Reverse Phase High Performance Liquid Chromatographic analysis of flavonoids in two *Ficus* species.

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Abstract: HPLC is gaining increasing importance for the analysis of plant extracts. The flavonoids are also thought to have antioxidant, anti-allergenic, and anti-inflammatory effects, thus contributing to human health. Reversed-phase HPLC has been used in a number of occasions for the analysis of flavonoids in plants. In present study two *Ficus* species were analyzed for their flavonoid contents. Kaempferol,rhamnetin, myricetin, isorhamnetin and quercetin were used as standards. Results showed that quercetin was most abundant flavonol present and it was extracted in diethyl ether layer after fractionation. How ever myricetin was also present in good amounts. It was observed that *Ficus bhengalensis* contained a very high amount of flavonoids as compared to *Ficus religiosa.* [New York Science Journal. 2009;2(5):32-35]. (ISSN: 1554-0200).

Key words: HPLC, Qualitative and quantitative HPLC, Flavonoids, Quercetin

INTRODUCTION

Plants have the ability to produce a large variety of secondary metabolites, such as terpenoids, phenylpropanoids, flavonoids, and alkaloids, which together account for over 200,000 compounds [Dixon, RA, .et al. 2003]. The National Cancer Institute has identified a host of compounds found in foods and plants that possess cancer preventing properties. Among these are antioxidants, phytosterols, carotenoids, triterpenes, saponins, tannins, and flavonoids. These phytochemicals may augment immune function, inhibit the formation of cancer-causing nitrosamines, hinder hormonal activity, as well as induce phase I or phase 2 detoxification enzymes, thus protecting the body against chronic diseases, such as cancer. Even so, a substantial amount of additional research is needed in order to obtain a better understanding of the role these agents play in cancer chemoprevention. Flavonoids, including the anthocyanins, flavonols and flavones, are among the most intensely studied secondary products with over 6,000 known compounds [Harborne, 2000]. Many of them play important roles as flower and fruit pigments, UV protectants, signaling molecules between plants and microbes, and regulators of auxin transport [Dooner, 1991][Dixon, 1991]. The flavonoids are also thought to have antioxidant, anti-allergenic, and anti-inflammatory effects, thus contributing to human health [Scalbert, 2005][Ross, 2002].

The qualitative analysis by HPLC 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.

Experimental

Chemicals

All reagents were of analytical grade and were used as received. Quercetin (3,3,4,5,7-tetrahydroxyflavonol), myricetin (3,3,45,5,7-hexahydroxyflavone), kaempferol, rhamnetin, and isorhamnetin were purchased from sigma Aldrich.

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]

