UDK 581.192

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TLC IDENTIFICATION OF FLAVONOIDS IN THREE MONGOLIAN MEDICINAL PLANTS AND THEIR FREE RADICAL SCAVENGING ACTIVITY

In this study we have identified chemical and photochemicals in plants, *Centaurea cyanus L, Pulsatilla flavescens (Zucc.) Juz and Pulsaltilla ambigua stricta* in Mongolia. In traditional Mongolian medicine, these plants are used for inflammatory diseases.

Flavonoids are one of the most important classes of phenol compounds in higher plants. The primary significant role in this pattern is the screening of these photochemical in the plants. Qualitative determination of flavonoid compounds in the extracts was performed by Thin Layer Chromatography (TLC) procedures. The all studies were compared with plant stems and flowers. The following flavonoid compounds were identified from the plants studied. They are as follows: quercetin Rf: 0.35, apigenine Rf: 0.37, chrisyn Rf: 0.56, flavonon Rf: 0.85, morin Rf: 0.14 and galangin Rf: 0.6.

The influence of ethyl acetate extracts of plants, in concentration range 250 – 1000 μg/mL, on 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radicals was investigated by spectrophotometric methods.

Key words: *Centaurea cyanus L, Pulsatilla, Flavonoids, TLC, DPPH.*

Introduction

Flavonoids are members of a class of natural compounds with widespread occurrence in the plant kingdom. They are one of the largest groups of natural products known. Over 4000 flavonoids have been identified to date, widely distributed in the leaves, seeds, bark and flowers of plants. In plants, these compounds provide protection against ultraviolet radiation, pathogens, herbivores and are anthocyanin copigments in flowers that attract pollinating insects [1].

These compounds not only able protect the plants from stress and oxidative reaction but also give protection to human being from free radicals cascade due to the pharmacological properties like antioxidants, hepatoprotective, antimicrobial, antiallergic, analgesic and anti- inflammatory, anticarcinogenic and anti-obesity activities [2].

In the last decades natural alternatives to synthetic antioxidants have been intensively studied and the effects of antioxidative substances obtained from natural sources elucidated. Among them, flavonoids are a particularly attractive class of polyphenols, as they often occur in significant concentrations (0.5-1.5 %) [3].

Materials and methods

Plant Materials and sample preparing

Centaurea cyanus L, Pulsatilla flavescens (Zucc.) Juz and *Pulsaltilla ambigua stricta,* Plant materials, whole plants were collected in the region of Tuv aimag, separated stems and flowers and dried in air. Dried plants of were extracted with 70 % methanol at room temperature for 24 h used by sonicator. Aliquot of the obtained extract was evaporated to dryness (methanol extract). The amount of 80 % v/v of the extract was concentrated under the reduced pressure and the obtained product was fractioned using different organic solvents: hexane, chloroform, ethyl acetate and *n*-butanol.

Methods

Biologically active compounds such as flavonoids, alkaloids, and lignans were identified in plant samples using spectrophotometric and volumetric methods.

Chloroform, Ethyl acetate fractions were subjected for identification of flavonoids by performing TLC fingerprinting. The sample of these fractions spotted on precoated silica gel G aluminum plates 60F254 with the glass capillary tubes. The spotted plate with proper handling was placed in mobile phase of solvent system (toluene: ethyl acetate: Glacial Acetic Acid 18:6:2.5 v/v/v solvent) [1]. The spotted plate after proper separation of chemical constituents was take out from solvent system, and then dried at room temperature. The color of spots were seen in UV light (254nm and 365nm) and Rf values of each spots in were calculated and were tabulated in table 2.

Free radical scavenging ability of the fractions was tested by DPPH radical scavenging assay as described by Brand-Williams et al., [4]. DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at

different concentrations (250–1000 μg/mL). The reaction mixture was vortexed thoroughly and left in the dark at 30 min. When the DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm.

Dried samples of plant stems and flowers were finely powdered for XRF analysis. The XRF, DC 6500 instrument was used for elemental composition.

Result and discussion

The results of Table 1 show that *Centaurea cyanus L* has the highest content of flavonoids, alkaloids and lignans in the stems, at 0.25%, 1.82% and 0.45% respectively. *Pulsatilla flavescens (Zucc.) Juz* was not analyzed for TLC due to its low flavonoid content.

