effect against *S. aureus*. As for methanol extract, the MBC value was slightly higher (0.781 mg/ml) compared to MIC value (0.391 mg/ml) that indicated the methanol have bacteriostatic effect on the bacteria.

The result of phytochemical screening of the extracts of *C. odontophyllum* leaves was shown in **Table 7**. The

Table 1. Extraction yield of *C. odontophyllum* leaves using various solvents.

Extraction solvent	Fresh sample weight (g)	Extract weight (g)	Percentage of yield (%)
Hexane	100	0.98	0.98
Acetone	100	2.83	2.83
Methanol	100	5.46	5.46
Water	100	4.0	4.0

Table 2. Antimicrobial activity of extracts from *C. odontophyllum* leaves (6.25 mg/ml - 50 mg/ml) against four bacterial species.

Extract	Extract concentration (mg/mL)	Diameter of inhibition zone (mm)			
		S. aureus	*B. cereus*	*E. coli*	*P. aeruginosa*
Hexane	50	–	–	–	–
	25	–	–	–	–
	12.5	–	–	–	–
	6.25	–	–	–	–
Acetone	50	24.33 ± 1.15	–	–	–
	25	20.33 ± 0.58	–	–	–
	12.5	17.33 ± 0.58	–	–	–
	6.25	12.00 ± 0.00	–	–	–
Methanol	50	22.33 ± 0.58	–	–	–
	25	18.67 ± 0.58	–	–	–
	12.5	12.33 ± 0.58	–	–	–
	6.25	7.00 ± 0.00	–	–	–
Water	50	–	–	–	–
	25	–	–	–	–
	12.5	–	–	–	–
	6.25	–	–	–	–
Control positive	Vancomycin (30 µg/ml)	15.00 ± 0.00	–	–	–
	Gentamisin (10 µg/ml)	–	22.00 ± 0.00	22.00 ± 0.00	29.00 ± 0.00
Control positive	Hexane	–	–	–	–
	Acetone	–	–	–	–
	Methanol	–	–	–	–
	Water	–	–	–	–

(−): No inhibition of bacterial growth; Positive control comprises vancomycin (30 µg/ml) for *S. aureus* ATCC 25923 and gentamicin (30 µg/ml) for *B. cereus* ATCC 6633, *E. coli* ATCC 27853 and *P. aeruginosa* ATCC 25922; Negative control comprises respective extraction solvent.

Table 3. Mean diameter of inhibition zones of methanol and acetone extracts from *C. odontophyllum* leaves against *S. aureus* ATCC 25923.

Concentration (mg/ml)	Diameter of inhibition zone (mm)	
	Methanol extract	Acetone extract
50	22.33 ± 0.58	24.33 ± 0.15
25	18.67 ± 0.58	20.33 ± 0.58
12.5	12.33 ± 0.58	17.33 ± 0.58
6.25	7.00 ± 0.00	12.00 ± 0.00
Vancomycin (30 µg/ml)	15.00 ± 0.00	

Table 4. Determination of MIC values of acetone and methanol extracts of *C. odontophyllum* leaves against *S. aureus* ATCC 25923.

Concentration (mg/ml)	*S. aureus* ATCC 25923			
	Extracts		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 5. Determination of MIC value of vancomycin against ATCC 25923.

Concentration (μg/ml)	*S. aureus* ATCC 25923		
	Vancomycin	Positive control	Negative control
7.5	−	+	−
3.75	+	+	−
1.875	+	+	−
0.938	+	+	−
0.469	+	+	−
0.234	+	+	−
0.117	+	+	−
0.059	+	+	−
0.029	+	+	−
0.015	+	+	−

(−): 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 6. Determination of MBC values of acetone and methanol extracts from *C. odontophyllum* against *S. aureus* ATCC 25923.

