International Journal of Research in Medical and Basic Sciences
Volume 5 Issue 6, June 2019 ISSN: 2455-2569 Impact Factor: 4.457
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

DETERMINATION OF FLAVONOIDS FROM SOME MEDICINAL PLANTS COMPOSITION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

D.M.Ismatov, N.Q.Muhadiev, O.F.Fayzullayev
Samarkand state university, Uzbekistan,

Abstract

Optimal conditions for separating and analyzing flavonoids from medicinal plants composition by High Performance Liquid Chromatography (HPLC) were found. It was found that Pimpinella anisum is rich in rutin and quercetin, codonopsis is rich in rutin, the amount of rutin is the same in Tanacetum Vulgare L. and Ferula moschata plants, the amount of quercetin in Ferula moschata is less by 3 times than in Tanacetum Vulgare L.

Keywords: flavonoid, plant, composition, HPLC, analysis, quality, quantity, rutin, quercetin.

Introduction. Flavonoids are in the first place on dissemination among biological active substances in plants and found in all plants composition in practice. Flavonoids consist of a large group of polyphenol compounds in C_6-C_3-C_6 – series (more than 5000). The basis of their molecule structure consists of flavan tricycle and two benzene rings (A and B) form heterocyclic ring (C) by connecting to each other through oxygen propane bridge [1-5]. As well as, flavonoids are classified on oxidized and reduced degree of their heterocycles into eight groups: flavones, flavonols, isoflavones, flavanones, catechins, anthocyanidins, leico-anthocyanidins (or flavandiols-3,4) and chalcones [6-9].

Flavonoids are found mainly in nature as combined with carbohydrates (glycosides). Monosaccharides (glucose, galactose, xylose, rhamnose, arabinose) and disaccharides (gentobiosis, sofos, rutinose) were disseminated mainly as carbohydrate residues. Three, four (upto six) carbohydrate residue derivatives combined less. Sugar combines to flavonoids without carbohydrates (aglicons) or on hydroxyl group 3 in states 7, 4', 3'. Formed bond goes through oxygen atom, these kinds of compounds are called O-glycosides and they get hydrolyzed easily. Flavonoids and their derivatives are physiological compounds and they

have a significant importance in medicine. Therefore determination of flavonoids from their composition is actual from the point of view of estimation of use possibility of medicinal plants.

Purpose of the research. High Performance Liquid Chromatographic study of the flavonoids composition of some medicinal plants.

Object and methods of the research. The above ground portion of the plants Pimpinella anisum, Codonopsis pilocula, Tanacetum Vulgare L. cultivated in the Oltinsoy district of Surkhandarya region and Urgut district of Samarkand region, as well as the underground part of plant Ferula moschata were taken for the research. The flavonoids compositions of the plants were studied using High Performance Liquid Chromatography (Shimadzu, Japan).

Experimental part. To extract flavonoids from plants composition, 5.0 g sample was grinded and extracted with 40 % ethanol solution using reflux (at $t=75^0C$) for 30 minutes. The obtained extracts were filtered after cooling, and the filtrates were used to determine flavonoids in plants composition.

In order to separate and identify flavonoids by High Performance Liquid Chromatography, a 4.6 x 150 mm column filled with the reversed phase C18 has been used. A solution of acetonitrile and orthophosphate acid (N_3PO_4) in water (pH = 3.5) in a ratio of 25:75 (or 1: 3) was used as eluent. The eluent consumption is 0.8 ml/min. Spectrophotometric detector, 255 nm. The sample amount was 5 µl. Chromatograms of the extracts obtained from plants under optimal conditions are shown in Figures 1-4.

International Journal of Research in Medical and Basic Sciences
Volume 5 Issue 6, June 2019 ISSN: 2455-2569 Impact Factor: 4.457
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

