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SHORT COMMUNICATION

Antiphatogenic and Anti Food Spoilage Activities of Ethylacetate and Methanol Extract of *Panax ginseng* var. *Notoginseng*

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Javanese ginseng is a traditional herb known to possess broad health benefits that have been clinically proven. The aim of this research was to analyze the antimicrobial activity of Javanese ginseng against pathogenic bacteria (*Escherichia coli, Salmonella typhimurium, Bacillus cereus, Staphylococcus aureus*), food spoilage bacteria (*Bacillus stearothermophilus* and *Pseudomonas fluorescens*) and food spoilage fungi (*Aspergillus flavus, Fusarium graminearum*, and *Penicillium citrinum*). The result may increase the utilization of ginseng not only for health purposes but also as a natural food preservative. It may also open new possibilities for the development of natural functional foods. Ethylacetate and methanol extracts, obtained by maceration, were fractionated employing vacuum liquid chromatography (VLC). Fractionation using methanol and ethylacetate as solvents produced six fractions from each solvent. Fractions 1 and 4 of methanol extract performed the highest growth inhibitory effects on *Bacillus cereus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria), whereas fractions 4 and fraction 5 of methanol extract effectively inhibited the growth of *Penicillium citrinum*.

Key words: antimicrobial activity, extract, Javanese ginseng, pathogenic bacteria, food spoilage

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Ginseng (*Panax* spp.) is a herb whose roots and rhizomes have been widely used as traditional medicine all over the world. More than 10 *Panax* spp. (Araliaceae) have been used, but the most popular is *Panax ginseng* (Asian ginseng).

Zhu *et al.* (2004) reported that almost 200 components of *P. ginseng* had been isolated and characterized, among others were ginsenosides, polyacetylenes, alkaloids, polysaccharides, oligosaccharides, oligopeptides, flavonoids, lipids, vitamins, and minerals; with ginsenosides (saponin triterpene) as the main fraction exhibiting biological activities.

Ginseng has been known to possess bioactive compounds and physicochemical activities that can decrease the risks of various diseases. According to a number of pharmacological studies undertaken over the last 20 years, *P. ginseng* extract could influence the cardiovascular system, the immune system, the endocrine system, and the nervous system (Oliveira *et al.* 2005); causing lowered blood pressure (Kim *et al.* 1994; Han *et al.* 1998; Jeon *et al.* 2000; Sung *et al.* 2000); and playing a role as an antioxidant (Kim *et al.* 1992). Empirically, ginseng had been used as supplement to cure fatigue (Attele *et al.* 1999), improve gastrointestinal symptoms, as a sedative, and as a tonic (Takagi *et al.* 1972 a, b; Nabata *et al.* 1973; Kaku *et al.* 1975). In fact, ginseng water extract, especially ginsenosides, had the ability to accelerate small intestine transit times which meant it was effective in directly suppressing intestinal motility on muscles and inhibited the cholinergic nervous system (Hashimoto *et al.* 2003).

Hexane extracts from ginseng (*Panax ginseng* var. *notoginseng*) rhizomes did not show antimicrobial activities against pathogenic bacteria (*Escherichia coli, Salmonella typhimurium*, and *Bacillus cereus*) and food spoilage bacteria (*Staphylococcus aureus, Bacillus stearothermophilus*, and *Pseudomonas fluorescens*) or food spoilage fungi (*Aspergillus flavus, Fusarium graminearum*, and *Penicillium citrinum*). On the other hand, ethylacetate extracts had the ability to inhibit the growth of all microbes tested, while methanol extracts were able to inhibit only the bacteria tested (unpublished data). Therefore, the goal of this research was to reexamine the antimicrobial activities of ethylacetate and methanol extract fractions against pathogenic and food spoilage bacteria, as well as food spoilage fungi.

