

Antimicrobial Activity of *Persea americana* Peel Extract from North Sumatera, Indonesia , against Gram Positive and Gram Negative Bacteria in Vitro

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Abstract

Lots of species of Indonesian plants are suspected to have potential as an antimicrobial, but not yet optimally utilized. Avocado peel (*Persea americana*) is one of the nutritional plant species can be used as an antimicrobial agent. The aim of the study was to determine the antimicrobial activity of ethanolic extract of *Persea americana* peel (EEPA) against several bacteria. The extraction was conducted by maceration and concentrated using rotary evaporator using ethanol. The indicator bacteria used in this study were *Staphylococcus aureus*, *Proteus vulgaris*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The concentration of EEPA were 0,4%; 0,78%; 1,56%; 6,25%; 12,5%; 50%; 75% and 100%. The antimicrobial activity was conducted using disc diffusion method. The result showed that EEPA have antimicrobial activity against Gram positive and negative bacteria with the strongest inhibition zone at 100% and the weakest inhibition zone at 0,4%.

Keywords: avocado peel; lime citrus peel; antimicrobial; Gram positive; Gram negative.

1. Introduction

According to WHO (World Health Organization), more than 80% of world's population rely in traditional medicinal plant for their needs or maintaining their health. The use of medicinal plants in Asia has had a long history of interaction between humans and the environment [1].

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The study related to antimicrobial activity of medicinal plant is growing due to the increasing cases of antibiotic resistance occurring in almost parts of the world. Traditional healing system around the world utilize herbal medicine have already produced compounds that are effective against antibiotic-resistant strain of bacteria [2]. Medicinal plants have immunomodulatory and antioxidant properties, leading to antimicrobial activity. Medicinal plants are known to have a versatile immunomodulatory activity by stimulating specific and non specific immunity [3].

Avocado (*Persea americana*) is known as a fruit that contains carbohydrates, protein, fibers and micronutrient necessary for humans including vitamins, minerals and polyphenols. Like other fruit, the peel of the fruit is generally discarded, while in a study reported that the peel of avocado has a high antioxidant content [4]. The aim of the study was to determine the antimicrobial activity of ethanolic extract of *Persea americana* peel (EEPA) against Gram positive and negative bacteria to investigate the potential of this peel fruit as a source of antimicrobial agent.

2. Experimental section

2.1 Materials

The materials used in this study were peel of avocado, bacteria indicators, ethanol 96%, nutrient broth, Mueller Hinton Agar, distilled water, ethanol 70%, DMSO and blank disc

2.2 Instrumentation

The instrumentation including glassware, rotary evaporator, autoclave, petri dish, sieve, incubator and micro pipette

2.3 Procedures

Sample preparation

The peel of avocado was removed from its fruit, and then washed using running water. The peel of avocado was air dried at room temperature for 5 days and then dried once more at 40°C in the oven for 24 hours. The peel then mashed into powder and macerated using ethanol 96% for 5 days. After 5 days the filtrate was collected. The filtrate was concentrated using rotary evaporator and continued with heating on the hot plate stirrer to remove the solvent. The concentrated extract then diluted into several concentrations (75%; 50%; 25%; 12.5%; 6.25%; 3.125%; 1.562%; 0.78% and 0.4%) using DMSO.

Antimicrobial activity of EEPA (Ethanolic extract of *Persea americana* peel)

The disc diffusion method was conducted to determine the antimicrobial activity of EEPA. Briefly, the indicator bacteria (*Staphylococcus aureus*, *Proteus vulgaris*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*) were inoculated into sterile nutrient broth, incubated at 37°C for 24 h. Mueller Hinton Agar was prepared according to the manufacturer's instructions. The indicator bacteria were swabbed on to the surface of

agar using sterile cotton swab. The blank disc was dipped into EEPA and put on the surface of agar. The agar then incubated in incubator at 37°C for 24 h. The inhibition zone around the discs was measured using caliper.

3. Result and discussion

Inhibition zone of Indicator Bacteria

The diameter of inhibition zone around the blank disc was shown in table 1

Table 1: Mean of Inhibition zone (I.H) of EEPA against Several Indicator Bacteria

Indicator bacteria		Concentration of EEPA (%)									
S.aureus		0,4	0,78	1,562	3.125	6,25	12,5	25	50	75	100
	I.H (mm)	6	6	6	6	9,86	12,58	14,55	15,26	15,66	16,40
E.coli	I.H (mm)	6	6	6	6	9,86	12,58	14,55	15,26	15,66	16,41
P.aeruginosa	I.H (mm)	6	6	6	6	6	12,31	13,60	14,23	15,66	16,26
P.vulgaris	I.H (mm)	6	6	6	6	9,76	12,47	14,68	14,8	15,58	17,38
E.faecalis	I.H (mm)	6	6	6	6	6	10,98	12,71	13,85	14,49	16,1

The antimicrobial activity was classified into 3 levels; low activity (inhibition zone $\leq 12\text{mm}$), moderate activity (inhibition zone between 12-20mm) and strong activity ($\geq 20\text{mm}$) [5].

Based on the table.1 the strongest antimicrobial activity of EEPA was shown at concentration 100% and the weakest was at 6,25% against S.aureus, P.vulgaris and E.coli. For P.aeruginosa and E.faecalis, the weakest antimicrobial activity was shown at concentration 12,5%. The avocado leaf, stem, fruit and peel of avocado have biological activities proven. Studies with seed demonstrated antioxidant activity and antimicrobial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas* spp. Vinha and his colleagues [6] have studied about the bioactive compounds (phenolics, flavonoids, carotenoids, ascorbic acid and vitamin E) of edible and non edible parts (pulp, peel and seed) of avocado variety 'Hass'. It was reported that the peel of avocado has a high content of total phenolic as $679 \pm 117,0 \text{ mg/100 g}$ fresh

weight. The peel of the fruit also had the highest carotenoid content, since this part is usually the fraction where these phytochemicals are concentrated [6]. Rodriguez-Carpena and his colleagues [7] reported that peel and seeds of avocado had higher amounts and a more intense in vitro antioxidant potential than the pulp. The in vitro antioxidant activity against the ABTS Radical of acetone peel extract of avocado were approximately $103,75 \pm 44,49$ and for Hass varieties and $242,26 \pm 28,31$ for Fuerte varieties. The in vitro antioxidant activity of avocado peel extract against the DPPH radical were approximately $88,94 \pm 48,22$ for Hass varieties and $199,61 \pm 33,15$ for Fuerte varieties. Antasionasti and his colleagues [8] in their study about antioxidant activities and phenolics content of avocado (*Persea americana* Mill.) peel in vitro, reported that methanol extract of avocado peel has the strongest antioxidant activity. The highest antioxidant activity in methanol extracts is due to the high levels of phytochemicals having reducing properties such as phenolics, flavonoids and vitamin C. The antimicrobial activities of EEPA might be due to the high content of flavonoids and phenolic compound. Flavonoids are ubiquitous in photosynthesizing cells and are commonly found in fruit, vegetables, nuts, seed, propolis and honey. This class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity [9]. Phenolics are a class of plant secondary metabolites that contain one or more hydroxyderivatives of benzene rings. Phenolics are widely distributed in plants and are used for defensive functions in many plant species [10].

4. Conclusion

From the obtained result it can be concluded that avocado peel has a wide range of antimicrobial activities against Gram positive or Gram negative bacteria, which encourage replacing the synthetic medicines in treatment of diseases caused by these bacteria. Further studies needed in evaluation of cytotoxicity effect of avocado peel.

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