1874-2106/20

Send Orders for Reprints to reprints@benthamscience.net

The Open Dentistry Journal

Content list available at: https://opendentistryjournal.com

CrossMark

RESEARCH ARTICLE

Antibacterial Activity of Buah Merah (*Pandanus conoideus* Lam.) Against Bacterial Oral Pathogen of *Streptococcus sanguinis* ATCC10556, *Streptococcus mutans* ATCC 25175, and *Enterococcus faecalis* ATCC 29212: An *in Vitro* Study

Lisda Damayanti[1], Ida Ayu Evaangelina[1], Avi Laviana[1], Yetty Herdiyati[1] and Dikdik Kurnia[2,*]

[1]*Department of Pediatric Dentistry, Faculty of Dentistry, University of Padjadjaran, Bandung, Indonesia.*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Science, University of Padjadjaran, Bandung, Indonesia*

Abstract:

Background:

Caries and periodontitis are dental diseases caused by bacteria of *S. sanguinis*, *S. mutans,* and *E. faecalis* with three main etiological factors of the host, substrate, and time.

Objective:

This study proposed to investigate the antibacterial effects of Buah Merah (*Pandanus conoideus* Lam.) against oral bacteria of *E.faecalis*, *S. mutans*, and *S. sanguinis*.

Materials and Methods:

The Buah Merah was extracted with different solvents to yield *n*-hexane, ethyl acetate, methanol, and H_2O extracts. The concentrations of single and mixture extracts were adjusted for antibacterial assay against bacteria of *E. faecalis*, *S. mutans*, and *S. sanguinis* strains through agar well diffusion assay with chlorhexidine, fosfomycin, and quercetin used as positive controls.

Results:

The ethyl acetate extract showed highest antibacterial activity against three oral bacterial of *E. faecalis*, *S. mutans*, and *S. sanguinis* with inhibition zones values of 9.3, 12.3, and 17.9 mm at 40%, respectively, together with their MIC and MBC values of 1250 & 2500, 0.312 & 0.625, and 0.312 & 0.625 ppm, respectively. For the formulation of extracts, combinations samples test gave various effects to different bacteria, with the best activity showed by methanol-ethyl acetate (M-Ea) extracts against *S. mutans* with an inhibition zone of 16.25 mm at 40 ppm. The strong and synergistic effect of methanol extract against *S. mutans* was supported by inhibition zones of the formulation of methanol extract-fosfomycin which showed an inhibition zone of 25.9 mm at 10 ppm.

Conclusion:

The extracts of Buah Merah demonstrated antibacterial activity against oral bacteria of *E. faecalis*, *S. mutans,* and *S. sanguinis* and gave important information for further *in vivo* clinical studies to determine the exact dosages and its effectiveness in practical application. These results prove the antimicrobial effects of Buah Merah extracts as alternative natural drugs with synergistic effects of active constituents.

Keywords: Buah Merah, *Pandanus conoideus* Lam., antibacterial compound, *S. sanguinis*, *S. mutans*, *E. faecalis*.

Article History	Received: December 21, 2019	Revised: February 19, 2020	Accepted: February 23, 2020

1. INTRODUCTION

Caries is a disease of teeth's hard tissue, which are enamel, dentin, and cementum. There are three main etiological factors of caries: host, substrate, and time. *Streptococcus mutans* is included as cariogenic bacteria because it is able to produce acid and carbohydrate quickly. The pathogenesis process of teeth caries is marked by the ability to grow in an acid surrounding and quick sugar metabolism characteristic for organic acid including lactic acid [1, 2].

[*] Address correspondence to this author at the Department of Chemistry, Faculty of Mathematics and Natural Science, University of Padjadjaran, Bandung, Indonesia; Tel: +062227794391; E-mail: dikdik.kurnia@unpad.ac.id

DOI: 10.2174/18742106020140113, 2020, *14*, 113-119

Caries and periodontitis are types of dental diseases caused by bacteria. Some bacteria are responsible for dental disease including *S. sanguinis*, *S. mutans,* and *E. faecalis* [3, 4]. All the three are interconnected to each other to cause infection in teeth. Two of them, *S. mutans* and *S. sanguinis*, contribute to form dental plaque which develops caries [5]. If it is not treated immediately, it will lead to further infection such as root canal infection. However, *E. faecalis* is one of the bacteria which exists in the root canal [6]. Systemic diseases will occur if infection in that step is not treated wisely [7].

