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Research Article

Antibacterial Potential of Edible Vegetables and Fruits Extracts against Oral Pathogen of *Enterococcus faecalis* ATCC29212: An *in vitro* study

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Abstract

Background and Objective: Elimination of microbial irritants inside an infected root canal is the ultimate goal of a root canal treatment. Most of re-treatment cases of a failed root canal treated teeth reveal the existence of *Enterococcus faecalis* in the pulp and periapical compound. *E. faecalis* has the ability to survive under harsh condition following endodontic treatment. The present work entailed preliminary screening to determine the potency of antibacterial activity of edible fruits and vegetables against pathogenic oral bacteria *Enterococcus faecalis* ATCC 29212. **Materials and Methods:** Different solvents were used to yield edible vegetables and fruits extracts through maceration and liquid-liquid partition methods. Extracts of edible fruits and vegetables (50 mg mL⁻¹) were prepared and evaluated for their antibacterial activities against *E. faecalis* ATCC 29212 strain through agar well diffusion assay with chlorhexidine (20 mg mL⁻¹) used as the positive control. **Results:** Each sample of vegetables and fruits were extracted with four different solvents, methanol, *n*-hexane, ethyl acetate and water, to separate their chemicals constituents based on polarities. Phytochemicals screening showed that all the extract samples contain mainly flavonoids. Inhibition zone appeared for all the extract specimens in the agar plate confirming their antibacterial activities. **Conclusion:** Kemangi leaf (*Ocimum basilicum* L.) and Strawberry (*Fragaria xanayasa*) were found active and possess active antibacterial flavonoid against *E. faecalis*. These findings may support the use of edible plants in herbal medicine and as a baseline for the development of new drugs and phytomedicine for pathogenic oral diseases.

Key words: Edible plants and fruits, *Enterococcus faecalis*, root canal, antibacterial compound, oral bacteria

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The rationale of root canal treatment is to debride and sterilize the root canal and eliminate pathogens where tooth infection occurs, as a thorough control of root canal sterilization is required for a predictable treatment¹. Following a root canal preparation, the canal should be considerably clean with diminished number of pathogens. Oral pathogens such as *E. faecalis* seldom found in failed endodontic treatments where infection of the root canal and periapical tissue re-occurs. This bacteria is able to survive in unfavorable conditions, forming biofilms which enables it to penetrate dentinal tubules^{2,3}. *Enterococcus faecalis* is not only found in the root canals and can also exist in both the root canals and in saliva⁴. Root canal treatment has a success rate of 85% which is considered high, but in long term, some have failed and became reinfected⁵. Beside the nature of the bacteria involved in a failed root canal treatment, morphology of the root canal, especially at the apical portions, contributes to the failures. Irregularities of the dentinal wall and the existence of the accessories canals often missed during the bio-mechanical preparation, leaving the biofilm unreached⁶.

As an alternative to antibacterial agents for root canals sterilization, search for new antibacterial and anti-inflammation agents, irrigants, medicaments and materials for endodontic treatment are in demand. Edible plants and herbs have emerged as a possible alternative, since screenings have shown their significant active phytotherapy compounds^{7,8}.

This study looks into natural substances which may have potential activities against pathogenic oral pathogens, focusing on active agents derived from edible fruits and vegetables in eliminating *E. faecalis*⁹⁻¹². Concentrated juice of lemon and limes, basil leaves are known to have antibacterial and antioxidant properties^{13,14}. Periodontal pathogens are reported sensitive to bromelain source in pineapple¹⁵. *Averrhoa bilimbi* possesses antibacterial property against Gram-negative bacteria¹⁶. Considering the antibacterial property shown by edible fruits and vegetables, this study emphasized in screening samples for their antibacterial activities against *E. faecalis*.

Herbal medicines have been accepted as an alternative to modern medicine available. For this research, choosing edible fruits and vegetables as the source for developing an antibacterial agent was done with an assumption that the process for drug development would be simpler. Toxicity of fruits and vegetables is negligible, since they are consumed in daily basis.

MATERIALS AND METHODS

Materials: Fresh vegetables, Kemangi (*Ocimum basilicum* L.), Belimbing Wuluh (*Averrhoa bilimbi* L.), Lobak (*Raphanus sativum* Linn.) and fruits; Nanas (*Ananas comosus* L.), Strawberry (*Fragaria × ananassa* Duch) and Jeruk Nipis (*Citrus × aurantiifolia*) were collected in June 2017 from a commercial market in Bandung, Indonesia. Extracts were prepared by an extraction method with organic solvents of methanol, *n*-hexane and ethyl acetate.

Instruments: Laminar airflow, incubator memmert, autoclave machine HVE-50 Hirayama and jars.

