FLAVONOID QUANTIFICATION AND ANTIOXIDANT ACTIVITIES OF SOME *PIMPINELLA* SPECIES

Zuhal Guvenalp[1],* Hilal Ozbek[1], Merve Yuzbasioglu[2], Ayşe Kuruuzum-Uz[2] and L. Omur Demirezer[2]

[1]*Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, 25240, Erzurum*
[2]*Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100, Ankara*

ABSTRACT

In this research, the flavonoid contents of the ethanol and acetone extracts of the aerial parts of *Pimpinella rhodantha*, *Pimpinella tragium* subsp. *lithophila*, *Pimpinella cappadocica* and *Pimpinella kotschyana* (Umbelliferae) were determined as rutin and hyperoside, and the antioxidant activity of the ethanolic extracts of these species was studied by using spectrophotometric assays. As a result, the maximum flavonoids containing plant, *Pimpinella cappadocica* was shown the highest antioxidant activity among the other researched *Pimpinella* species.

Key words: *Pimpinella* sp. Flavonoid quantification, DPPH free radical

1. INTRODUCTION

The genus *Pimpinella* (Umbelliferae) has 23 species, 2 subspecies, 2 varieties, 27 taxon growing in Turkey and 5 of them are endemic /1/. Some *Pimpinella* species are used as carminative, appetitive, sedative,

*Corresponding author.
Tel: +90-442-2315226
Fax:+90-442-2360962
E-mail address: guvenalp@atauni.edu.tr

antidepressant, antiseptic, diüretic and to increase lactation /2,3/. The essential oils of the fruits of some *Pimpinella* species are used in perfumery and drug industry. The biological activity studies of some *Pimpinella* species indicated that they have antimicrobial /4/, antibacterial /5/, antispasmodic /3/, estrogenic /6/, acariside /7/, anticonvulsant /8/, expectorant /9/, antifungal and antimalarial /4/ activities. According to the previous studies on *Pimpinella* species; phenylpropanoids /4,10/, sesquiterpenes /4/, phenolic compounds /11/, flavonoids /12/, coumarins /13/ and sterols /13/ were isolated. In our previous study, we isolated some flavonol glycosides such as kaempherol, quercetin and isorhamnetin glycosides from *P. kotschyana* /14/.

Natural products have been shown to be a tremendous and consistent resource for the development of new drugs /15/. Free radicals play a critical role in many diseases, such as cancer and cardiovascular diseases /16-17/. The intake of antioxidants such as polyphenols has been effective in the prevention of these diseases /18-19/. Flavonoids are major polyphenolic compounds. Their physiological and pharmacological functions may originate from their antioxidant properties /20,21/.

This study is aimed for determination of the flavonoid content of *Pimpinella rhodantha*, *Pimpinella tragium* subsp. *lithophila*, *Pimpinella cappadocica* and *Pimpinella kotschyana* and antioxidant properties of their extracts /22-24/.

2. MATERIALS AND METHODS

2.1. Plant Material

Pimpinella cappadocica was collected from Erzurum Ilıca-İspir road, the junction to Güllüce Village, in July 2009 (2240 m). A voucher specimen (HUEF 10001) has been deposited in the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Pimpinella kotschyana was collected from the road to Mardin-Midyat Ömerli Village in June 2009. A voucher specimen (HUEF 09011) has been deposited in the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Pimpinella rhodantha was collected from 3 km west of Zigana Tunnel on July 2009 (1650 m). A voucher specimen (HUEF 10002) has been deposited in the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Pimpinella tragium subsp. *lithophila* was collected from Kayabaşı Bridge

(20. km of Erzurum Köprüköy-Hınıs Road) on July 2009 (1720 m). A voucher specimen (HUEF 10006) has been deposited in the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

2.2. Procedure

2.2.1. Determination of the Flavonoids Content over Rutin

The air-dried aerial parts of the plant material were crumbled into small pieces and 1 g of it was extracted with ethanol (70 %) at 60°C under reflux condenser for 1 hour. The extraction was repeated twice at the same conditions. The extracts were filtered through filter paper into a 100 ml volumetric flask and adjusted to the mark with ethanol (70 %) (Solution A).