S. mutans is able to metabolize all kind of sugar and glycosides, such as glucose, fructose, sucrose, lactose, and others. Because of the presence of extracellular glucose and sucrose, *S. mutans* is able to synthesize Intracellular Polysaccharide Glycogen (IPSs) and produce mutation (bacteriocin) which is considered as an important factor of teeth biofilm's colonization and production [1].

One of the methods to maintain teeth with big and infected caries on the pulp surface is by doing a root canal or endodontic treatment. Approximately 24-77% of endodontic treatment failures are caused by *E. faecalis* bacteria. This microbe is able to produce biofilm, go through the dentin tubule, survive in low pH levels, and resist many intracanal medicines [8 - 10].

Intracanal medicament for every visit is recommended to reduce whole bacteria inside the root canal. Root canal medicament must have antibacterial characteristics, able to reduce the rest of microbial biofilm, not irritate periapical tissue, help the regeneration of periapical tissue, and also easy to clean and applicate [10, 11].

Teeth surface is covered with biofilm layer: a layer containing millions of bacteria, polymer saliva, and food leftovers and not every teeth covered with biofilm shows the signs of caries [10 - 12]. The prevention of caries and periodontal diseases are based on the control of bacteria and plaques and the production of plaques begins with the formation of pellicle on the teeth surface.

The first bacteria contacted by the pellicle is *S. sanguinis* which is able to facilitate nutrition and surrounding for another new bacteria inside the oral cavity. The ongoing accumulation of plaques on the upper area of gingival margin, interproximal, pit, and fissure is able to cause caries. The plaque production can be inhibited through adhesion, proliferation, and bacterial aggregation [12].

As there is the bacteria resistance, the discovery of antibacterial agents is still ongoing. Medicinal plants and other natural products are a source of new antibacterial agents that have to be explored [13, 14]. Recently, many researchers have given more concern for herbal medicine because of the variety of safety and synergic effects [15]. A large number of plants that can be used as herbal medicine are available in nature. One of them is Buah Merah.

Herbal based chemical materials are developed in order to find effective alternative materials to control biofilm formation by pathogenic bacteria [13]. The utilization of medicinal plants is also increasing because traditional medicines are cheaper, easy to get, and have a relatively smaller side effect [13, 16].

Buah Merah, known as *Pandanus conoideus* Lam., is an indigenous plant from Papua, Indonesia. Local people usually consume it directly as food or use its oil for treatment to cure diseases including stroke, HIV, and cancer [17]. In the previous studies, Buah Merah showed that it has antibacterial activity against *C. albicans*, *S. aureus,* and *M. gypseum* [16]. Moreover, Oil of Buah Merah has been used as a major content in hand soap formulation [18]. Based on the studied data that Buah Merah showed potential antibacterial activity, it was suggested to contain antibacterial agents for dental disease.

The acceptance of Herbal medicines as an alternative to modern medicine has led researchers to investigate antibacterial agents of medical plants [19]. Selection of Buah Merah as the source for an antibacterial agent was done with an assumption that the process for drug development would be simpler because the toxicity of Buah Merah is negligible since it is consumed on a daily basis.

2. MATERIALS AND METHODS

2.1. Materials

Fresh Buah Merah (*Pandanus conoideus* Lam.) was collected in June 2017 from West Papua, Indonesia. Sample extracts were prepared by an extraction method with organic solvents of methanol, *n*-hexane, and ethyl acetate.

2.2. Instruments

Laminar airflow, incubator (Memmert, IN55), anaerobic jar (Oxoid, AG0025A), autoclave, microplate reader (Biochrom EZ read 400, 80-4001-40), micropipette (Eppendorf, 3120000062 and 3120000054), colony counter (Schuett-Biotec, 3081502).

2.3. Preparation of the Buah Merah Extracts

The fresh fruit of Buah Merah was extracted with methanol (1:3 m/v) for 72 hours, and filtered and evaporated *in vacuo* at 40°C yielded crude extract. The methanol extract was subsequently partitioned between *n*-hexane-water and ethyl acetate-water resulting in *n*-hexane, ethyl acetate, and water extracts, respectively.