Preparation of the vegetables and fruits extracts: Each sample of vegetables and fruits (2 kg) were extracted with methanol for 3 × 24 h, filtered and evaporated in vacuo at 40°C to give methanol extracts. Then, the extracts of vegetables and fruits were subsequent partitioning between *n*-hexane-water and ethyl acetate-water resulted of their *n*-hexane, ethyl acetate and water extracts, respectively as shown in Table 1.

Preliminary phytochemical screening: Screening for alkaloids, terpenoids and flavonoids secondary metabolites were performed to methanol, *n*-hexane, ethyl acetate and water extracts of previously mention vegetables and fruits^{17,18}.

Microorganism assay: *Enterococcus faecalis* ATCC 29212 were used for antibacterial test on Muller Hinton broth and Muller Hinton agar as medium, chlorhexidine (purchased from Merck Co. Ltd. and Sigma Aldrich) as positive control and anaerobic jar antibacterial assay.

Antibacterial activity: Susceptibility of vegetables and fruits extracts against *E. faecalis* ATCC 29212 can be evaluated from their inhibition zone of sample on bacteria growth by Kirby-Bauer method. The susceptibility test of sample was conducted at concentrations of 50 mg mL⁻¹, with chlorhexidine as positive control and MeOH and H₂O as negative controls which did not generate any inhibition zones. This determines the sensitivity or resistance of *E. faecalis* to compounds based on CLSI protocols^{19,20}. All samples were diluted with methanol-water (1:1), except chlorhexidine (control) with water. Paper discs (7 mm) were impregnated with 20 µL of each sample and then placed on the surface of the agar. Tests were performed in duplicate.

RESULTS

Vegetables and fruits extracts: All samples were extracted with MeOH for 3x24 h by subsequent partitioning between *n*-hexane-water and ethyl acetate-water to yield concentrated extracts as shown in Table 1.

Phytochemicals screening of the vegetables and fruits extracts: Data of the presence of secondary metabolite constituents of samples by phytochemical analysis indicated that phenolic and flavonoid compounds were found in all of the specimens; Kemangi contains steroid, saponin and tanin; Strawberry contains triterpenoid and tanin; Lobak contains saponin and tanin and Belimbing Wuluh contains triterpenoid; whereas none of the sample tested contain alkaloids as shown in Table 2.

Table 1: Data of weight extracts of fruits and vegetables

Samples	Extracts weight (mg)		
	<i>n</i> -hexane	Ethyl acetate	H ₂ O
Kemangi leaf (<i>O. basilicum</i> L.)	440	221	86
Belimbing Wuluh (<i>A. bilimbi</i> L.)	280	520	250
Lobak (<i>R. sativum</i> L.)	80	42.5	200
Nanas (<i>A. comosus</i> L.)	57.5	80	231
Strawberry (<i>Fragaria</i> × <i>ananassa</i> Dutch)	27.5	195	286

Table 2: Data of phytochemical analysis the extracts of fruits and vegetables

		Samples					
Secondary metabolites	Reagent	Kemangi (leaf)	Strawberry (fruit)	Nanas (fruits)	Lobak (fruit)	kemangi (bark)	Belimbing (fruits)
Phenolic	FeCl ₃ 5%	+	+	+	+	+	+
Flavonoid	HCl (p.a)+Mg	+	+	+	-	+	+
	H ₂ SO ₄ 2N	+	+	-	-	+	+
	NaOH 10%	+	+	-	+	+	+
Steroid	Lieberman burchard	+	-	-	-	+	-
Triterpenoid	Lieberman burchard	-	+	-	-	+	+
Saponin	HCl+H ₂ O	+	-	-	+	-	-
Tanin	FeCl ₃ 1%	+	+	-	+	+	-
Alkaloid	Dragendorff	-	-	-	-	-	-
	Wagnerr	-	-	-	-	-	-

Table 3: Antibacterial activity the extracts of fruits and vegetables at 50 mg mL⁻¹ against pathogenic oral bacteria *E. faecalis* ATCC 29212

Plants and fruits	Inhibition zones (mm)					
	Extracts			Standard and control		
	H ₂ O	EtOAc	<i>n</i> -hexane	CHx	MeOH (+)	H ₂ O (-)
Strawberry	0	6.8	7.0	17.7	0	0
Nanas	0	0	0	17.3	0	0
Lobak	0	0	0	17.3	0	0
Kemangi	7.1	7.5	7.0	17.4	0	0
Belimbing Wuluh	0	0	0	18.3	0	0

Antibacterial activity: The test showed that Nanas, Lobak, nor Belimbing Wuluh were not active against *E. faecalis*. However, H₂O, ethyl acetate and *n*-hexane extracts of Kemangi were active with inhibition zone values of 7.1, 7.5 and 7.0 mm, respectively; The ethyl acetate and *n*-hexane extracts of Strawberry showed inhibition zones of 6.8 and 7.0 mm, respectively as shown in Table 3.