Table 1

The content of biological active compounds of the plants in Mongolia

Biological active compounds, %	*Centaurea cyanus L*		*Pulsatilla flavescens (Zucc.) Juz*		*Pulsaltilla ambigua stricta*	
	Stems	Flowers	Stems	Flowers	Stems	Flowers
Flavonoid	0,25	0,11	0,11	0,10	0,12	0,20
Alkaloid	1,82	0,37	0,50	0,71	1.,37	1,46
Lignan	0,45	0,34	–	–	–	–
Cumarin	–	–	–	–	–	–

TLC chromatograms of the chloroform and ethyl acetate fractions are presented in Table 2. Spots were characterized by Rf values and color under UV light and after spraying with 5% sulfuric acid in methanol.

Table 2

The Rf values of flavonoids in ethyl acetate and chloroform extracts of the plants

Flavonoids	Rf*	*Centaurea cyanus L*				*Pulsaltilla ambigua stricta*			
		Ethyl acetate		Chloroform		Ethyl acetate		Chloroform	
		Stem	Flower	Stem	Flower	Stem	Flower	Stem	Flower
Quercetin	0,35	–	–	0,35	–	–	–	–	–
Apigenine	0,37	–	–	–	0,37	–	–	–	–
Chrisyn	0,56	0,56	–	–	–	–	–	–	–
Flavonon	0,85	–	0,85	–	–	0,85	0,85	0,85	–
Morin	0,14	–	–	–	–	0,14	0,14	0,14	–
Galangin	0,60	–	–	–	0,6	0,60	0,60	–	0,6

* Spots Rf were compared with reference [1].

The results of TLC analyses show that different flavonoids are present in the investigating extracts. A largest number of flavonoids were found in ethyl acetate fraction of *Pulsaltilla ambigua stricta.* The ethyl acetate and chloroform extracts of *Pulsaltilla ambigua stricta* contain flavonon, morin and galangin flavonoids.

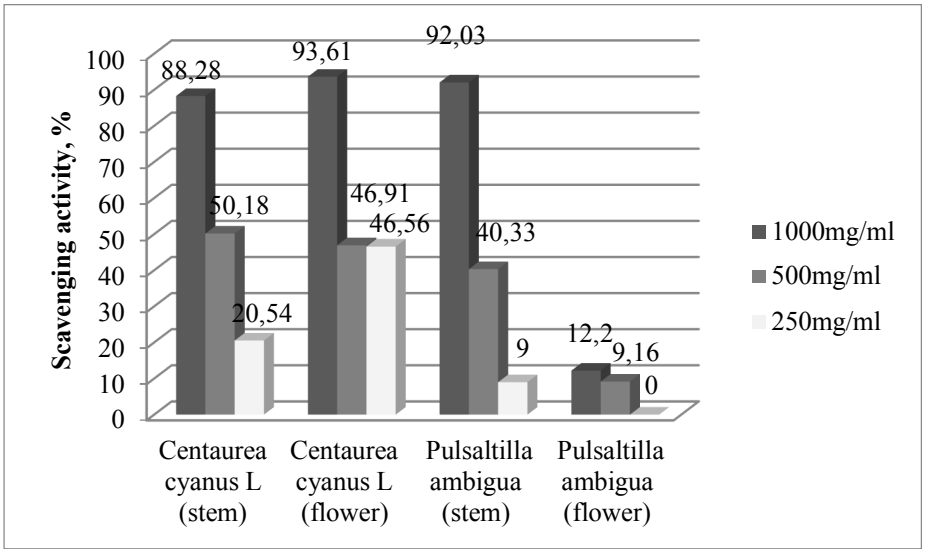

Figure 1. DPPH free radical scavenging activity of ethyl acetate extracts of plants

Figure 1 a shows the free radical scavenging activity of the ethyl acetate extracts of *Centaurea cyanus L* and *Pulsaltilla ambigua stricta.* Among the extractives, ethyl acetate extracts of stem of *Pulsaltilla ambigua stricta* possessed the highest activity. At a concentration of 1000 μg/mL, 500 μg/mL, 250 μg/mL, the scavenging activity of ethyl acetate extracts of flowers of *Centaurea cyanus L*, were 93.61%, 46.91% and 46.56%, respectively.