2.4. Preparation of the Combination Extracts and References Compound

Each extract and fraction of *M. pendans* were made in concentrations 40, 20, 10, and 5% (all samples made in 1 ml). The antibacterial activity of the sample was divided into three categories. First, to evaluate the antibacterial activity of a single fraction, concentrations used are 40, 20, and 10%. Second, extract at concentration 5% was used to evaluate the antibacterial activity of the combined fraction. Samples were combined following Table **1**, 10 µl of each fraction were combined to another fraction to provide six mixtures. Finally, all samples were combined with positive control of Quercetin, Fosfomycin, and Chlorhexidine to give twelve combinations. Each sample in each category (20 µl) was added into the paper disc.

2.5. Preliminary Phytochemical Screening

Screening for alkaloids, terpenoids, and flavonoids secondary metabolites was performed to methanol, *n*-hexane, ethyl acetate, and water extracts previously reported [20 - 22].

2.6. Microorganism Assay

The bacteria of *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10566 were used for antibacterial test on Muller Hinton broth and Muller Hinton agar as a medium, chlorhexidine (purchased from Merck Co. Ltd. and Sigma Aldrich) as a positive control, Brain Heart Infusion broth (Oxoid, CM1135), Muller Hinton agar (Oxoid, CM0337), paper disc 6 mm (Sigma-Aldrich, Z741310), aquabidest (Ikapharmindo Putramas), microplate 96 well (Iwaki, 3820 024), filter tips (Biologix, code 22-0010, 22-0200, and 22-1000), parafilm (Sigma-Aldrich P7688-1EA).

2.7. Antibacterial Activity Assay

Antibacterial effects of Buah Merah extracts against *E. faecalis*ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10566 were observed using Kirby-Bauer disk diffusion. This determination of the sensitivity or resistance of *E. faecalis, S. mutans, and S. sanguinis* to compounds was based on CLSI protocols (CLSI, 2012) [23, 24]. All samples were diluted with methanol except chlorhexidine (control) with water. The concentration used for all samples and control was 40, 20, and 10%. Paper discs (6 mm) were impregnated with 20 µL of each sample and then placed on the surface of the agar. Tests were performed in duplicate.

The MIC and MBC activities of compounds **1** and references of antibiotics against *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10566 were determined by the micro-dilution method in 96-well microplates (CLSI, 2012). The bacterial cells were precultured in Muller Hinton broth at 37°C under aerobic conditions and incubated in the presence of compounds with the concentrations obtained by serial two-fold dilution at 37°C, without shaking in the same broth for 24h on the micro. Their MICs were estimated as the lowest concentrations, where the bacterial cells were not observed visually as reported previously and were given based on triplicate experiments. Water or methanol used for dissolving compounds had no effect on the bacterium. The positive control, chlorhexidine and fosfomycin, were dissolved in water and the tests were performed in duplicates.

3. RESULTS

3.1. Buah Merah Extracts

The fresh fruit of Buah Merah (3 kg) was extracted with 15 L of methanol (1:3 m/v) for 72 hours, and filtered and evaporated *in vacuo* at 40°C yielded 820 g of crude extract. The methanol extract was subsequent partitioned solvent between *n*-hexane-water (1:1, v/v) and ethyl acetate-water (1:1, v/v) resulting in *n*-hexane (525 g), ethyl acetate (22 g) and water (18 g) extracts, respectively.

3.2. Phytochemicals Screening of the Buah Merah Extracts

Data of secondary metabolite constituents samples by phytochemical analysis as shown in Table **1**, indicated that phenolic and flavonoid compounds were found in all of the fractions; *n*-hexane fraction contains steroid and triterpenoid; ethyl acetate and H_2O fraction contain phenolic, flavonoid, steroid, triterpenoid, alkaloid, and tannin. The H_2O fraction contains all secondary metabolites of phenolic, flavonoid, steroid, triterpenoid, saponin, tannin, and alkaloid, while in *n*-hexane fraction only steroid and triterpenoid. In the ethyl acetate and methanol fractions, similar major secondary metabolites of flavonoids were identified [25].

The analysis data for secondary metabolites constituents of Buah Merah (*P. conoideus* Lam.) as shown in Table **1**, showed that all factions contained different components. Research data gave support that a previous study indicated flavonoids as an important antimicrobial compound [26].

Table 1. Data of phytochemical analysis of the extracts of Buah Merah.