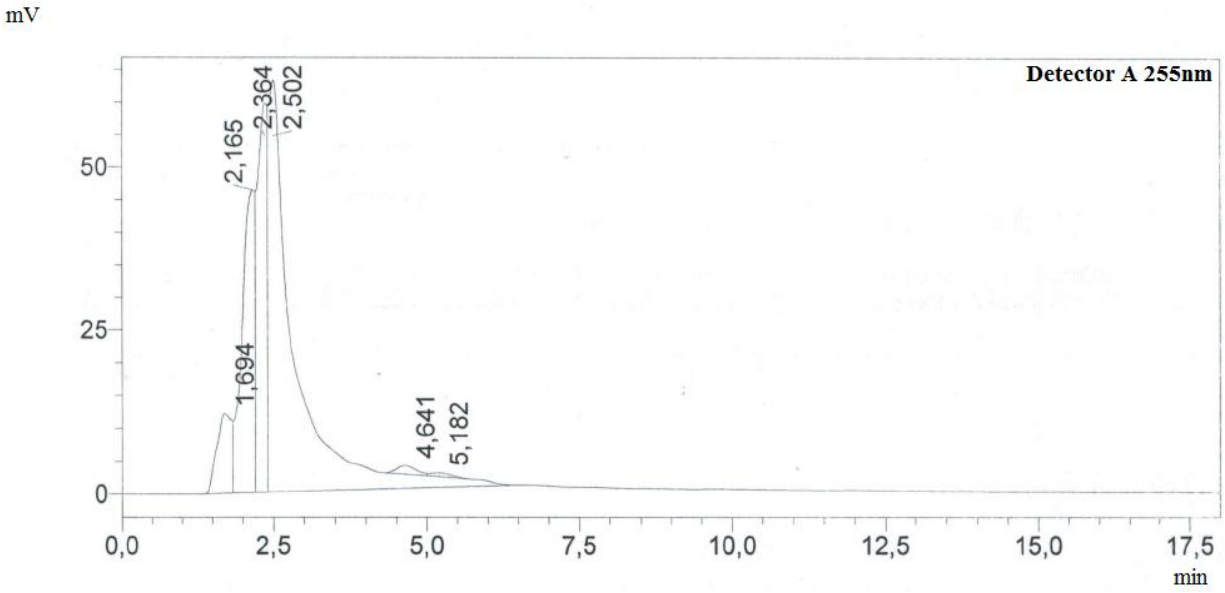

Figure 1. Pimpinella anisum plant extract chromatogram

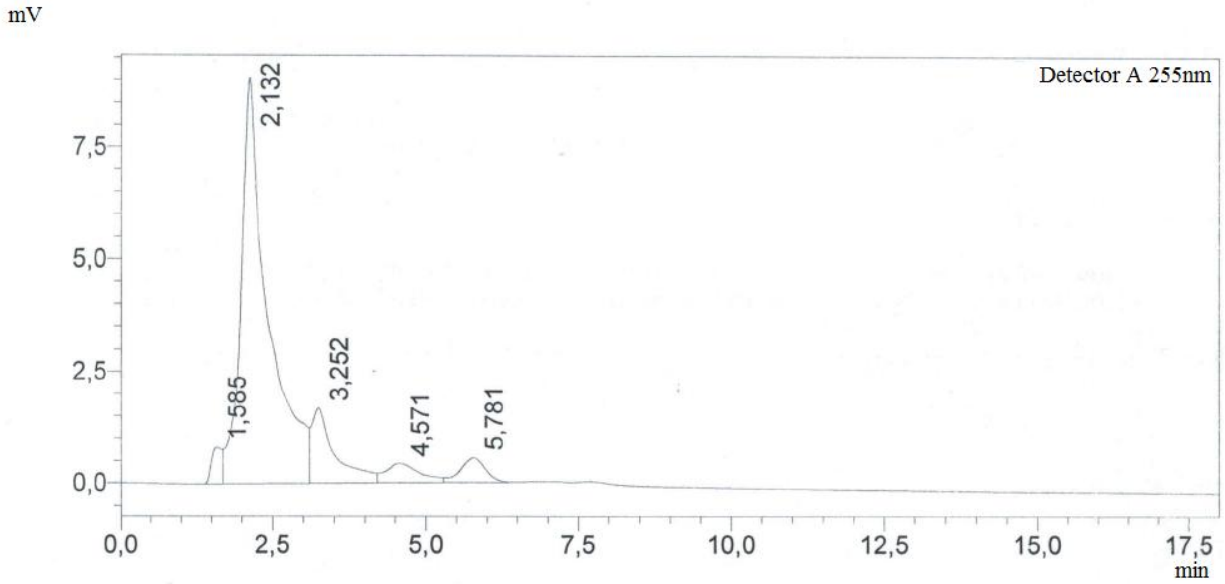

Figure 2. Tanacetum Vulgare L. plant extract chromatogram

International Journal of Research in Medical and Basic Sciences
Volume 5 Issue 6, June 2019 ISSN: 2455-2569 Impact Factor: 4.457
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

