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RESEARCH ARTICLE

ANTI-INFLAMMATORY ACTIVITY OF PARTIALLY PURIFIED BIOACTIVE MATERIALS FROM *PROTAETIA BREVITARSIS* SEULENSIS LARVAE.

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Abstract

The goal of this study was to establish function of the larvae via analysis of anti-inflammatory effect from *Protaetia brevitarsis* seulensis larvae. As a result of extraction depending on each solvent, the methanol extract showed the highest anti-inflammatory activity. Although all the fractions obtained from fractionation according to methanol concentration were detected by higher activities, especially, the activity was highly detected in both the supernatant and precipitated pellet of 50% methanol concentration. As the results of partition in organic solvents with 50% methanol fraction, the activity was detected in water phases of both chloroform and ethyl acetate from the supernatant and precipitated pellet of 50% methanol concentration. Therefore, since anti-inflammatory activity was detected from the water phases in the supernatant and precipitated pellet of 50% methanol concentration among *P. brevitarsis* larva extracts, it was estimated that the active substances have a tendency of both strong and weak hydrophilicities.

Introduction:-

The chemical composition of *Protaetia brevitarsis* larvae accounts for the highest percentage as crude protein content of 50.7~58.8% among 100 g of the larva powder based on dry weight. Among amino acids, the total content of essential amino acids such as methi onine, threonine, valine, isoleucine, leucine, phenylalanine, histidine and lysine had been reported as 178.5 ~ 206.7 mg/100 g (Chung et al., 2013; Noh et al., 2015). In particular, histidine contains the most abundance, which classify as an essential amino acid in the early childhood due to alleviating allergic symptoms and helping blood production. In addition, from a result of analyzing the contents of cysteine, aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine, arginine and proline to play an important role in growth of biological tissues and regeneration of muscle cells and to promote metabolism, glutamic acid known as a

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useful amino acid for improving cognitive ability and memory maintains the most amount (Chung et al., 2013; Noh et al., 2015).
As crude fat is 16.1~18.5%, ratio of the unsaturated fatty acid is 75.5~76.4%, which oleic acid (C18:1) accounts for more than 60% among them (Chung et al., 2013; Yeo et al., 2013; Noh et al., 2015). Ash and total dietary fiber contents have been reported by 8.36% and 5.31%, respectively. The content of minerals has been highly detected by potassium (K), phosphorous (P) and magnesium (Mg) (Chung et al., 2013; Noh et al., 2015).

For extraction according to each solvent, hot water extraction was treated at 100℃ for 90 min after adding the distilled water of 9 folds to the sample amount. Ethanol and methanol extraction were treated at 4℃ for 1 h after adding absolutely solvent of 9 folds to the sample amount, and then the supernatants were recovered by centrifugation at 2,000 rpm for 20 min. The extracted solutions were filtered through whatman No. 1 filterpaper and 0.2 um filter, and then applied for the study after concentration by 10 fold. For methanol fractionation, the hot water-extracted solution was adjusted into 30, 50 and 70% methanol concentrations, reacted at 4℃ for 1 h, separated into the supernatant and precipitated pellet by centrifugation at 2,000 rpm for 20 min, and then applied for the study after adjusting into the same volume with the originally used amount. Chloroform and ethyl acetate for partition were mixed with the same volumes of the supernatant and precipitated pellet of 50% methanol fractionation, phase separation was performed by centrifugation at 2,000 rpm for 20 min, and then applied for the study after adjusting into the same volume with the originally used amount.

Anti-inflammatory activity was assayed by BV-2 cell line, and the cell culture medium was composed of DMEM-low glucose medium (Sigma-Aldrich Korea, Seoul, South Korea), glucose 4 g/l, sodium bicarbonate 3.7 g/l, 10% FBS (fetal bovine serum) and antibiotics (100 unit/ml penicillin and 100 μg/ml streptomycin). The mixed medium was adjusted into pH 7.2~7.4, and then sterilized by 0.22 um filter. For analysis of cytotoxicity with MTS assay, BV-2 cells were inoculated into each well of a 96-well plate of 0.5×10^5 cells, cells were attached to the bottom of each well for 12 h, LPS was treated for 24 h, and then cytotoxicity was evaluated by MTS assay kit (Promega, Madison, WI, USA).