No.	Secondary metabolites	Reagent	Samples fraction			
			H_2O	*n*-Hexane	EtOAc	MeOH
1	Phenolic	$FeCl_3$ 5%	+	-	+	-
2	Flavonoid	a. HCl (p.a)+ Mg	+	-	+	+
		b. H_2SO_4 2N	+	-	+	+
		c. NaOH 10%	+	-	+	+
3	Steroid	Lieberman Burchard	+	+	+	+
4	Triterpenoid	Lieberman-Burchard	+	+	+	-
5	Saponin	HCl + H_2O	+	-	-	-
6	Tanin	$FeCl_3$ 1%	+	-	+	-
7	Alkaloid	a. Dragendroff	+	-	+	+
		b. Wagnerr	+	-	-	-

3.3. Antibacterial Activity

3.3.1. Antibacterial Activity of the Extracts

To determine the potential of Buah Merah as natural sources of antibacterial agents, the extracts to inhibit bacterial growth were tested against *E. faecalis, S. mutans,* and *S. sanguinis*. Susceptibility of Buah Merah extracts against bacteria can be evaluated from their inhibition zone of sample on bacteria growth by Kirby-Bauer method, and the sample was conducted at concentrations of 40, 20, and 10%, with chlorhexidine as positive control and methanol & H_2O as negative controls. Based on the assay data in Table **2**, this study found that only ethyl acetate extract was active against *E. faecalis, S. mutans,* and *S. sanguinis* with different inhibition zone value for each different bacteria, while the most active against *S. sanguinis* with inhibition zone values of 14.5, 16.1, and 17.9 mm at a concentration of 10, 20, and 40%, respectively. Further analysis for their MIC values was conducted for ethyl acetate extract against *E. faecalis, S. mutans,* and *S. sanguinis*, and the assay data showed that MIC and MBC were the same values against *S. mutans* and *S. sangunis*, while less active for *E. faecalis*.

Table 2. Antibacterial activity of the extracts of Buah Merah against pathogenic oral bacteria *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556.

No.	Extracts	Inhibition Zones (mm) at concentration (%)			MIC (ppm)	MBC (ppm)
		40	20	10		
			E. faecalis			
1	MeOH	0	0	0	-	-
2	*n*-Hexane	0	0	0	-	-
3	EtOAc	9.3	8.6	7.5	1250	2500
4	H_2O	-	-	-	-	-
5	CHX 2%	16.6	15.7	15.7	0.003	0.006
			S. mutans			
1	MeOH	0	0	0	-	-
2	*n*-Hexane	0	0	0	-	-
3	EtOAc	12.3	1.2	10.4	0.312	0.625
4	H_2O	0	0	0	-	-
5	Chx 2%	21.0	21.0	20.5	< 0.001	< 0.001
			S. sanguinis			
1	MeOH	0	0	0	-	-
2	*n*-Hexane	0	0	0	-	-
3	EtOAc	17.9	16.1	14.5	0.312	0.625
4	H_2O	6.9	0	0	-	-
5	Chx 2%	19.3	19.6	19.6	< 0.001	< 0.001

Note: Chx: Chlorhexidine

3.3.2. Antibacterial Activity of the Combinations Extracts

In order to determine activity effects of active constituents in the single and combinations extracts, the formulated mixtures extracts were made and their antibacterial activity was re-evaluated against bacteria of *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556, respectively.

Table 3. Antibacterial activity of the combination extracts of Buah Merah (*P. conoideus* Lam.) at a concentration of 5% against pathogenic oral bacteria *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556.

No.	Samples	Inhibition Zones at concentrations of 5%		
		E. faecalis	*S. mutans*	*S. sanguinis*
1	M+Hex	9.75	12.2	10.35
2	M+Ea	9.85	16.25	12.5
3	M+H_2O	8.6	15	10.8
4	*n*-Hex+Ea	0	14.9	10.8
5	*n*-Hex+H_2O	0	7.15	13.4
6	Ea+H_2O	7.75	14.9	12.4

Note: M: methanol, Hex: n-Hexane, Ea: Ethyl acetate

Antibacterial activity of combinations extracts as shown in Table **3**, indicated that all combinations extract were active against two oral bacteria of *S. mutans* ATCC 25175 and *S. sanguinis* ATCC 10556 with different inhibitions zones values, while against bacteria of *E. faecalis* ATCC 29212, two combinations extracts of *n*-Hex+Ea and *n*-Hex+H_2O were inactive.

3.3.3. Antibacterial Activity of the Combinations Extracts and References Compounds

Further analysis determines activity effects of active constituents in the single and references compounds of chlorhexidine, fosfomycin, and quercetin, the formulated mixtures extracts were made and their antibacterial activity was re-evaluated against bacteria of *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556, respectively.

