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EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF THE TURKISH MEDICINAL PLANT *Sambucus ebulus*

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Sambucus ebulus *L. (Caprifoliaceae) herbs are widely used in Turkish folk medicine for the treatment of rheumatic pain. Anti-inflammatory and anti-arthritic effects of the extracts as well as fractions obtained from the aerial parts are investigated by using* in vitro *(PLA_2-inhibitory activity) and* in vivo *test models (carrageenan- and serotonin-induced hind paw edema in mice, and adjuvant-induced chronic arthritis in rats). Through the fractionation of the MeOH extract by successive extractions with hexane, chloroform, and n-butanol, an anti-inflammatory principle is isolated from the butanolic extract and its structure is elucidated as chlorogenic acid.*

In Turkish folk medicine, the aerial parts of *Sambucus ebulus* L. (Caprifoliaceae) are widely used for the treatment of rheumatic pain as a bath form. This study deals with the anti-inflammatory activity of the aerial parts of the plant and isolation of the active principle.

Both MeOH and aqueous extracts prepared from the herbs of *S. ebulus* showed potent anti-inflammatory activity when tested against the carrageenan-induced edema model as well as arthritic activity against adjuvant-induced chronic arthritis model in oral and topical administrations.

Through the fractionation of the MeOH extract, an active anti-inflammatory principle is isolated. By the interpretation of ^{1}H- and ^{13}C-NMR spectra as well as that of DEPT and 2D-NMR (H-H COSY, C-H COSY, C-H LR COSY; C-H NOESY, C-H COLOC) the structure is determined as 3-[[3-(3,4-dihydroxyphenyl)-L-oxo-2-propenyl]-oxy]-1,4,5-trihydroxy cyclohexane-carboxylic acid (chlorogenic acid).

This is the first report for chlorogenic acid to have an anti-inflammatory activity. Nevertheless, the potent anti-inflammatory activity of other caffeic acid derivatives has been reported previously. Kushihara et al. [1] reported that caffeic acid exhibits strong and specific inhibitory activity toward 5-lipoxygenase (5-LO), and Sugiura et al. [2] synthesized various caffeic acid derivatives of selective inhibitor for 5-LO. They claim that two adjacent hydroxy groups attached to the benzene ring, as well as hydrophobic alkyl side chain were required for its strong binding to 5-LO. This description for a strong activity is also compiled with our isolate.

EXPERIMENTAL

Extraction and Fractionation of the Material. Dried herbs (3 kg) are extracted with MeOH (50 liters) and evaporated to dryness. After dissolving the residue in H_2O, the extract is fractionated by successive solvent extractions with hexane, chloroform, and n-butanol satd. with H_2O. Each extract is then evaporated to dryness and weighed; hexane (87.5 g), chloroform (172.0 g), butanol (180.0 g), and final aqueous extracts (186.0 g). Butanol extract is further fractionated by precipitation from cold diethylether. The anti-inflammatory activity of each fraction is tested by using the serotonin-induced hind paw edema model in mice.

For the fractionation of the active fraction, the precipitate obtained from butanol extract is then submitted to a Diaion HP-20 column and eluted with H_2O, H_2O:MeOH (1:1), and MeOH, respectively. The activity is observed only in H_2O eluents

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and this fraction is further fractionated from the same column with H_2O and H_2O:MeOH (7:3). The former group of eluents showed the activity and this is applied to Sephadex LH-20 column for further fractionation and eluted with MeOH. Fractions are combined into four groups by TLC control (chloroform:MeOH:H_2O; 7:4.2:1); fractions 10-13, 14-19, 20-26, 27-34.

Fractions 27-34 obtained from Sephadex LH-20 column showed significant anti-inflammatory activity and purification of the active principle is first done by using a flash chromatography column on Wako silicagel 70FM using nitrogen gas and eluted with chloroform/MeOH/H_2O (7:4.2:1) and then C-18 RP cartridge using MeOH:H_2O (7:3).

Structure Elucidation of the Active Principle. NMR Spectra; Bruker-NMR. ^1H at 300.135 MHz, ^{13}C at 75.469 MHz, in DMSO-d_6, internal standard TMS; ^1H-NMR (ppm): 6.74, 7.49, 7.0, 6.18, 6.9, 5.32, 5.29, 3.65, 3.63, 1.87, 1.61, 1.54.

^{13}C-NMR (ppm): 165.7, 148.1, 145.4, 144.4, 125.5, 121.0, 115.7, 114.7, 70.35, 70.13, 69.09, 36.34, 35.93.

Anti-inflammatory Activity Studies

Carrageenan and Serotonin-Induced Edema. A minimum of seven animals are used in each test group. For carrageenan- and serotonin-induced hind paw edema tests male albino mice weighing 22 $\pm$ 2 g are used.

Details of both methods have been described previously [3].

Topical Effects of Extracts on Carrageenan-Induced Paw Edema. An ointment is prepared from each test sample using white Vaseline and applied on the right hind paw of each mouse, while contralateral paw is treated with the same amount of Vaseline. Details were given previously [4].

Adjuvant-Induced Chronic Arthritis. A method described by Newbould [5] is used with some modifications.

Assay for Phospholipase A2-Inhibitory Activity. The methodology was described previously [4].

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