ABSTRACT

YUSPIHANA FITRIAL. The Potential Analysis of Seed and Root of Water Lily (Nymphaea pubescens Willd) as a Functional Food Prebiotic and Antibacterial Enteropathogenic Escherichia coli K1.1. Under supervision of MADE ASTAWAN, SOEWARNO T. SOEKARTO, KOMANG G. WIRYAWAN and TUTIK WRESDIYATI.

The purposes of this study were (1) to determine the antibacterial activities of water lily seed and root, both against pathogenic bacteria causing diarrhea and beneficial bacteria; (2) to identify phytochemical components in water lily seed and root, and to evaluate their activities as antibacterial against pathogenic bacteria causing diarrhea; (3) to measure and evaluate water lily seed’s and root’s prebiotic activities of carbohydrate fraction in vitro; (4) to evaluate biological activity of water lily seed’s and root’s flour and extract as antidiarrheal and prebiotic sources; (5) to evaluate the potential of water lily seed and its extract as immunomodulator.

Extraction of antibacterial components used multistage maceration extraction method based on solvent polarity level, i.e. hexane, ethyl acetate and ethanol. Each extract’s activities were tested using diarrhea-causing bacteria, Enteropathogenic E. coli K.1.1 (EPEC K1.1) and S. Typhimurium with agar well diffusion method. The MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values were calculated with the highest antibacterial activities. Fractination was performed on extract with the largest antibacterial activity using thin-layer chromatography. The activities of each fraction were tested qualitatively by bio-autography method. Qualitative phytochemical tests were performed on all extracts.

Carbohydrate fractions (glucose, fructose, sucrose, raffinose and stacchiose) in water lily seed and root were analyzed by HPLC. Carbohydrate fractions which were separated in previous stage were considered as sugar growth medium by modifying the growth medium of lactic acid bacteria and bifidobacterium in vitro.

In vivo test was carried out on Sprague Dawley male rats for 28 days. There were two groups of rats to be studied, i.e. normal (healthy) rats group and another rats group which were intervened by Enteropathogenic E.coli K.1.1. The rats group that was intervened by EPEC K1.1, after 2 weeks feeding treatments, were orally infected with EPEC K1.1 in a week. In this stage, a basal diet was made with casein as the feed protein source. Male rats were fed a basal diet (control group) or the same diet containing of water lily seed flour or the same diet containing of FOS (isocalory and isonitrogen) or a basal diet with water lily seeds extract (given orally) for 4 weeks. Biological activities of water lily seed and extract were evaluated by observing total count of E.coli in cecal content and on cecum mucosa; total count of both aerobe and anaerobe lactic acid bacteria, small intestine histology and immunohistochemistry IgA of test rat’s small intestine.

Results of this study showed that the water lily’s seed and root had an antibacterial activity against EPEC K1.1 and Salmonella Typhimurium, especially in ethyl acetate extract. Ethanol extract had the same, yet lower activity. Ethyl acetate and ethanol extract of the seed did not show inhibition against the growth of lactic acid bacteria (Lactobacillus sp) and Bifidobacterium bifidum.

The MIC and MBC values of the ethyl acetate extract on EPEC K1.1 were 0.89 (mg/mL) and 1.33 (mg/mL), respectively, while similar values of that on S. Typhimurium were 1.11 (mg/mL) and 1.33 (mg/mL), respectively. On the other
hand, MIC and MBC values of the root’s ethyl acetate extract on EPEC K1.1 were 1.11 (mg/mL), while similar values of that on S. Typhimurium were 1.11 and 1.55 (mg/mL), respectively.

Phytocompounds in water lily seed’s ethyl acetate extract were alkaloids, flavonoids, tannins, glycosides, saponins, and triterpenoids. Meanwhile, compounds within root’s ethyl acetate extract were alkaloids, tannins, saponins, glycosides and steroids. All fractions in the ethyl acetate extract had antibacterial activities against EPEC K1.1 and S. Typhimurium. These fractions were thought to be able to inhibit the growth of test-microbes by synergic action of the components.

Stachiose and raffinose were carbohydrate fractions of water lily’s seed and root that could possibly be considered as a prebiotic sources. They could be fermented by Lactobacillus acidophilus and Bifidobacterium bifidum. The level of stachiose and raffinose was 0.60 mg/g of dry weight and 10.69 mg/g of dry weight in the seed and root, respectively.

Water lily seed and its ethyl acetate extract at the level of ≈ 1.87 g water lily seed or ≈ 13.23 mg/g rat weight or 17.80 mg/ml ethyl acetate extract significantly prevented and inhibited the growth of pathogenic bacteria like EPEC K1.1 causing diarrhea in rats. Water lily seed had higher ability of preventing and inhibiting the growth of these bacteria than its ethyl acetate extract. Water lily seed and its extract served as immunomodulator (meaning to stimulate the production of IgA) as a defense against pathogenic bacteria attack in the digestion tract. Both water lily seed and root had antibacterial and prebiotic activities, but water lily seed had more potency as antibacterial, while its root had more potency as a prebiotic source.

Key words: water lily, seed, root, antibacterial, ethyl acetate extract, anti-diarrheal, prebiotic, phytocompound