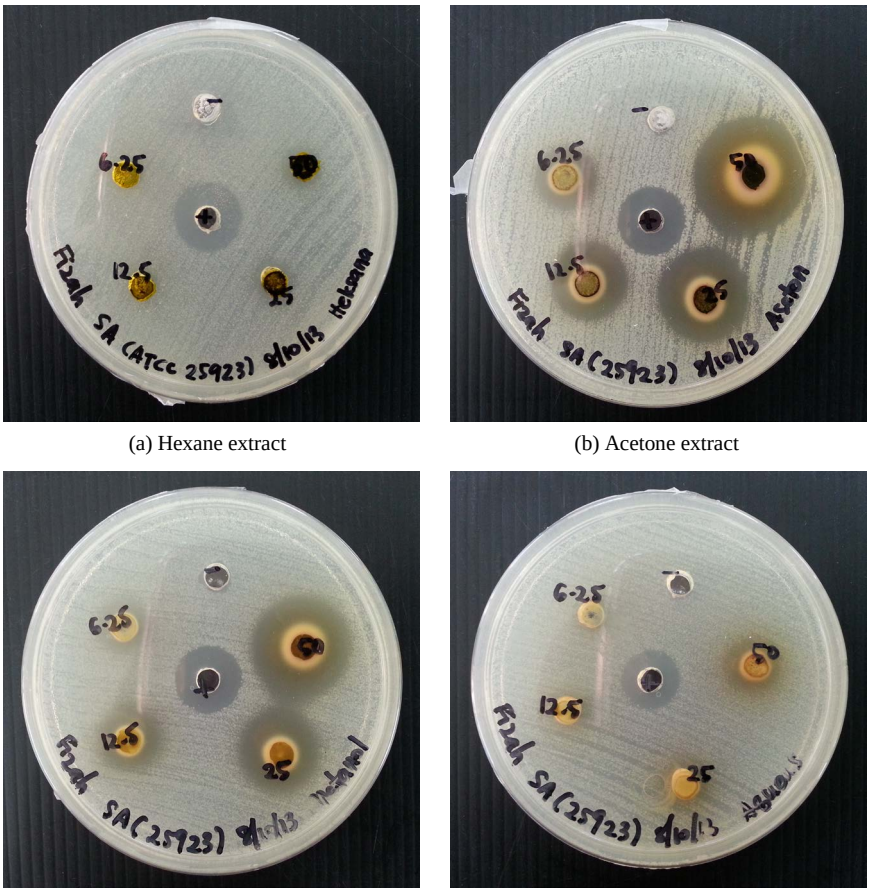
Bacteria	Extracts	Concentration of the extracts (mg/ml)									
		25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098	0.049
S. aureus ATCC 25923	Acetone	−	−	−	−	−	−	−	+	+	+
	Methanol	−	−	−	−	−	−	+	+	+	+

(−): Absence of growth; (+): Presence of growth.

Table 7. Result of phytochemical constituents screening of extracts of *C. odontophyllum.*

Test	Methanol extract	Acetone extract	Aqueous extract
Alkaloid	–	–	–
Flavonoid	+	+	+
Saponin	+	+	–
Tannin	+	+	+
Terpenoid	+	+	+
Phenolic compound	+	+	+

(+): Presence of constituent; (−): Absence of constituent.



(a) Hexane extract

(b) Acetone extract

(c) Methanol extract

(d) Aqueous extract

Figure 1. Plates showing diameter zone of inhibition by the hexane, acetone, methanol and aqueous extract of *C. odontophyllum* leaves against *S. aureus.*

screening test revealed the presence of terpenoid, tannin, flavonoid and phenol in acetone, MeOH and aqueous extracts. However, only the aqueous extract did not show the presence of saponin. Alkaloid was not detected in all three extracts from *C. odontophyllum* leaves.

4. Discussion

Solvent with increasing level of polarity namely hexane, acetone, methanol and water were used in this study to extract a wide range of active compounds from the plant. In general, the percentage yield of extraction was

higher in polar solvent than nonpolar solvent. Nonpolar solvent (hexane) produced a yield of less than 1% which comprises mostly lipophilic compounds whereas a value of 5.46% represented the yield obtained when methanol which was the more polar solvent was used. The semipolar solvent used in this study was acetone and it resulted in a medium yield of 2.83%. Methanol resulted in a higher yield when compared to other solvents which was in agreement with [19] which reported that methanol extraction showed a higher yield of polar compounds from *Cistus creticus* compared to using dicholoromethane. This suggests that there are more polar compounds in *C. odontophyllum* leaves than nonpolar compounds.

Despite higher polarity index for water compared to methanol, aqueous extract however produced lower extraction yield than methanol in this study. This is due to the effect of other solvent properties such as viscosity and vapor pressure on the extraction. According to [20], solvent with low viscosity (methanol = 0.6) can easily penetrate into plant texture due to its low density and high diffusivity compared to liquid with higher viscosity (water = 0.89). In addition to this, the higher vapor pressure of methanol allows the cavitational bubbles formed as a result of its low viscosity, to require less force to collapse, hence less energy is released to disrupt plant tissue [21]. Methanol has a low viscosity compared to water which eases the diffusion of methanol into the pores of the plant materials to extract the compound more effectively compared to other extraction solvent which have the same polarity [22]. Generally, the percentage yield of *C. odontophyllum* leaves decreased in the following order; methanol > water > acetone > hexane. Since the yield of hexane extract was the lowest and showed no antibacterial activity, phytochemical screening was not done on hexane extract from *C. odontophyllum* leaves. Analysis of phytoconstituent testing was performed on methanol, aqueous and acetone leaf extracts from *C. odontophyllum*.