The effects of additional reference compounds on the antibacterial activity of extracts are shown in Table **4**. All references compounds give different effects depend on each bacteria. The assay data contained some different inactive combination extracts against all bacteria. On the other hand, the activities represented by inhibition values were in the range of 6.85 to 25.9 mm.

Table 4. Data of antibacterial activity of the combination extracts of Buah Merah (*P. conoideus* Lam.) and reference compounds at a concentration of 10% against pathogenic oral bacteria *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, and *S. sanguinis* ATCC 10556.

No.	Samples	Inhibition zones at concentrations of 10%		
		E. faecalis	*S. mutans*	*S. sanguinis*
1	M+Chx	10.15	0	9.75
2	M+F	21.6	25.9	6.9
3	M+Q	0	7.05	10.15
4	Hex+Chx	0	0	13.25
5	Hex+F	20,7	23.9	0
6	Hex+Q	6,85	0	12.7
7	Ea+Chx	7.7	12.8	11.85
8	Ea+F	19.65	21.9	11.3
9	Ea+Q	7.7	6.85	11.85
10	H_2O+Chx	12.15	18.7	11.15
11	H_2O+F	22.1	22.1	0
12	H_2O+K	0	0	0

Note: M: methanol, Hex: n-Hexane, Ea: Ethyl acetate, Chx: Chlorhexidine, Q: Quercetin, F: Fosfomycin

4. DISCUSSION

Recently, potential challenges for the sustainability of modern new drug discovery for antimicrobial drug resistance have increased markedly over the last decades. Current treatment for carries disease caused by infection of some pathogenic oral bacteria of *E. faecalis, S. mutans,* and *S. sanguinis* mainly used 2% chlorhexidine as the gold standard [27, 28], but it may cause discoloration of the teeth and drug resistance. Previous papers reported some alternatives for the prevention of dental plaque related diseases and the improvement of dental health by complement or substitute active antibacterial agents *i.e.* probiotics, xylitol and sea salt [29 - 35]. Therefore, to solve this problem, there is a need to find and develop new antibacterial compounds that are more selective, effective, and efficient with no or a very limited negative side effect.

Natural products are potential sources that synthesize diverse bioactive compounds that act as antifungal and antibacterial agents for therapeutically viable new antibacterial agents. This study presents data on the antibacterial activity of edible plants selected based on the fact that they were consumed as daily health food [36, 37].

Pandanus conoideus Lam., also known as Buah Merah, is an edible plant used traditionally as healthy food for daily life as natural multivitamins. The plant was already reported to cure some diseases together with pharmacological activities including anticancer, antibacterial, antioxidant, *etc*. According to the phytochemical analysis data, this study showed a correlation between their pharmacological activities with chemical constituents. Table **1** showed that Buah Merah contained secondary metabolites of phenolic, flavonoid, steroid, triterpenoid, saponin, tannin, and alkaloid which distributed into different extracts according to their chemical properties, while the information of their bioactive constituent as antibacterial compounds against pathogenic oral bacteria was not yet reported.

The susceptibility potential of Buah Merah as natural sources of antibacterial agents to inhibit bacterial growth against *E. faecalis, S. mutans* and *S. sanguinis* was evaluated from their inhibition zone by Kirby-Bauer method. The assay data represented that only ethyl acetate extract was active against *E. faecalis, S. mutans,* and *S. sanguinis* with different inhibition zone values for each different bacteria, while the most active against *S. sanguinis* with inhibition zone value of 14.5, 16.1, and 17.9 mm at a concentration of 10, 20, and 40%, respectively.

Referring to the phytochemical analysis, of ethyl acetate that contained phenolic, flavonoid, steroid, triterpenoid, tannin, and alkaloid, the high values of antibacterial activity is considered as a result of the synergic effect mixture of their bioactive compounds, but further advance assay and specific research should be conducted to support this assumption.

Further detail analysis to determine susceptibility categories, their MIC and MBC values were conducted for ethyl acetate extract against *E. faecalis, S. mutans,* and *S. sanguinis*. The assay data showed that MIC and MBC were same values against *S. mutans and S. sangunis*, while the least active for *E. faecalis* explained that ethyl acetate extract has the potential as an antibacterial against *S. mutans and S. sangunis* but cannot replace drugs used as positive controls, and ethyl acetate extract has no potential to *E. faecalis*, respectively. Compared to the reported data, the MIC & MBC values of ethyl acetate extract from Buah Merah were 0.312 & 0.625 ppm against *S. mutans and S. sangunis*.