Sample material used in this research was Javanese ginseng rhizome powder obtained from Semarang (Central Java). Bacteria tested were *E. coli, S. typhimurium, B. cereus, S. aureus, B. stearothermophilus*, and *P. fluorescens* obtained from the Food Microbiology Laboratory, Center for Food and Nutrition Studies, Institut Pertanian Bogor; fungi tested were *A. flavus, F. graminearum*, and *P. citrinum* all from the Microbiology laboratory, Indonesian Institute of Sciences (Lembaga Ilmu Pengetahuan Indonesia), Bogor.

Test bacteria were prepared in 10 ml nutrient broth media, incubated at 37 °C for 24 h. The test-bacteria culture (10^5 CFU ml^{-1}) was for use in the antimicrobial activity test of ginseng extract. Fungal cultures were subcultured on potato-dextrose-agar medium and incubated at 28 °C for 4-7 days until spores were produced. Spore suspensions were prepared by adding physiological saline solution containing 0.5% (vol/vol)Tween 80 to give a concentration of 10^5 spores ml^{-1}. This spore suspension would be used in antimicrobial activity test of ginseng extract.

Ethylacetate and methanol extracts of Javanese ginseng, which were known to have antimicrobial activities, were obtained through multistage extractions without heating. The extraction process was conducted with the ratio of material to solvent 1:3 and repeated several times until a colorless extraction was produced. Each extract was evaporated using rotavapor at 45 °C to remove solvent residue. Concentrated extracts were placed into dark bottles, redried using freeze drying method, and then blown with N_2. The extracts would be used for antimicrobial tests employing a well-diffusion method (Garriga *et al.* 1993).

In antibacterial activity test, the four wells with a 6.2 mm diameter were prepared in one Petri dish, 60 µl of Javanese ginseng extract was put into two wells (duplicate) and the other two wells were each filled with 60 µl dimethyl sulfoxide (DMSO), and another with 60 ml solvent (both as a negative control). Then the agar plate was incubated for 24 h at 37 °C.

Fungal antimicrobial activity tests were conducted by inoculating 60 µl Javanese ginseng extract into two wells, and the other 2 wells containing 60 µl DMSO, and 60 µl solvent (as a negative control) respectively. The agar plate was incubated for 48 h at 28 °C (room temp.).

The fractionation of ethylacetate and methanol extracts of Javanese ginseng, were known to possess high antimicrobial activities and broad spectrum inhibition against all tested microbes, was conducted employing vacuum-column-chromatography with silica gel 60 GF_{254} with a particle size at less than 45 µm. The objective was to find the fractions that played a role in the inhibition of bacterial growth. Initial fractionation was conducted to determine the optimal solvent system using thin layer chromatography (TLC). Solvents used consisted of proanalysis grade diethylether, petroleum ether, and chloroform. The fractions obtained were evaporated using a rotavapor at 45 °C, dried using N_2, and then weighed. A solution of each fraction (5%, wt/vol) was made by dissolving in DMSO for later use in the antimicrobial activity test.

Ethylacetate extracts and methanol extracts were subfractioned into six fractions. All six fractions from the ethylacetate extract did not show growth inhibitory abilities against the fungi tested. The fractions 1, 2, 4, 5, and 6 from the ethylacetate extract were able to inhibit the growth of *B. cereus*. In addition fraction 1 was able to inhibit the growth of all bacteria tested except for *B. stearothermophilus*, while fractions 3-6 inclusive were able to inhibit the growth of *E. coli* and *P. fluorescens* (Table 1).

Fraction 1-4 inclusive of methanol extract were able to inhibit the growth of all tested bacteria, whereas fraction 5 was only able to inhibit the growth of *B. cereus* (4.87 mm), *B. stearothermophilus* (4.36 mm), *E. coli* (3.65 mm), *S. typhimurium* (2.30 mm), and fraction 6 was able to inhibit the growth of *B. stearothermophilus* (3.00 mm) and *S. aureus* (0.70 mm). All six fractions of methanol extracts did not show growth inhibitory abilities against the fungus *F. graminearum*. In addition, fractions 1 and 2 were able to inhibit the growth of the fungus *A. flavus* (1.20 mm and 0.60 mm respectively), while fractions 4 and 5 were only able to inhibit the growth of *P. citrinum* (3.35 mm and 4.55 mm respectively) (Table 2).