For assay of nitric oxide (NO), BV-2 cells were pre-treated with the same as MTS assay, and then the supernatant of culture was employed for NO assay. To relatively compare the amount of NO secreted by BV-2 cells to be induced into inflammatory response, the recovered culture broth was mixed by the same volume with Griess reagent (Enzo Life Sciences, Inc., Farmingdale, NY, USA), and then OD value was measured at 540 nm by Microplate reader (Synergy HT).

The significant test of experimental results was carried out by ANOVA (one-way analysis of variance) and Duncan's method in InfoStat software (version 2012) or SPSS statistical program (IBM, Armonk, New York, USA). From experimental results, the bars of each graph represented the mean and standard error of the results through at least three-replicated experiments, and the different letters indicated the significant differences ($p < 0.05$).

As shown in Fig. 1 the anti-inflammatory activity by the extraction solvent showed the highest activity in methanol, but the lowest value in hot water extract. According to these results, since it was estimated that hot water extract has relatively low activity owing to maintain various impurities, in the next purification, we did methanol fractionation according to each concentration after hot water extraction. As shown in Fig. 2, all methanol fractions were observed by high activity. Especially, since both the supernatant and precipitated pellet in 50% methanol concentration presented higher activities, partition of the next purification step carried out as a 50% methanol fractions.

Organic solvent partition was carried out by chloroform and ethyl acetate. As shown in Fig. 3, all the activities were shown in the water phases of chloroform and ethyl acetate in supernatant and precipitated pellet of the 50% methanol fraction.

Therefore, according to the results of this purification procedure, since anti-inflammatory activity was detected from 50% methanol supernatant fraction and water phase of organic solvent partition, it was assumed that the substances to have the activity maintain a high polarity. On the other hand, science anti-inflammatory activity appeared strongly from the precipitated pellet of 50% methanol fractionation and water phase of organic solvent partition, it was estimated that somewhat lower polarity substances are also involved in the activity.

Taken all together, we suggest that anti-inflammatory activity in BV-2 cell is induced by *Protaetia brevitarsis* larvae extract, and the activity is presented from high polar and middle polar bioactive substances.

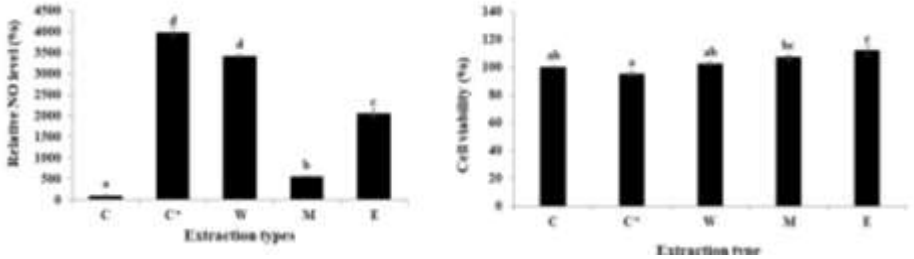

Fig. 1:- Antiinflammatory activity of bioactive materials in *Protaetia brevitarsis* larvae depending on extraction solvent types. Antiinflammatory activity was assayed by nitric oxide (NO) level in BV-2 cell. C; treated for 24 h by PBS buffer, C*, W, M, and E; treated for 24 h by 1 ug/ml LPS, W, M, and E; extracted by absolute water, methanol and ethanol, respectively.