The role of single and combinations extracts contributing to antibacterial activity was then evaluated against all bacteria. By this assay, sample formulation could be determined the study of the synergistic effects of the extracts. As shown in Table **3**, the antibacterial activity of combinations extracts described all combination extracts were active against two oral bacteria of *S. mutans* ATCC 25175 and *S. sanguinis* ATCC 10556 with different inhibition zone values, while against bacteria of *E. faecalis* ATCC 29212, two combination extracts of *n*-Hex+Ea and *n*-Hex+H_2O were inactive. This finding predicted that the addition of ethyl acetate extract with other extracts caused synergistic effect their antibacterial activity against *S. mutans* ATCC 25175 and *S. sanguinis* ATCC which identified by increasing of their inhibition zones values. On the other hand, it was conversely observed that antagonistic effect resulted from combination extracts between *n*-Hex+Ea and *n*-Hex+H_2O against *E. faecalis* ATCC 29212.

According to the main target of this study for discovering new antibacterial agents as complemented or substituted antibacterial drugs, the effect of combinations between extracts and reference compounds against their antibacterial activity was also evaluated. In this study, chlorhexidine, fosfomycin, and quercetin were used as reference compounds. Chlorhexidine has substantivity for a period of 10-12 hours. It has long-lasting antibacterial activity with a broad spectrum of action [27]. Fosfomycin is bactericidal with putative activity against several bacteria, including multidrug-resistant Gram-negative bacteria, by irreversibly inhibiting an early stage in cell wall synthesis [28]. Quercetin is a polyphenolic flavonoid with potential chemoprotective properties. The present studies show the effectiveness of quercetin as an antibacterial agent on selected organisms [29].

The effects of extracts addition into reference compounds on their antibacterial activity as shown in Table **4**, showed that different combinations resulted in increasing antibacterial activity against each bacteria. The highest antibacterial activity against *E. faecalis, S. mutans,* and *S. sanguinis* was combination of H_2O+F, M+F, and Hex+Chx with vales of 22.1, 25.9, and 13.25, respectively. This fact suggested that the bioactive constituents in methanol and H_2O extract together with fosfomycin were synergically combined to increase their activity to inhibit bacterial growth of *E. faecalis and S. mutans.* Since fosfomycin was known as inhibitor enzyme murA, the H_2O+F, M+F extracts were active as antibacterial agents by inhibiting the formation of the bacterial cell wall of *E. faecalis and S. mutans* [30]. Then, the increasing antibacterial activity caused by the addition of Hex+Chx extracts presented the possible mechanism that bacterial growth of *S. sanguinis* was inhibited *via* damage to the membrane of bacteria [38]. Though there has not been any thorough research into the mechanisms underlying the antibacterial activity for extracts, previous research suggested that different compounds would target components of bacterial cells differently. Based on the data in this study, the results showed that the edible plant of Buah Merah (*Pandanus conoideus* Lam.) has potential antibacterial agents. The different activity values of single and combination extracts together with the added effect of reference antibacterial drugs were given important biomarkers as antibacterial constituents according to oral bacteria species. The structure and identity of antibacterial lead compounds in active extracts could be determined in single or pure compounds by guided combination phytochemical screening and antibacterial activity data.

CONCLUSION

The edible plant of Buah Merah (*Pandanus conoudeus* Lam.) demonstrated *in vitro* antibacterial activity against pathogenic oral bacteria of *E. faecalis* ATCC 29212, *S. mutans*

ATCC 25175, and *S. sanguinis* ATCC 10556. This finding is an important database for further *in vivo* clinical studies to determine the exact dosages and its effectiveness in practical application. Since the plant was used as daily consumption, toxicity studies should be conducted to determine the safety aspect. Further work is required to isolate and determine the lead antibacterial compounds that play the role in inhibiting or eliminating bacteria. Discovery of new antibacterial agents from promising of the edible plant may open the possibilities of finding new clinically effective antibacterial compounds against dental caries and other bacterial oral pathogens.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS:

Not applicable.

FUNDING

Research Grant of Riset Kompetensi Dosen Unpad (RKDU) 2017-2018 from Universitas Padjadjaran, Bandung - Indonesia.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors are grateful to Universitas Padjadjaran for Research facilities.