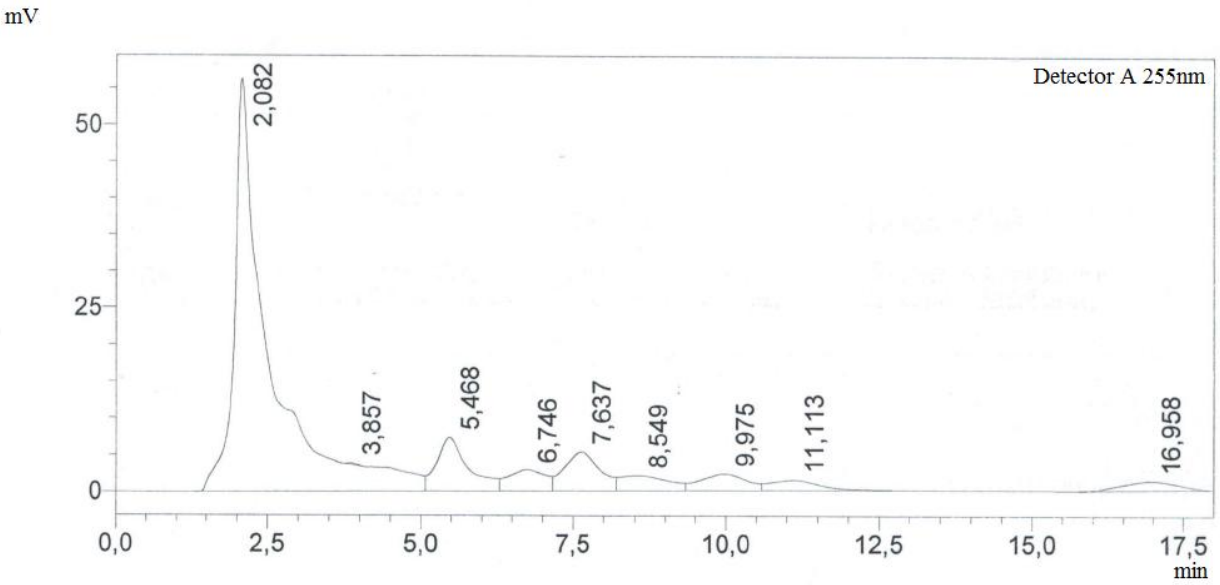

Figure 3. Codonopsis pilocula plant extract chromatogram

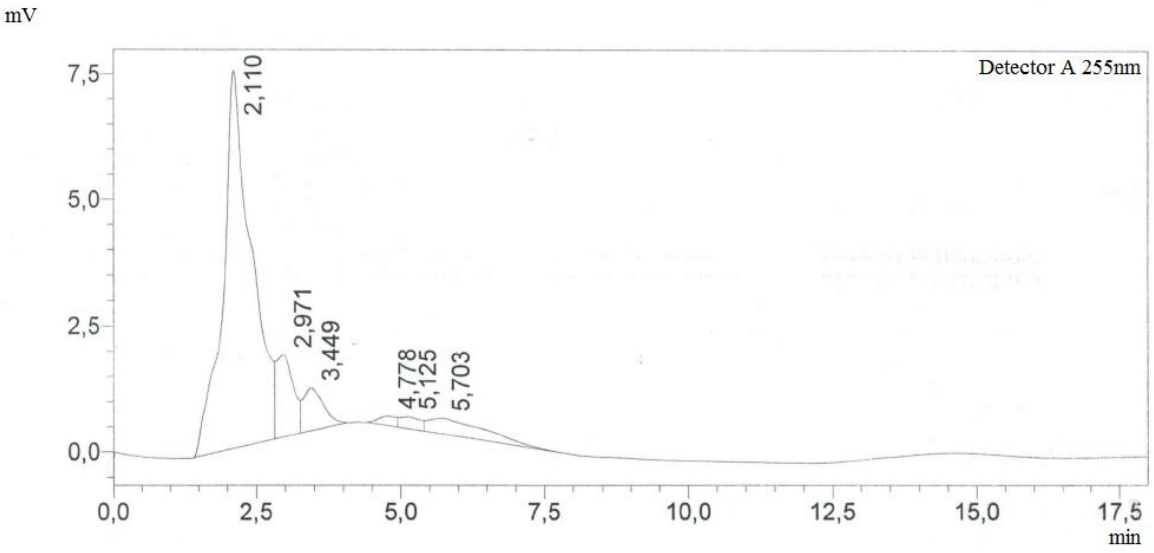

Figure 4. Ferula moschata plant extract chromatogram

Standard solutions of quercetin and rutin flavonoids were prepared for the determination of their amount and also a number of standard solutions were prepared by diluting them. For this purpose, 1.8 mg quercetin and 1.6 mg rutin were weighed in analytical weight and all of which were dissolved in 4 ml of acetonitrile and the resulting solution was diluted by 2, 3, 4, 5 times respectively, and other standard solutions were prepared. The sample amount fed to the chromatograph was 5 µl. The samples of standard solutions were

injected to chromatograph, their chromatographic characteristics were determined (retention time, height of chromatographic peak, surface etc.).

The dependence plots of the concentration of the standard solution on the chromatographic peak height (surface) were created separately for quercetin and rutin, and the dependence degree of the calibration curve was estimated by calculating the square