DISCUSSION

In an attempt to pursue active antibacterial activities of edible fruit and vegetables, this study had confirmed that *n*-hexane, ethyl acetate and H₂O extracts of Kemangi (*O. basilicum* L.) and both *n*-hexane and ethyl acetate extracts of Strawberry (*Fragaria* × *ananassa* Dutch) were active against *E. faecalis* ATCC 29212.

Antimicrobial drug resistance has increased markedly over the last decade and challenges the sustainability of modern drugs discovery. Among the bacteria that causes infection of the oral soft and hard tissue is *E. faecalis*, Current treatment for pulpal and periapical lesions uses mainly 2% chlorhexidine as gold standard^{21,22}. Chlorhexidine causes discoloration of the teeth and induces drug resistance, therefore, there is a need to find and develop new

antibacterial compounds which are more selective, effective and efficient with no or a very limited of negative side effects.

Natural products are the source of therapeutically viable antibacterial agents. Higher plants synthesize diverse bioactive compounds that act as antifungal and antibacterial agents. This study presents preliminary data of antibacterial activity of some edible vegetables and fruits selected based on the fact that they are consumed in daily diet²³⁻²⁵.

The preliminary phytochemical screening described in Table 2 showed that all samples of vegetables and fruits contain phenolic and flavonoid constituents. This supports a previous study which indicated that flavonoids as an important antimicrobial compound²⁶.

Based on the assay data in Table 3, this study found that only Kemangi and Strawberry showed inhibition zones against *E. faecalis*. Strawberry (*Fragaria* × *ananassa* Duch.) fruits contained various phenolic compounds^{27,28}, including ellagic acid composed of 50.9% the total phenolic compounds in ripe fruits. The fruits also contained p-coumaric acid, trans-cinnamic acid, gallic acid, caffeic acid, p-benzoic acid, kaempferol, morin, myricetin, quercetin, resveratrol and these phenolic compounds have higher antimicrobial activity compared to other phenolic compounds²⁹⁻³¹. Strawberry extract was reported to inhibit the growth of Gram-negative bacteria; *Escherichia coli*, *Salmonella enterica* ser. Typhimurium, *Campylobacter jejuni* and *Candida albicans*³²⁻³⁵. In addition, the antimicrobial effect on 1D TLC bioassay plates for ethanol extract of unripe fruit was greater than that of the extract of ripe fruit³⁶. There were none of study that has reported the antibacterial activity of the Strawberry extract against *E. faecalis* has yet been reported paper³⁷.

Kemangi (*Ocimum basilicum* L.) also known as basil, is an aromatic herb used extensively for its distinctive aroma and for food flavoring. The essential oil of *O. basilicum* associated with existing standard antibiotics may increase their antibacterial activity, resulting in a synergistic activity against bacterial strains of clinical importance. The antibacterial activity of *O. basilicum* essential oil may be associated with linalool. The essential oil showed antibacterial activity against strains of *S. aureus*, *E. faecalis*, *L. monocytogenes*, *E. aerogenes*, *E. coli*, *S. enteric* and *S. typhimurium*³⁸. The extract of *O. sanctum* showed antibacterial activity against *S. mutans*¹³, while the activity of the leaf extract of *O. basilicum* against *E. faecalis* has not been reported. The antibacterial activity of the *n*-hexane, ethyl acetate and water extracts of *O. basilicum* is covered in this study.

Referring to the phytochemicals analysis, it is strongly suggested that the active antibacterial constituent is the flavonoid group. Flavonoids interfere with cell functions in the

eukaryotic system. Although there has not been any thorough research into the mechanisms underlying the antibacterial activity of flavonoids, previous research had suggested that different compounds would target components of bacterial cells differently³⁹.

The results showed that extracts of Kemangi and Strawberry have potentials antibacterial agents. The lead compounds of the extracts maybe useful as an intracanal antiseptic to cure disease caused by *E. faecalis*.

CONCLUSION

Screening of some edible vegetables and fruits extracts led the study to conclude that Strawberry and Kemangi extracts have potential as antibacterial agents due to their strong antibacterial activity against *E. faecalis in vitro*. Further work is required to isolate and determine the lead antibacterial compounds that play role in inhibiting or eliminating bacteria.

SIGNIFICANCE STATEMENT

This is a preliminary search for an alternative oral drug derived from edible fruits and vegetables that worked as potential antibacterial agents. None of previous study has reported the potential of antibacterial activities of extracted Strawberry and Kemangi against *E. faecalis*. The research will be subsequently followed by a thorough series of bioactivity-guided purifications that may lead to isolates which can be further developed and synthesized for pharmaceutical use.

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