REFERENCES

[1] Karpinsky T, Szkaradkiewicz A. Microbiology of dental caries. J Biol Earth Sci 2013; 3(II): 21-4.

[2] Forssten SD, Björklund M, Ouwehand AC. Streptococcus mutans, caries and simulation models. Nutrients 2010; 2(3): 290-8. [http://dx.doi.org/10.3390/nu2030290] [PMID: 22254021]

[3] Könönen E, Asikainen S, Saarela M, Karjalainen J, Jousimies-Somer H. The oral gram-negative anaerobic microflora in young children: Longitudinal changes from edentulous to dentate mouth. Oral Microbiol Immunol 1994; 9(3): 136-41. [http://dx.doi.org/10.1111/j.1399-302X.1994.tb00049.x] [PMID: 7936718]

[4] Haapsalo M, Udnaes T, Endal U. Persistent, recurrent, and acquired infection of the root canal system post-treatment Endo topics. Denmark: Blackwell Munksgaard 2003; pp. 29-56.

[5] Ge Y, Caufield PW, Fisch GS, Li Y. Streptococcus mutans and Streptococcus sanguinis colonization correlated with caries experience in children. Caries Res 2008; 42(6): 444-8. [http://dx.doi.org/10.1159/000159608] [PMID: 18832831]

[6] Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006; 32(2): 93-8. [http://dx.doi.org/10.1016/j.joen.2005.10.049] [PMID: 16427453]

[7] Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev 2000; 13(4): 547-58. [http://dx.doi.org/10.1128/CMR.13.4.547] [PMID: 11023956]

[8] Singh H, Kapoor A. Comparative evaluation of antibacterial efficacy of "active point" and combi point as intra-canal medicaments against Enterococcus faecalis: An ex vivo study. JOHDM 2014; 13(2)

[9] Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006; 32(2): 93-8. [http://dx.doi.org/10.1016/j.joen.2005.10.049] [PMID: 16427453]

[10] Flemming HC, Neu TR, Wozniak DJ. The EPS matrix: The "house of biofilm cells". J Bacteriol 2007; 189(22): 7945-7. [http://dx.doi.org/10.1128/JB.00858-07] [PMID: 17675377]

[11] Ambikathanaya UK. Intracanal antiseptic medications; A Review. J Unique Med Dent Sci 2014; 02(03): 136-42.

[12] Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm virulence 2011; 2(5): 435-4.

[13] Chandra Shekar BR, Nagarajappa R, Suma S, Thakur R. Herbal extracts in oral health care - A review of the current scenario and its future needs. Pharmacogn Rev 2015; 9(18): 87-92. [http://dx.doi.org/10.4103/0973-7847.162101] [PMID: 26392704]

[14] Romero CD, Chopin SF, Buck G, Martinez E, Garcia M, Bixby L. Antibacterial properties of common herbal remedies of the southwest. J Ethnopharmacol 2005; 99(2): 253-7. [http://dx.doi.org/10.1016/j.jep.2005.02.028] [PMID: 15894135]

[15] Mundy L, Pendry B, Rahman M. Antimicrobial resistance and synergy in herbal medicine. J Herb Med 2016; 6(2): 53-8. [http://dx.doi.org/10.1016/j.hermed.2016.03.001]

[16] Indrawati I. Sensitivity of pathogenic bacteria to Buah Merah (*Pandanus conoideus Lam*) American Institute of Physics 2016; 1-9. [http://dx.doi.org/10.1063/1.4953502]

[17] Rohman A, Windiarsih A. Characterization, biological activities, and authentication of red fruit (Pandanus conoideus Lam.) oil. Food Res 2017; 2(2): 134-8. [http://dx.doi.org/10.26656/fr.2017.2(2).152]

[18] Sahamastuti AAT, Hartrianti P, Hartiadi LY. Formulation and antimicrobial assay of Red Fruit (*Pandanus conoideus Lam*.) extract and oil as antibacterial hand soap World J Pharm Pharm Sci 2018; 7(10): 288-98.

[19] Karteek P, Jahnavi V, Keerthi DV, Sravanthi KC. Evaluation of antibacterial activity of herbs. Int Res J Pharm 2012; 3(8)

[20] Harbone JB. Phytochemical methods: A guide to modern technique of plant analysis. 2nd ed. New York, NY: Chapman and Hall 1973.