In the screening of antibacterial activity using agar-well diffusion technique, only *S. aureus* was found to be susceptible towards the *C. odontophyllum* leaf extracts. In other words, out of the four bacterial species studied, only *S. aureus* was found to be susceptible towards the methanol and acetone extracts of *C. odontophyllum* leaves. The methanol and acetone extracts were capable of inhibiting the growth of *S. aureus* whereas no antimicrobial activity against *S. aureus* was recorded by the hexane and aqueous extracts from the leaf of *C. odontophyllum*. This results demonstrated that the non-polar and the most polar solvent were incapable of extracting active anti-*S. aureus* component from the leaves of *Canarium* species. The leaf from *Canarium schweinfurthii* demonstrated inhibitory effect on the growth of *S. aureus* [23] by the ethylacetate extract but not by dicholoromethane. This is further supported by [24] that the hexane extract of *C. patentinervium* leaves showed no antimicrobial activity. As far as aqueous extract is concerned, this study also implies that the tested *S. aureus* isolates were not very sensitive to the polar compounds of *C. odontophyllum*. This is in accordance with [25] that compared the aqueous extract of the stem bark of *Combretum molle* with acetone extract against *Helicobacter pylori* activity, whereby the anti-*H. pylori* compounds in *C. molle* were not extracted by polar solvents. It was also reported [26] that absence of antimicrobial activity of the aqueous extract of other plant indicating that water may not be a suitable solvent for the extraction of antimicrobial compounds from the plants despite its high availability and relatively no toxicity. Our finding with acetone extract clearly showed a significantly ($p < 0.05$) higher inhibitory effect against *S. aureus* compared to methanol extract as observed from the size of the diameter of the inhibition zone at different concentration between the extracts. The diameter of inhibition zones seemed to decrease with an increase in the polarity of the solvent from acetone to methanol. This was probably because the total amount of phenolic compounds such as flavonoid extracted by acetone is much higher compared to methanol despite lower extraction yield compared to methanol. This is in accordance with [27] that acetone was the best solvent for extracting flavonoids from bitter melon out of five solvents (ethanol, methanol, n-butanol, acetone and water). The antimicrobial activity of the methanol and acetone extracts from *C. odontophyllum* leaves was concentration-dependent towards the growth inhibition of *S. aureus* ATCC 25923 and this could correlated to the presence of flavonoids in the extracts.

The equal MIC values for both methanol and acetone extracts against *S. aureus* indicated that both extracts exhibited equal antimicrobial potency against the bacteria. However, the MIC values of both extracts did not correlate well with the screening result using agar well diffusion technique. This is because although acetone extract showed a significantly stronger inhibitory effect against *S. aureus* compared to methanol extract, it would be predicted that the MIC value of the acetone extract is lower than the latter but this is not the case. Our observation is in line with [28] whereby the MIC value of methanol and ethyl acetate extracts from *Thuja orientalis* leaves against *Pseudomonas aeruginosa* were found to be same despite discrepancy in the inhibitory activity of these extracts in agar well diffusion screening assay. As expected, the MIC values of acetone and metha-

nol extract of *C. odontophyllum* leaves were dramatically lower than that of vancomycin. The difference may be due to the impurity of these crude extract that might contained a mixture of compound compared to pure compound in standard drug [29].

In screening of new antimicrobial agent, it is very important to characterize the type of antibacterial activity displayed by plant extract. This can be done by comparing MBC value with MIC value of each extract. Current study showed that the MIC and MBC values of acetone extract against *S. aureus* were the same whereas the MBC values of methanol extract was twice its MIC value. This indicated that acetone and methanol crude extract might contain bioactive constituent that respectively, exhibited bactericidal and bacteriostatic action against *S. aureus*. The bactericidal effect of the acetone extract from the leaves of *C. odontophyllum* was in agreement with [25] which confirmed that acetone extract from *Combretum molle* exhibited considerable bactericidal activity against *Helicobacter pylori*. Phytochemical screening from this study showed the presence of flavonoid, terpenoid, tannin, phenoland saponin in both the leaf extracts from *Canarium odontophyllum*. The anti-*S. aureus* activity of *Canarium odontophyllum* was possibly due to its phenolic constituents as suggested by [30] that there is a correlation between total phenolic content and antibacterial activity of the aqueous acetone from the extract of *Anogeissus leiocarpus* leaves. The leaf extracts from *Canarium schweinfurthii* also revealed the presence of tannin and saponin which could contributed to its antimycobacterial activity against *Mycobacterium tuberculosis* [31]. In another study, *Canarium album* leaves had a relatively high level of total phenolic and extractable condensed tannin [32] whereas ethanol extract of leaves and barks from *Canarium patentinervium* accumulate substantial amounts of tannin and flavonoid [24]. Flavonoids are known to be synthesized by plants in response to microbial infection [33] [34]. Saponin which was present in the acetone and methanol extract studied, could also account for the antimicrobial activity against *S. aureus*. This is confirmed by [35] that saponin fractions of the leaves of *Solanum xanthocarpum a*nd *Centella asiatica* inhibited the growth of Gram positive bacterium *S. aureus*. It is pertinent to mention here that saponin was not detected in the aqueous extract from *C. odontophyllum* leaves studied and this could justify the absence of antimicrobial activity in this extract. This is because the active components in the crude extract may be acting in synergism to produce greater antimicrobial effects [36].

5. Conclusion

In conclusion, the acetone extract of *C. odontophyllum* leaves have the potential to be developed as an alternative treatment against *Staphylococcus aureus*-associated skin and soft tisue infections. However, identification work is necessary to determine the bioactive compound in the extract responsible for the antibacterial activity. Moreover, evaluation of antimicrobial activity potency against a wider range of microorganisms such as clinical isolates of resistant organisms, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) is also recommended to obtain a more accurate evaluation of the therapeutic potential of *C. odontophyllum* leaves.

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