Macro- and micro-element identification experiments were carried out on plant stems and flowers (Table 3). Experiments have shown that, most of the elements are contained in the stems and flowers differently.

Table 3

Content of macro- and micro-elements of plants

№	Elements, μg/g	*Centaurea cyanus L*		*Pulsaltilla ambigua stricta*	
		Stem	Flower	Stem	Flower
1	Potassium, K	5 152,1	4 698,1	5 782,9	6 903,7
2	Calcium, Ca	5 407,1	6 062,1	5 135,4	2 856,1
3	Titanium, Ti	–	27,1	9,0	311,6
4	Copper, Cu	24,9	11,7	8,0	28,9
5	Zinc, Zn	50,5	50,7	62,7	98,6
6	Gallium, Ga	8,9	7,3	7,2	11,6
7	Arsenic, As	–	3,0	6,8	–
8	Rubidium, Rb	21,9	18,2	18,1	28,2
9	Strontium, Sr	92,7	117,5	75,5	38,4
10	Yttrium, Y	9,9	12,4	9,0	7,2
11	Zirconium, Zr	162,2	145,2	162,3	187,2
12	Niobium, Nb	117,7	109,5	105,0	105,9
13	Silver, Ag	73,4	74,5	66,1	84,9
14	Tin, Sn	1,4	1,6	1,2	2,3
15	Lead, Pb	61,2	29,0	60,2	76,3

The analytical methods allow the determination of 15 elements (K, Ca, Ti, Cu, Zn, Ga, As, Rb, Sr, Y, Zr, Nb, Ag, Sn, and Pb) in plants. The potassium was highest in flower of *Pulsaltilla ambigua stricta* at

6903.7μg/g, while calcium in flower of *Centaurea cyanus L* was highest at 6062.1μg/g. In some cases, the stems or flowers of these plants do not contain arsenic.

Conclusion

On the basis of the above experiments, it is concluded that, the biologically active compounds of plants are contained in different amounts in the stems and flowers.

Also, it is shown that ethyl acetate extracts of plants contain large amounts of flavonoid compounds and exhibits high free radical scavenging activities. Therefore, it is recommended to use ethyl acetate extract in further studies to separate flavonoid compounds from these plants with high yield and purity.

Acknowledgement

The authors wish to thank the other members of the Laboratory of Functional Food and Natural Products, Department of Chemistry, School of Arts and Sciences, National University of Mongolia. This research financially supported by the National University of Mongolia.

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TLC-ИДЕНТИФИКАЦИЯ ФЛАВОНОИДОВ В ТРЕХ МОНГОЛЬСКИХ ЛЕКАРСТВЕННЫХ РАСТЕНИЯХ И ИХ АКТИВНОСТЬ В ПРОЦЕССЕ ОЧИСТКИ ОТ СВОБОДНЫХ РАДИКАЛОВ

В представленном исследовании мы идентифицировали химические и фотохимические вещества в растениях Centaurea cyanus L, Pulsatilla flavescens (Zucc.) Juz и Pulsaltilla ambigua stricta в Монголии. В традиционной монгольской медицине эти растения используются при воспалительных заболеваниях.

Флавоноиды являются одним из наиболее важных классов фенольных соединений у высших растений. Основная значимая роль в этом паттерне – скрининг этих фотохимических веществ в растениях. Качественное определение флавоноидных соединений в экстрактах проводили методом тонкослойной хроматографии (TLC). Все исследования проводились со стеблями растений и цветами. Следующие соединения флавоноидов были идентифицированы из изученных растений. Они следующие: кверцетин Rf: 0,35, апигенин Rf: 0,37, хризин Rf: 0,56, флавонон Rf: 0,85, морин Rf: 0,14 и галангин Rf: 0,6.

Влияние этилацетатных экстрактов растений в диапазоне концентраций 250-1000 мкг/мл на свободные радикалы 2,2'-дифенил-1-пикрилгидразила (DPPH) исследовали спектрофотометрическими методами.

Ключевые слова: *Centaurea cyanus L, Pulsatilla, флавоноиды, TLC, DPPH.*