value of the correlation coefficient (Figures 5 and 6). The obtained results show that the value of r^2 is greater than 0.99. This is notice for using the calibration curves to determine the amount of quercetin and rutin from various samples composition.

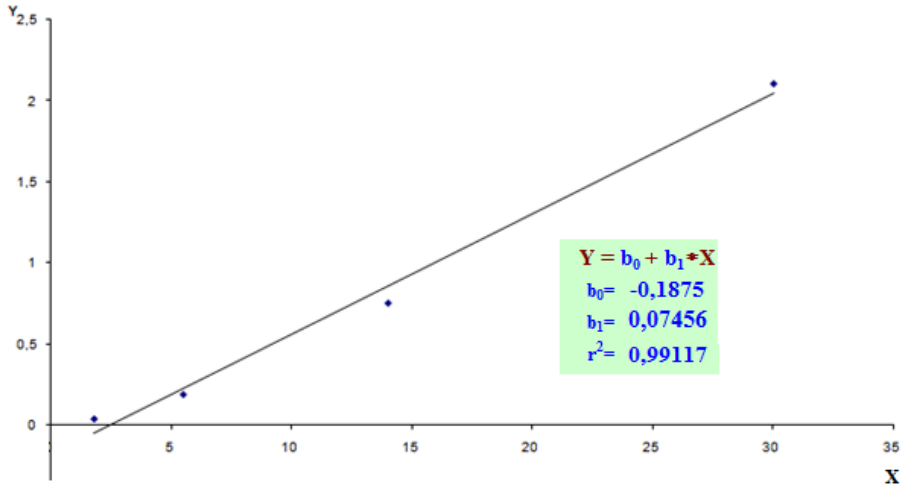

Figure 5. Calibration curve for quercetin

The extracts, obtained using the abovementioned method were used to identify flavonoids from the vegetative organs of the above ground and underground parts of the plant. The volume of the extracts was increased to 100 ml using 40% ethanol. The amounts of quercetin and rutin in the samples were determined by extrapolation method using a calibration curves. Other flavonoids and their quantitative composition found in chromatograms were determined based on the results presented in literatures. Repetition of the analyses is 5.

International Journal of Research in Medical and Basic Sciences
Volume 5 Issue 6, June 2019 ISSN: 2455-2569 Impact Factor: 4.457
Journal Homepage: http://mbsresearch.com, Email: mbsresearchp@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal

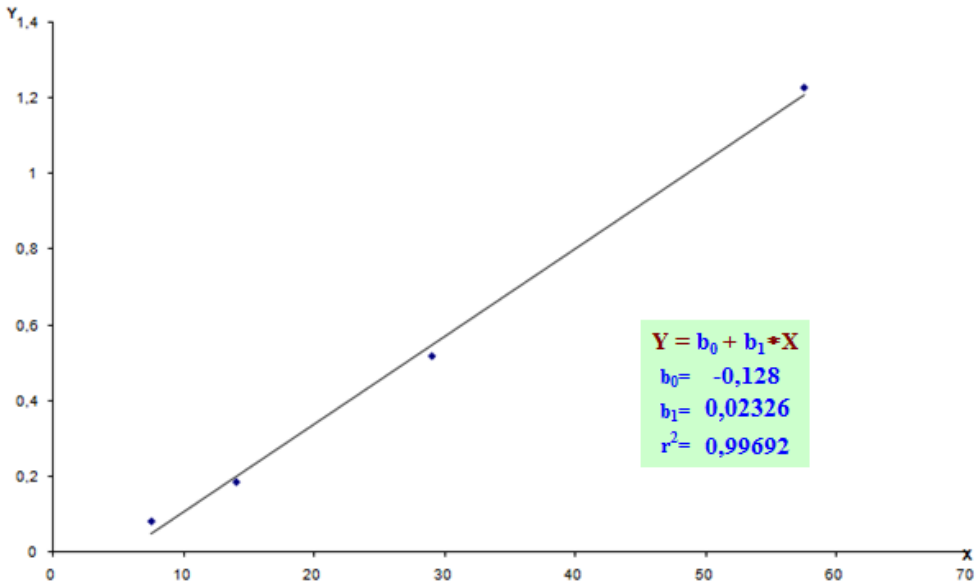

Figure 6. Differential Curve for Rutin

The amount of quercetin and rutin determined from composition of studied medicinal plants, are given in table 1.

Table 1

The amount of quercetin and rutin determined from composition of some medicinal plants

№	Plant	Flavonoids, mg/g	
		Rutin	Quercetin
1	Pimpinella anisum	50,415±3,216	9,977±0,657
2	Tanacetum Vulgare L.	9,264±0,732	1,084±0,082
3	Codonopsis pilocula	63,762±5,212	
4	Ferula moschata	7,595±0,614	0,354±0,028

According to the data in the table, Pimpinella anisum is rich in rutin and quercetin, and codonopsis is rich in rutin, the amount of rutin in plants of Tanacetum Vulgare L. and Ferula moschata almost the same, the amount of quercetin in Ferula moschata is less 3 times than in Tanacetum Vulgare L.

Conclusions

1. Optimal conditions for separating and analyzing flavonoids from medicinal plants composition by High Performance Liquid Chromatography (HPLC) were found.

2. It was found that Pimpinella anisum is rich in rutin and quercetin, codonopsis is rich in rutin, the amount of rutin is the same in Tanacetum Vulgare L. and Ferula moschata plants, the amount of quercetin in Ferula moschata is less by 3 times than in Tanacetum Vulgare L.

The research was fulfilled under sponsorship of the State grant OT-F7-83.

References

1. Krasilnikova L.O., Avksentyeva O.O., Zhmurko V.V. Biochemistry of Roslin. - Kharkiv: Kolorit, 2007.- P. 27-144. (in Ukrainian)

2. Levitsky A.P., Makarenko O.A., Sukmansky O.I. Phytoestrogens (biochemistry, pharmacology, use in medicine). - Odessa: Moryak, 2002. - 95 p. (in Russian)

3. Tutelyan V.A., Baturin A.K., Martinchik E.A. Flavonoids: content in Food products, consumption and Bioavailability // Nutrition Issues. - 2004.- 73, № 6. - P. 43-48. (in Russian)

4. Chekman I.S., Zavalko I.V. Flavonoids: pharmacotherapeutic aspect // Phytotherapy. Chasopie. - 2008. - № 1. - p. 3-11. (in Russian)

5. The Science of flavonoids / [Ed. by E. Grotewold] - New York: Springer Science, 2006. - 273 p.

6. Levitsky A.P. Bioflavonoids as modulators of estrogenic and osteogenic activity // VSN. pharmacology and pharmacy. - 2004. - № 2. - p. 2-4. (in Russian)

7. Hollman P.C., Arts C.W. Flavonols, flavons and flavanols - nature, occurrence and dietary burden // J. Sci. Food Agr. - 2000. - 80. - p. 1081-1093.

8. Santos-Buelga C., Scalbert A. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects and health // J. Sci. Food Agr. - 2000. - 80. - P. 1094-1117.

9. Tomas-Barberan F.A., Clifford M.N. Flavanones, chalcones and dihydrochalcones - nature, occurrence and dietary burden // J. Sci. Food Agr. - 2000. - 80. - p. 1073-1080.