2 ml of solution A, 4 ml of ethanolic $AlCl_3$ (10 %) solution and 0.1 ml of dilute HCl were placed into a 50 ml volumetric flask and adjusted to the mark with EtOH (95%). The reference solution was prepared with 2 ml of solution A and 0.1 ml of dilute HCl in a 50 ml volumetric flask and adjusted to the mark with EtOH (95%).

0.05 g rutin and 10 ml EtOH (95%) was placed into a 50 ml volumetric flask and dissolved on a water bath at 80°C, and adjusted to the mark with EtOH (95%) (Solution A). 1 ml of solution A was placed into a 50 ml volumetric flask, treated with 4 ml $AlCl_3$ solution (10%) and 0.1 ml dilute HCl, and adjusted to the mark with EtOH (95%). The blind solution was prepared without $AlCl_3$ at the same conditions

The absorbances of the solutions were measured after 20 minutes on a spectrophotometer at 412 nm and the flavonoid contents were calculated over rutin by means of the following equation

$$\text{The flavonoid content calculated over rutin in percent} = \frac{\text{Absorbance of the test solution x mass of rutin (g) x 100 x 50 x 100 x 100}}{\text{Absorbance of rutin x the raw material mass (g) x 2 x 50 x 50 x 100}}$$

2.2.2. Determination of the Flavonoids Content over Hyperoside

The air-dried aerial parts of the plant material were crumbled into small pieces and 0.6 g of this material was placed into a flask. 1 ml hexametilentetramin (0.5%), 20 ml acetone and 2 ml HCl (25%) were added to the flask and extracted under reflux condenser for 30 minutes. After the

filtration of the extract through cotton, the residue was extracted with 10 ml acetone for 10 minutes twice. At the end of the extraction the filtrates were combined in a 100 ml volumetric flask and adjusted to the mark with acetone.

20 ml of the solution and 20 ml water were placed into a separating funnel and extracted with 15 ml ethyl acetate once, and with 10 ml ethyl acetate three times. The ethyl acetate phases were combined in a separating funnel and extracted with 50 ml water twice. The rinsed ethyl acetate phases were placed into a 50 ml volumetric flask and adjusted to the mark with ethyl acetate.

10 ml of this solution and 1 ml 2% $AlCl_3$:Glacial acetic acid-methanol reagent were placed into a 25 ml volumetric flask and adjusted to the mark with glacial acetic acid- methanol (5%). On the other hand for the blind solution, 10 ml test solution was diluted to 25 ml with methanol-glacial acetic acid.

The absorbances of the solutions were measured after 30 minutes on a spectrophotometer at 425 nm and the flavonoid contents were calculated over hyperoside by means of the following equation.

$$\text{The flavonoid content calculated over hyperoside in percent} = \frac{1.25 \times \text{absorbance of the test solution}}{\text{The raw material mass (g)}}$$

2.2.3. DPPH Radical Scavenging Activity:

For quantitative assays preparing plant extracts were also used for free radical scavenging activity measurement /22-24/. The air-dried aerial parts of the plant materials (*PR, PTL, PC* and *PK*) was crumbled into small pieces and 1g of it was extracted with ethanol (70 %) at 60°C under reflux condenser for 1 hour. The extraction was repeated twice at the same conditions. The extracts were filtered through filter paper into a 100 ml volumetric flask and adjusted to the mark with ethanol (70 %) (Solution A). 2 ml of this solution was used for the flavonoid content calculated over rutin. The rest of solution was evaporated to dryness.

The antiradical activity of the extract was assessed on the basis of the radical-scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Different concentrations (200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 10 µg/ml) of *Pimpinella rhodantha*, *Pimpinella tragium* subsp.

lithophila, *Pimpinella cappadocica* ve *Pimpinella kotschyana* species were examined for DPPH free radical scavenging activity /24/. All extracts and DPPH radical (0,022%) were prepared in methanol. After incubation of the radical and extracts for 30 minutes in room temperature, all samples were read spectrophotometrically at 517 nm. Control solution included all reagents except inhibitors. Ascorbic acid was used as a reference. Inhibition of free radical DPPH in percent (I%) was calculated as given below.

$$I\,\% = [(A_{Control} - A_{Extract}) / A_{Control}] \times 100$$

3. RESULTS AND DISCUSSION

In the present study, the flavonoid contents of *Pimpinella rhodantha*, *Pimpinella tragium* subsp. *lithophila*, *Pimpinella cappadocica* and *Pimpinella kotschyana* were determined by the spectrophotometric aluminium chloride method and results were calculated over rutin and hyperoside. Their radical scavenging effects were measured by using spectrophotometric DPPH method.

