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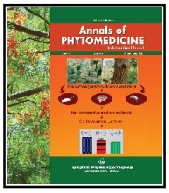

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Studying the stimulating properties of *Asarum europaeum* L. growing in Republic of Uzbekistan

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Abstract

The stimulating properties of *Asarum europaeum* L. extract on seeds of wheat (Tatiana variety), cucumber (Orzu variety) and melon (Mirzachul variety) were studied. Growth-stimulating activity was established in the range of 0.1% - 0.001%. The composition of the extract was studied using chromatographic methods of analysis, the content of flavonoids was determined: rutin (0.1344 mg/g) and quercetin (0.0211 mg/g). Using the mass-spectrometric method, the presence of trace elements was determined, such as: Li, Be, B, Na, Mg, Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu , Zn, Ga, Ge, As, Se, Rb, Sr, Zr, Nb, Mo, Ag, Cd, In, Sn, Sb, Cs, Ba, Ta, W, Re, Hg, Tl, Pb, Bi, U.

1. Introduction

The family Asarum L. includes 5-9 genera and more than 600 species. They are distributed mainly in the tropical and subtropical regions of North America, Japan, China, Korea, Bhutan, Nepal, India, Vietnam, Taiwan, and Russia. Since ancient times, various types of Asian ungulates had been used in traditional medicine in China, Japan, Korea, and other countries as medicines. Biologically active substances accumulated by various types of ungulates, have a wide spectrum of pharmacological activity (Murav'eva, 2001; Ho Chi Minh, 2020). Infusions and decoctions of *Asarum europaeum* L. are used in folk medicine for lung diseases: silicosis, bronchitis, bronchial asthma, pneumonia, acute respiratory infections, tuberculosis; in the early stages of pregnancy with heart and vascular diseases, including hypertension; in diseases of the gastrointestinal tract, liver diseases, enteritis, acute and chronic gastritis, diarrhea, inflammation of the biliary tract, intoxication, to improve digestion; with disorders of the central nervous system, epilepsy, neurotic conditions, hysteria, migraine; in children with heart disease and seizures; with other disorders of migraine, fever, malaria, gout, rheumatism, dropsy, with edema (Paseshnichenko *et al*., 2012; Vinay Sharma, 2021).

In veterinary practice, the rhizome with roots was used as a laxative and emetic; whole vegetable juice and tincture are used externally against scabies and lichen in horses (Teslova *et al*., 2010; Yamina Bouatrous, 2019). The rhizomes were used as a spice, when rubbed, it emits a smell similar to that of allspice and camphor. Essential oil from the underground part was locally used in dental practice, perfumery, and the food industry. Shchurevich and his team had studied phenolic compounds in the composition of *A. europaeum* using HPLC; 21 substances were isolated, from which 14 were identified (Shchurevich, *et al*., 2010).

In connection with the above, the purpose of this investigation was to study the chemical composition of the extract of the leaves of European hoofed deer, grown in the Republic of Uzbekistan, using the methods of high-performance liquid chromatography with a diode-matrix detector (HPLC-DAD) and a tandem chromatography-mass spectrometer (HPLC-Q-TOF-MS/MS).

2. Materials and Methods

2.1 Materials and reagents

Leaves of *A. europaeum* harvested during the flowering of the plant (2020) in the Toshkent Botanical Garden, in 70% ethanol solution, 0.1% formic acid solution, acetonitrile, chloroform, *etc*. High-performance liquid chromatography was performed using a PHPLCDIONEX chromatograph (Germany). Mass spectrometric studies of the isolated polyphenols were carried out on the Q-TOF LC-MS Agilent Technologies 6520B series device (Milevskaya *et al*., 2015).