Inhibition of several test bacteria by the ethylacetate extract was lower than that of the methanol extracted fractions, despite an indication from a previous research (unpublished data) that ethylacetate extract possessed antimicrobial activities against all tested bacteria. Therefore, it is safe to say that many fractions soluble in ethylacetate do not contain the active components needed to inhibit the growth of the test microbes.

Table 1 Activities of fractions of ethylacetate extract of Javanese ginseng against several tested microbes

Tested microbes	Zone of inhibition (mm) of fraction					
	1	2	3	4	5	6
B. cereus	0.95	1.01	0	0.85	1.00	2.18
B. stearothermophilus	0	0	0	0	0	0
E. coli	0.90	0	0.60	0.10	0.40	0.35
S. typhimurium	0.55	0	0	0	0.70	0
S. aureus	0.55	0	0	0.40	0	0
P. fluorescens	0	0	0.48	1.38	0.28	0.88
A. flavus	0	0	0	0	0	0
P. citrinum	0	0	0	0	0	0
F. graminearum	0	0	0	0	0	0

Table 2 Activities of fractions of methanol extract of Javanese ginseng against several tested

Tested microbes	Zone of inhibition (mm) of fraction					
	1	2	3	4	5	6
B. cereus	5.04	4.22	2.97	5.17	4.87	0
B. stearothermophilus	4.78	4.72	4.17	4.91	4.36	3.00
E. coli	3.85	3.30	3.13	5.77	3.65	0
S. typhimurium	4.22	4.78	1.01	3.67	2.30	0
S. aureus	4.53	4.19	1.90	3.02	0	0.70
P. fluorescens	1.98	3.13	2.13	3.93	0	0
A. flavus	1.20	0.60	0	0	0	0
P. citrinum	0	0	0	3.35	4.55	0
F. graminearum	0	0	0	0	0	0

Fractions of methanol extract of Javanese ginseng contained more active components. As a result, they had more effective inhibitory activity against the bacteria tested. In general, the methanol extract contains phenols, hydroquinones, alkaloids, flavonoids, steroids, triterpenoids, saponins, and tannins (Hiserdt 1998). Extracted phenolic components can easily penetrate bacterial cell walls, which might explain the high inhibitory activity against bacteria tested. This mechanism could be caused by the presence of accumulated lipophilic components in their cell wall or cell membrane which changed the composition of the cell wall components.

It is suspected that bacterial inhibition mechanism by methanol extract was the result of a reaction between the extract and cell membrane or components in cytoplasm. Phenolic compounds could react with bacterial cell, disturb transportation processes, cause coagulation of cytoplasmic components, and disturb the proton motive force that plays a role in the cell's energy production.

Based on the data of the antimicrobial activity test conducted on each six fractions of methanol and ethylacetate extracts, we can conclude that the fractions of methanol extract had broader antimicrobial capabilities compared to that of ethylacetate extract. Fraction 1, fraction 2, fraction 3, and fraction 4 of the methanol extract had a broad inhibitory spectrum against all tested bacteria with the highest inhibitory ability differently for each kinds of bacteria tested. Antifungal activity of the methanol-extract-fractions was shown only by fraction 1, fraction 2, fraction 4, and fraction 5, while all six fractions the methanol extract had no antifungal capabilities against the fungus *F. graminearum*. Ethylacetate fractions had low inhibitory abilities and inhibited few as tested bacteria, they were also unable to inhibit the growth any of the tested fungi.

This research has resulted in useful scientific information to widen the utilization of ginseng, not only for health purposes, but also as natural food preservative. By combining its benefits for health and its ability to preserve food naturally, it is possible to develop a functional drink formula for special purposes without the need to add synthetic preservative agents.

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