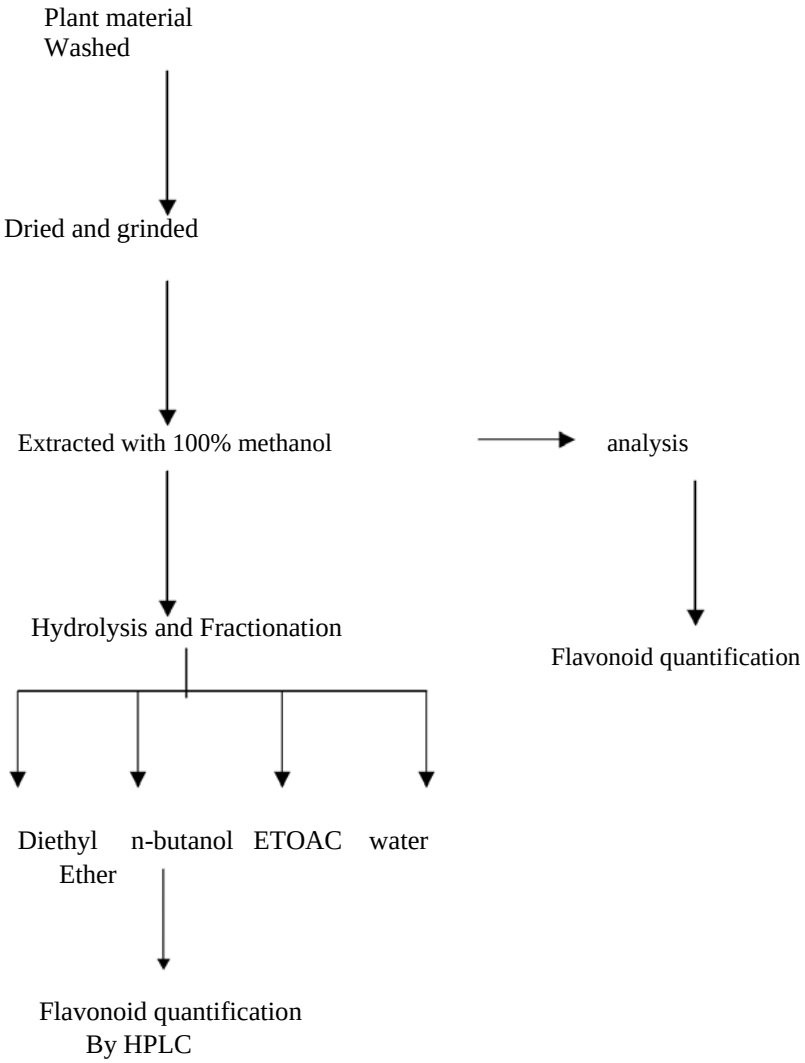
HPLC conditions

The HPLC system (Waters) consisted of a pump (1500 series), a UV detector (2487) was used in the study. Column was a C18, 250 x 4.6 mm, 5 mm particle sizes. Acetonitrile was from Merck. Water was HPLC grade. 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* and the elute was monitored at 254 nm. The filtered samples were injected under these conditions, as well as a mixture of authentic standards of leuteolin, myrcetin, quercetin, Kampherol,rhamnetin and isorhamnetin was also injected.

Sample preparation

Plants were collected from the university campus a voucher specimen were deposited at LCWU Herbarium. These were then dried at room temperature and for analysis the weighed portions of the dried sample were homogenized into powder. Ultrasonic extraction was performed using 100 % methanol. For the extraction 0.5 g of the ground plant was weighed and 5 mL of the extraction solvent was added. The sample was left at room temperature for 60 min and in an ultrasonic bath at room temperature for 20 min. The extract was filtered through a 0.45 µm filter and stored at + 4 °C in dark. The methanol extracts were then fractionated using diethyl ether, n-butanol, ethyl acetate and water to evaluate the most suitable solvent for separation.

Scheme for sample preparation:



Qualitative 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.

Quantitatification of flavonols:

Quantitative studies of flavonols were made by comparing with standard solutions of known concentration.

RESULTS AND DISCUSSION

Table-1: Percentage of methanol extracts of experimental plants

Names of Plants	**Code**	**Wt. of Fresh plant**	**%age Concentration of MEOH extract**
Ficus bhenghalensis	FB	1 Kg	7.056%
Ficus religiosa	FR	1Kg	6.966%

Table-2: Percentage of Flavanoids in different extracts of needles of *Ficus benghalensis* and *Ficus religiosa.*

Fraction	Myricetin	Kampherol	Rhamnetin	Isorhamnetin	Quercetin
FB Methanol	**2.857±0.1mg/kg**	0	0	0	**21.426±0.2 mg/kg**
FB- Diethyl ether	**2.07±0.3 mg/kg**	0	0	0	**5.712±0.1 mg/kg**
FB –n-butanol	0	0	0	0	**15.714 ±0.3 mg/kg**
FB -ethyl acetate	0	0	0	0	0
FB -Water	0	0	0	0	0
FR- Methanol	**1.0±0.5 mg/kg**	0	0	0	**4.29 ± 0.4 mg/kg**
FR -Diethyl ether	**0.08±0.3 mg/kg**	0	0	0	**2.857±0.1 mg/kg**
FR - n-butanol	0	0	0	0	**1.428± 0.5 mg/kg**
FR - Ethylacetate	0	0	0	0	0
FR -Water	0	0	0	0	0

Both plants were extracted with methanol and their methanolic extract weights were noted [table: 1]. These methanolic extracts were then hydrolyzed to convert glycosides into aglycones. Then fractionated with diethyl ether, n-butanol and ethyl acetate. These fractions were again subjected to HPLC analysis. It

was observed that most aglycone were present in diethyl ether layer after hydrolysis. Also it was clear from the study that quercetin was the most abundant flavonol in both species of *Ficus*[Table:2].

CONCLUSION

Ficus religiosa and *Ficus bhengalensis* are rich in flavonoids. The most important one Quercetin has been reported to have interesting biological activities including the inhibition of the cancer, heat shock protein-9 (Hsp90) [Nagai et al. 1995][Hansen et al. 1997][Kudo et al. 1999][Wu & Yu 2000].

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