[21] Kokate CK. A textbook of practical pharmacognosy. 5th ed. VallabhPrakashan 2005; pp. 105-11.

[22] Clinical and laboratory standards institute (CLSI - formerly NCCLS), 2012 Performance standards for antimicrobial disk susceptibility tests; Approved standard. 11th ed. PA, USA: Clinical and laboratory standards institute: Wayne 2012.

[23] Clinical and laboratory standards institute document M7-A8, 2012 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard. 9th ed. PA, USA: Clinical and laboratory standards institute: Wayne 2012.

[24] Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. Curr Med Chem 2015; 22(1): 132-49. [http://dx.doi.org/10.2174/0929867321666140916113443] [PMID: 25245513]

[25] Wang H, Ren D. Controlling Streptococcus mutans and Staphylococcus aureus biofilms with direct current and chlorhexidine. AMB Express 2017; 7(1): 204. [http://dx.doi.org/10.1186/s13568-017-0505-z] [PMID: 29143221]

[26] Yousefimanesh H, Amin M, Robati M, Goodarzi H, Otoufi M. Comparison of the antibacterial properties of three mouthwashes containing chlorhexidine against oral microbial plaques: An *in vitro* Study. Jundishapur J Microbiol 2015; 8(2)e17341 [http://dx.doi.org/10.5812/jjm.17341] [PMID: 25825646]

[27] Prasanna SGV, Lakshmanan R. Characteristics, uses and side effects of chlorhexidine- a review. J Dent Med Sci 2016; 15(6): 57-9.

[28] Dijkmans AC, Zacarías NVO, Burggraaf J, *et al.* Fosfomycin: Pharmacological, clinical and future perspectives. Antibiotics (Basel) 2017; 6(4): 1-17. [http://dx.doi.org/10.3390/antibiotics6040024] [PMID: 29088073]

[29] Cantore S, Ballini A, Mori G, *et al.* Anti-plaque and antimicrobial efficiency of different oral rinses in a 3-day plaque accumulation model. J Biol Regul Homeost Agents 2016; 30(4): 1173-8. [PMID: 28078871]

[30] Campanella V, Syed J, Santacroce L, Saini R, Ballini A, Inchingolo F. Oral probiotics influence oral and respiratory tract infections in pediatric population: A randomized double-blinded placebo-controlled pilot study. Eur Rev Med Pharmacol Sci 2018; 22(22): 8034-41.

[31] Inchingolo F, Dipalma G, Cirulli N, *et al.* Microbiological results of improvement in periodontal condition by administration of oral probiotics. J Biol Regul Homeost Agents 2018; 32(5): 1323-8. [PMID: 30334433]

[32] Cantore S, Ballini A, De Vito D, *et al.* Clinical results of improvement in periodontal condition by administration of oral probiotics. J Biol Regul Homeost Agents 2018; 32(5): 1329-34. [PMID: 30334434]

[33] Cantore S, Ballini A, Saini R, *et al.* Efficacy of a combined sea salt-based oral rinse with xylitol against dental plaque, gingivitis, and salivary *Streptococcus mutans* load. J Biol Regul Homeost Agents 2018; 32(6): 1593-7. [PMID: 30574771]

[34] Ballini A, Cantore S, Fotopoulou EA, *et al.* Combined sea salt-based oral rinse with xylitol in orthodontic patients: Clinical and microbiological study. J Biol Regul Homeost Agents 2019; 33(1): 263-8. [PMID: 30724059]

[35] Ballini A, Cantore S, Saini R, *et al.* Effect of activated charcoal probiotic toothpaste containing *Lactobacillus paracasei* and xylitol on dental caries: A randomized and controlled clinical trial. J Biol Regul Homeost Agents 2019; 33(3): 977-81. [PMID: 31035741]

[36] Jaisinghani RN. Antibacterial properties of quercetin. Microbiol Res (Pavia) 2017; 8(6877): 13-4.

[37] Sarkar P, Yarlagadda V, Ghosh C, Haldar J. A review on cell wall synthesis inhibitors with an emphasis on glycopeptide antibiotics. MedChemComm 2017; 8(3): 516-33. [http://dx.doi.org/10.1039/C6MD00585C] [PMID: 30108769]

[38] Mathur S, Mathur T, Srivastava R, Khatri R. Chlorhexidine: The gold standard in chemical plaque control. Natl J Physiol Pharm Pharmacol 2011; 1(2): 45-50.