Isolation of flavonols from Euphorbia *wallichii* by preparative High Performance Liquid Chromatography.

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Abstract: Flavonoids are a diverse group of natural products found in all plants. In present study three flavonols namely quercetin, kaemferol and myricetin were identified and isolated in Euphorbia *wallichii* in different solvents. Study was carried out on acid hydrolyzed methanolic extracts which was further fractionated into diethyl ether, n-butanol, ethyl acetate and water extracts. Quercetin was found to be the most abundant flavonol present in Euphorbia *wallichii*. [Nature and Science. 2009;7(8):86-88]. (ISSN 1545-0740).

Key words: Antioxidants, Flavonoids, Euphorbia *wallichii*, prep- HPLC

1. Introduction:

Interest in the role of antioxidants in human health has prompted research in the fields of food science and horticulture to assess fruit and vegetable antioxidants (Kalt et al., 1999). The majority of the antioxidant capacity of a fruit or vegetable may be from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins rather than from vitamins C, E or B-carotene (Wang et al., 1996; K.hk.nen et al., 1999). Many of these phytochemicals may help to protect cells against the oxidative damage caused by free radicals (Wada and Ou, 2002).

Many of them play important roles as flower and fruit pigments, UV protectants, signaling molecules between plants and microbes, and regulators of auxin transport (Doone, 1991, Dixon, 1995). The flavonoids are also thought to have antioxidant, anti-allergenic, and anti-inflammatory effects, thus contributing to human health (Scalbert, 2005, Ross, 2002).

HPLC is gaining increasing importance for the analysis of plant extracts. The qualitative analysis which produces a "fingerprint" chromatogram obtained under standard conditions can be very useful for quality control of phytochemicals. Although TLC is a powerful and simple technique used for this purpose, there are situations in which it can produce doubtful results. HPLC can also be a useful tool in chemosystematics helping, for example, to characterize species on the basis of their secondary metabolite contents.

Reversed-phase HPLC has been used in a number of occasions for the analysis of flavonoids in plants. In one study it was used to distinguish species based on the quantitative variation of flavonoids among them.

2. Materials and method:

All reagents were of analytical grade. Quercetin , myricetin
and kaemferol were purchased from Sigma Aldrich.

2.1 Extraction Method:

The Euphorbia *wallichii* was collected from Murree hills Pakistan in June. 2008 and a voucher

specimen was deposited at LCWU Herbarium. The plant material (1.00 kg) were dried away from the sunlight, powdered and exhaustively extracted with methanol using Soxhlet extraction method to give solvent free crude methanolic extract (7.056%). The methanolic extracts were then acid hydrolyzed and tested for flavonoid contents using standard myricetin, kampharol, and quercetin by HPLC. The methanol extracts was then fractionated using diethyl ether, n-butanol, ethyl acetate and water to evaluate the most suitable solvent for separation.

2.2 Acid Hydrolysis:

Controlled acid hydrolysis was carried out with 10% acetic acid under reflux for 3.5 hours. These fractionated samples were then analyzed by HPLC without any further separation [Filippo Imperato]

2.3 HPLC Conditions:

The prep HPLC system (Waters) consisted of a UV detector (2487). Column was a C18, (250 x 4.6 mm, 5 mm particle sizes). Acetonitrile was purchased from Merck. Water was HPLC grade and acidified with 1 % acetic acid. Qualitative analysis was made with samples, in isocratic mode, with acetonotrile/water 1:1 at a flow-rate of 1 mL min-1. The injection volume was 10 uL (analytical mode) and 10 mL(prep mode) and elute was monitored at 254 nm. The filtered methanol extracts (0.5 microns) of Euphorbia wallichii and its fractions was injected under these conditions and compared with authentic standards of myricetin, quercetin and kaemferol, injected under similar conditions.

2.4 Qualitative HPLC analysis:

The method developed for HPLC fingerprinting provided a quick analysis of the methanolic extract and fractions obtained after fractionation. The conditions used led to a good separation of the peaks which could be identified by comparing the chromatogram with the chromatogram of the reference compounds obtained under the same conditions. This way a qualitative analysis was made in analytical mode of HPLC.

2.5 Quantitative HPLC analysis:

Quantitative isolation of flavonols was made by HPLC in prep mode. Flavonols were easily isolated using fraction collector according to retention time of different peaks.

2.6 Vacuum evaporation:

Fractions collected containing single compounds were subjected to vacuum evaporation and after complete solvent removal, weights were noted. These compounds were then confirmed by comparison with standards in analytical mode of HPLC.

3. Results and discussion:

Myricetin and kaemferol were not detected in all the fractionated extracts of Euphorbia wallichii while quercetin was the most abundant flavonoid aglycone (20.626%) present in methanol extract, most of it went into n-butanol extract (14.714 %) and the rest in diethyl ether extract (5.712%) when fractionated [table-1]. Quercetin has been reported to have interesting biological activities including the inhibition of the anticancer drug target, heat shock protein-9 (Hsp90) [Nagai et al. 1995; Hansen et al. 1997; Kudo et al. 1999; Wu & Yu 2000]. Euphorbia *wallvichii* extract in methanol presented a better source of Quercetin having antihypertensive properties.

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Table 1. Percentage of Flavanols isolated different extracts of *Euphorbia wallichi*

Sr.No	Extract	Myrcetin	Kampherol	Quercetin
1	Euphorbia Methanol	1.640%	nd	20.626%
2	Euw - Diethyl ether	1.250%	nd	5.712%
3	Euw -n-butanol	nd	nd	14.714%
4	Euw -ethyl acetate	nd	nd	nd
5	Euw -Water	nd	nd	nd

Nd= Not Detected

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