The flavonoid contents of *PC, PTL,, PK,* and *PR* were found as 2.571, 1.740, 1.350, 1.142 % over rutin (Table 1) and 0.816, 0.706, 0.366, 0.225 % over hyperoside (Table 2), respectively. Maximum flavonoid content belonged to *Pimpinella cappadocica* among the working species.

In this study, to evaluate the antioxidant activity of our products we used DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC_{50} values of *Pimpinella cappadocica, Pimpinella kotschyana*, *Pimpinella rhodantha* and *Pimpinella tragium* subsp. *lithophila* were found to be 30.93 μg/ml, 33.95 μg/ml, 54.28 μg/ml and 62.80 μg/ml, respectively (Figure). The scavenging effects of DPPH radical rose with increasing concentration of the extracts. The highest radical scavenging effect was observed on *Pimpinella cappadocica* extract.

Table 1

The Flavonoid Content Calculated as Rutin for *Pimpinella rhodantha*, *Pimpinella tragium* subsp. *lithophila*, *Pimpinella cappadocica* and *Pimpinella kotschyana.*

Plant material	Wavelength (nm)	Absorbance	The flavonoid content calculated as rutin
Pimpinella cappadocica	412	1.198	2.571
Pimpinella tragium subsp. *lithophila*	412	0.134	1.740
Pimpinella kotschyana	412	0.104	1.350
Pimpinella rhodantha	412	0.088	1.142

Generally, antioxidant activity depends on the number and positions of hydroxyl groups especially *o*- position. Glycosidation of flavonoids reduced their antioxidant activities /25-27/. These results suggested that flavonoids of *Pimpinella tragium* subsp. *lithophila* maybe have less *o*-hydroxyl group or more glycosidation than the other studied species. Therefore, our further extensive investigations were planned to find out the active antioxidative principles present in these antioxidant *Pimpinella* species.

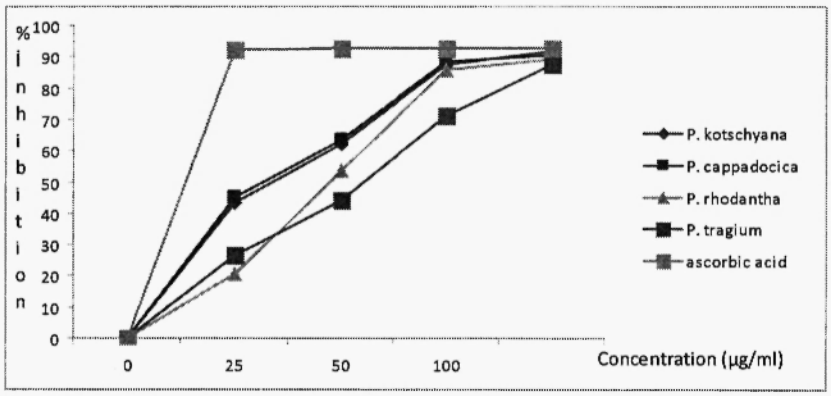

Figure: Antioxidant activities of *Pimpinella rhodantha*, *Pimpinella tragium* subsp. *lithophila*, *Pimpinella cappadocica* and *Pimpinella kotschyana* by DPPH test

Table 2

The Flavonoid Content Calculated as Hiperoside for *Pimpinella rhodantha*, *Pimpinella tragium* subsp. *lithophila*, *Pimpinella cappadocica* and *Pimpinella kotschyana*

Plant material	Wavelength (nm)	Absorbance	The flavonoid content calculated as hiperoside
Pimpinella cappadocica	425	0.392	0.816
Pimpinella tragium subsp. *lithophila*	425	0.339	0.706
Pimpinella kotschyana	425	0.176	0.366
Pimpinella rhodantha	425	0.108	0.225

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