2.2 Extraction of raw materials with chloroform

100 g of crushed air-dry raw material was placed in a flask with a capacity of 1.0 l, equipped with a reverse refrigerator. 0.8 l of chloroform was poured and extracted in a water bath at 50-55°C with regular stirring for 2 h. After that, the chloroform extractions were filtered through a Buchner funnel, and the raw material was added with a new portion of the extractant. After triple extraction,

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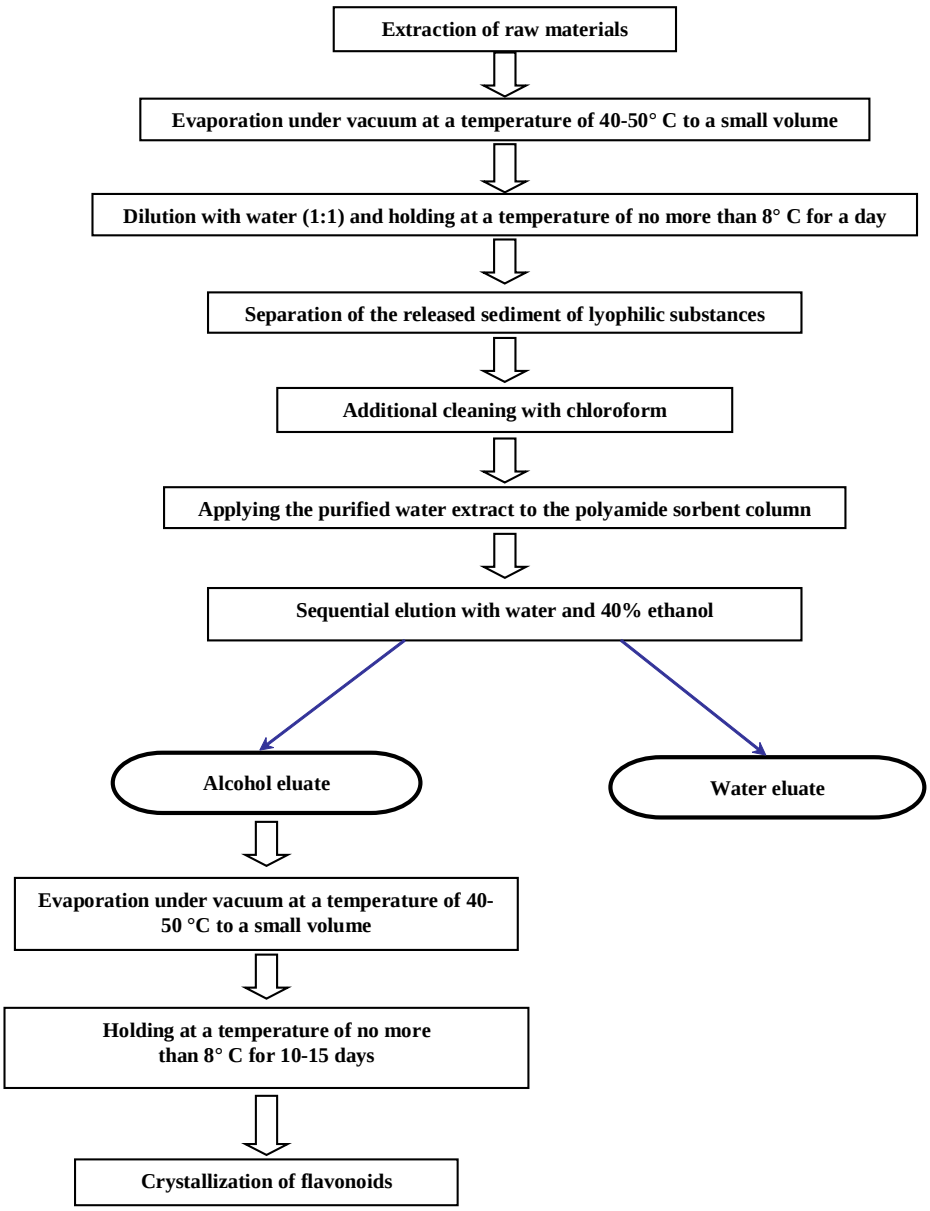
the raw material was dried under traction until traces of solvent were removed (Shchurevich *et al.*, 2012).

