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Isolation and Identification of a Flavonol Glycoside Using High Speed Counter Current Chromatographic Technique from the Leaves of *Salvadora Persica*

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This paper introduces the results of separation of a crude flavonoid sample, separated and identified as Kaempferol 3-α-L-rhamnosyl-7-β-xylopyranoside **1** alongwith Quercetin and Kaempferol from the 10% EtOH extract of the leaves of *Salvadora persica* through centrifugal partition chromatography (Pharma-Tech CCC-1000 Instrument) using upper phase as mobile phase and solvent system $CHCl_3$; MeOH; EtOH; H_2O (5:3:3:4). Detection was carried out at 254nm with flow rate 3ml/min and a rotational speed 800rpm. Three flavonoid peaks were resolved and eluted out in 3.5 h. The results obtained with the present CCC method are substantially far better than those from the conventional Column chromatography in terms of peak resolution and separation time, furthermore, separations are much improved.

Key words: High speed Counter Current Chromatograph (HSCCC), *Salvadora persica*, Kaempferol 3-rhamnoside-7-β-xyloside.

Introduction

Plant: *Salvadora persica* L (Salvadoraceae) commonly known as tooth brush tree in the Middle East, is widely cultivated in China, India, Persia, Middle East and Southern Africa. Different parts of the plants are used for treating swellings, ulcers and blisters, scorpion stings and for regulating menstruation (Ghazanfar 1994). Earlier report (Malik *et al* 1987) shows the presence of an alkaloid only. We now wish to report the separation and identification of flavonoids including a rare flavonol glycoside (Neeru *et al* 1990) from the leaves of *Salvadora persica.*

HSCCC: Counter Current Chromatography (CCC) is an efficient technique of partition chromatography that totally eliminates the use of solid supports. The name was derived from two classical partition methods: counter current distribution (CCD) and liquid chromatography (LC). These instruments are especially suitable for separating natural products in both analytical and preparative purposes. High speed counter current chromatography (HSCCC) is an advanced design of counter current chromatography that uses centrifugal forces to achieve high retention of the stationary phase and vigorous mixing between the stationary and mobile phases as the mobile phase elutes through a series of columns. The operating principles of HSCCC is based on the hidrodynamic equilibrium system (HDES) which was defined by Dr. Ito of NIH (Ito 1996).

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Experimental

Mps; uncorr. UV spectra were run in MeOH and IR spectra in KBr disc. ^{1}H-NMR spectra were run at 400 MHZ. Chemical shifts are given in δ (ppm) with TMS as internal standard. TLC was performed using silicagel $60F_{254}$ (Merck). Whatman No.1 paper was used in paper chromatography (PC). The TLC spots were visualized in UV light (365nm), after spraying with natural products-polyethylene glycol reagent (NP/PEG). For HSCCC two phase solvent system, composed of chloroform: methanol:ethanol: water at a 5:3:3:4 volume ratio was exclusively used. Yellowish brown plant extract used for CCC separation was obtained from dried *Salvadora persica* leaves by 10% ethanol extraction. The final solution was prepared by dissolving the above extract in the solvent mixture at a concentration of 2%. The yield of **1** was found (0.57%) with respect to 10% ethanol extract.

Acid Hydrolysis of 1: The glycoside was refluxed in 2M HCl-MeOH (5ml), for 2 h, then H_2O was added and the mixture was extracted with EtOAC. The aqueous layer was neu-

tralized with Ag_2CO_3, the ppt. filtered and the filtrate evaporated to give a residue. The aglycone in the EtOAC fraction was crystallized from $CHCl_3$-MeOH as yellow needles, m.p. 280-281° and identified as Kampferol by spectral and chromatographic comparison with an authentic sample.

Acetylation of 1: Compound **1** was acetylated with Ac_2O and pyridine (1:1) at room temperature for 48 hours and worked up in usual way. ^{1}H-NMR (400MHz, $CHCl_3$) δ7.82, (2H, d j=9Hz, H-2',6'); 7.30 (2H, d, J=9Hz, H-3'.5'), 7.04 (1H,d, 2.4Hz, H-6), 5.19 (1H, d, J=8.3Hz, H-1 Xyl), 5.59 (1H, d, J=1Hz, H-1-Rham); 1.22 (3h, d, J=6. 1Hz, Rham-Me), 3.75-5.70 (1 OH, m, Gly-H), 2.2. (3H, s, OAc-5) 2.28 (3H, s, OAc-4) 1.98-2.20 (18 H, m, aliphatic MeCO).