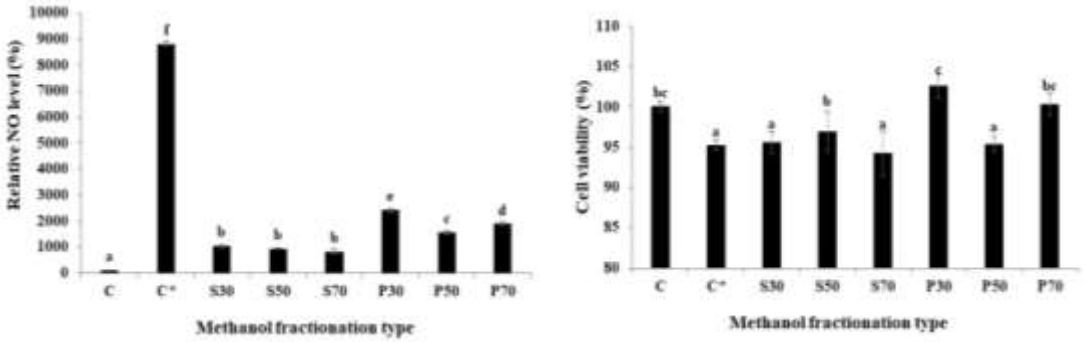

Fig. 2:- Antiinflammatory activity of bioactive materials in *Protaetia brevitarsis* larvae depending on methanol fractionation. Antiinflammatory activity was assayed by nitric oxide (NO) level in BV-2 cell. C; treated for 24 h by PBS buffer, C*, W, M, and E; treated for 24 h by 1 ug/ml LPS. S30, S50, and S70 indicate the supernatants obtained by centrifugation in 30%, 50%, and 70% methanol concentration, respectively. P30, P50, and P70 indicate the precipitated pellets obtained by centrifugation in 30%, 50%, and 70% methanol concentration, respectively.

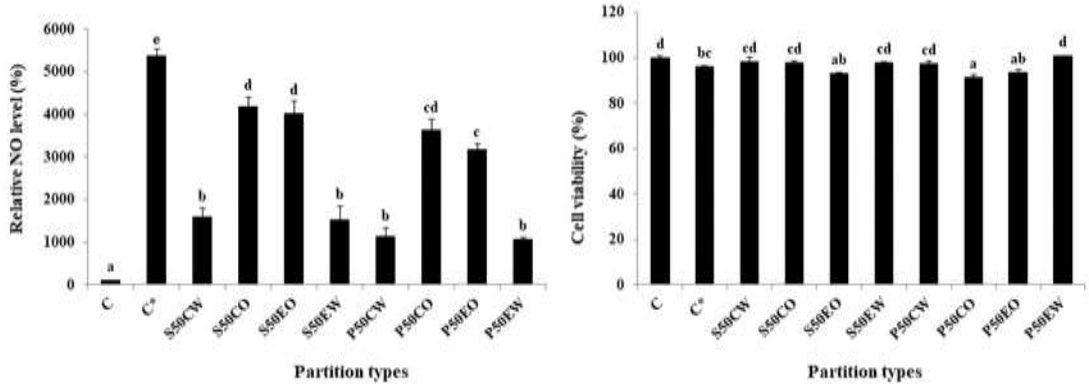

Fig. 3:- Antiinflammatory activity of bioactive materials in *Protaetia brevitarsis* larvae depending on organic solvent partition. Antiinflammatory activity was assayed by nitric oxide (NO) level in BV-2 cell. C; treated for 24 h by PBS buffer, C*, W, M, and E; treated for 24 h by 1 ug/ml LPS. S50CW and S50CO indicate water and organic phases originated from chloroform partition, respectively, by the supernatant of 50% methanol fractionation. S50EO and S50EW indicate organic and water phases originated from ethyl acetate partition, respectively, by the supernatant of 50% methanol fractionation. P50CW and P50CO indicate water and organic phases originated from chloroform partition, respectively, by the precipitated pellet of 50% methanol fractionation. S50CW and S50CO

indicate organic and water phases originated from ethyl acetate partition, respectively, by the precipitated pellet of 50% methanol fractionation.

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