Procedure. Separation of crude flavonoid sample was performed by HSCCC instrument using the upper phase as the mobile phase. Separation with multilayer coil plant centrifuge was performed as follows: The coiled column was first filled with stationary lower phase followed by injection of sample solution through the sample port. Then, the apparatus was rotated at the optimum revolutional speed 800 rpm while the upper mobile phase was pumped into the head and of the column at 200 ml/hr flow rate. Effluent from the tail of the coiled column was continuously monitored with a UV detector at 254nm and fractionated into test tubes with fraction collector. Aliquot of each fraction was diluted with methanol and the abosorbance was determined at 254nm with spectrophotometer.

Separation of a flavonol glycoside from *Salvadora persica* by CPC; Instrument Pharma-Tech CPC 1000; solvent system $CHCl_3$-MeOH-EtOH-H_2O(5:3:3:4); detection 254nm, mobile phase-upper phase; flow rate 3ml/min, rotational speed 800 rpm, sample 200 mg.

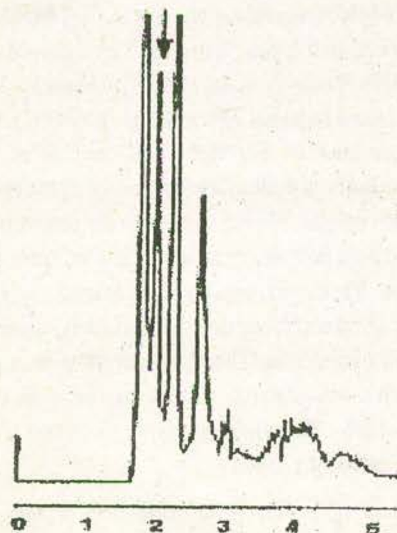
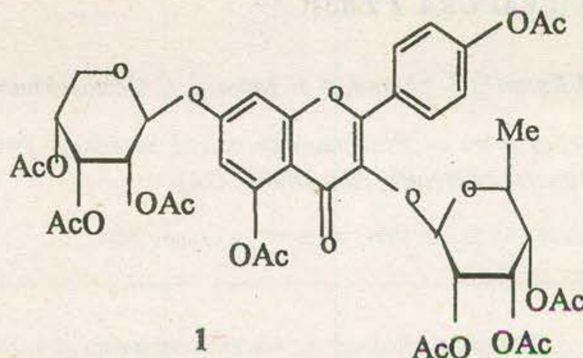


Fig 1.



Scheme I

Results and discussion

Compound **1** analysed for $C_{26}H_{28}O_{14}$. Its IR spectrum showed strong absorption bands at 3420 (OH), 1655 (C=O), 2950 (C-H), 1620 (C=C, aromatic) and a broad band at 1120-1000cm^{-1} (C-O). Colour reactions (dull ochre to fluorescent yellow in UV+NH_3), R_f values and UV spectral data with diagnostic shift reagents (Mabry *et al* 1970 and Harborne *et al* 1975) suggested it to be a 3,4-disubstituted flavonol glycoside with free hydroxyl groups at the C-5 and C-4 positions on a 3,7-disubstituted flavonol glyglycoside frame work. Total acid hydrolysis of 1 with 2M-HCl yielded an equimolar mixture of L-rhamnose, D-xylose (PC and GLC) and Kaempferol (spectral and chromatographic comparision Neeru et al). Enzymatic hidrolisis of 1 with β-xylosidase gave the same products as above. However, a bathochromic shift of 12nm in band II with NaOAC (absent in glycoside) indicated that D-xylose was attached at the C-7 position in 1 (Neeru *et al* 1990). On the basis of above findings, **1** was identified as Kaempferol 3-α-rhamnopyranoside 7-β-xylopyranoside, a rare natural product being reported for the first time from Salvadora persica. The constituents quercetin and kaempferol were identified by standard procedures and direct comparison with authentic samples.

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