To conduct qualitative reactions for flavonoids, they were extracted from the leaves of European ungulates according to the following method: 2 g of dried and crushed raw material (0.3 mm) was pretreated with chloroform in a ratio of 1:10 for an hour to remove pigments. The mixture was filtered and the chloroform was evaporated in a water bath. The treated suspension was placed in a flask with a capacity of 100 ml and 20 ml of 70% ethanol was added. The flask was connected to a reverse refrigerator and heated in a water bath within 10 min. After that, liquid-cooled and filtered (Aboel Dahab *et al.*, 2009). After extraction, following qualitative reactions for flavonoids were conducted: cyanidin reaction, boron-lemon reaction, reactions with an alkali solution, Wilson reaction, with an alcohol solution of $AlCl_3$, with a solution of ferric chloride, with a solution of lead acetate, a solution of vanillin in sulfuric acid, an azo combination reaction.

2.3 Quantitative determination of micro and macro-elements was conducted using inductively coupled plasma mass spectrometry (ICP-MS NEXION-2000)

0.05-0.5 g of the test substance was weighed on an analytical balance and transferred to Teflon autoclaves. Then, the autoclaves are filled with the appropriate amount of purified concentrated mineral acids (nitric acid (x / h) and hydrogen peroxide (x/h)) (Zimina *et al.*, 2013). Autoclaves were closed and placed on a microwave decomposition device. Determine the decomposition program based on the type of test substance, indicate the degree of decomposition, and the number of autoclaves (up to 12 pcs).



Scheme: Scheme for the isolation of flavonoids from the leaves of *Asarum europaeum* L.

2.4 Mass spectrometric analysis of the fractions of isolated polyphenols

Mass spectrometric studies of the isolated polyphenols were performed on a Q-TOF LC-MS agilent technologies 6520 V series instrument under the following conditions: ionization source-ESI, drying gas flow - 5 l/min, drying gas temperature - 300°C, voltage at skimmer cone - 20 V, fragmented - 125 V, mass range: in MS 100-2000 m/z mode, and targeted MS/MS50 - 2000 m/z mode, collision energy - 35, 50 eV. Ionization method: negative. The samples were entered into the mass spectrometer using an agilent technologies 1200 series chromatograph, zorbax SBC18 column, 3 µm, 0.5×150 mm. Mobile phase: A-0.1% formic acid solution, B-acetonitrile + 0.1% formic acid. Elutions were performed on an agilent technologies series 1260 cap pump device at a flow rate of 15 µl/min. Solution concentration gradient in minutes: 0-5 min -20%, 20 min -25%, 25 min-30%, 25.1-30 min -60%, 35 min -20%. The solutions were degassed on an agilent technologies 1260 µ-degasser device. The samples were applied to the column using the agilent technologies micro WPS device in 1 µl from a solution of polyphenols with a concentration of 0.1 mg/ml.

2.5 High performance liquid chromatography

Chromatography was performed on a PHPLCDIONEX chromatograph (Germany). We used a semipreparative column × select CSHP rep C18. 5 µl, 10 × 250 mm (Waters, USA), for chromatography and analytical purposes-phenomenex C 18.5 µl, 4.6 × 250 mm (Waters, USA). Solutions A-0.1% TFUC, B-acetonitrile. acetonitrile concentration gradient: 0 min -15%, 28 min -25%, 33-38 min-60%, 43 min-15%. The flow rate is 3 ml/min (for analysis and chromatography, 1 ml/min). Spectra of rutin and quartzine were taken by an EMC -30 PC-UV spectrophotometer with an absorption – at 269 nm. The spectra of rutin and quartzite were taken with an EMC-30PC-UV spectrophotometer.

3. Results

Chromatographic methods of analysis were used to separate and identify individual flavonoids obtained from the original and hydrolyzed extracts in 10% H_2SO_4 solution. The alcohol-water extract from the raw material isolated by the method described above was divided into fractions by the method of selective extraction (Berestetsky *et al.*, 2018). The extract was evaporated to dryness, diluted with hot water, filtered, and lipophilic substances (fatty oils, resins, chlorophyll) were additionally removed from the aqueous phase of a separating funnel with dichloroethane. Then, flavonoid aglycones were successively extracted from the aqueous phase with diethyl ether, monosides with ethyl acetate, and biosides with n-butanol.

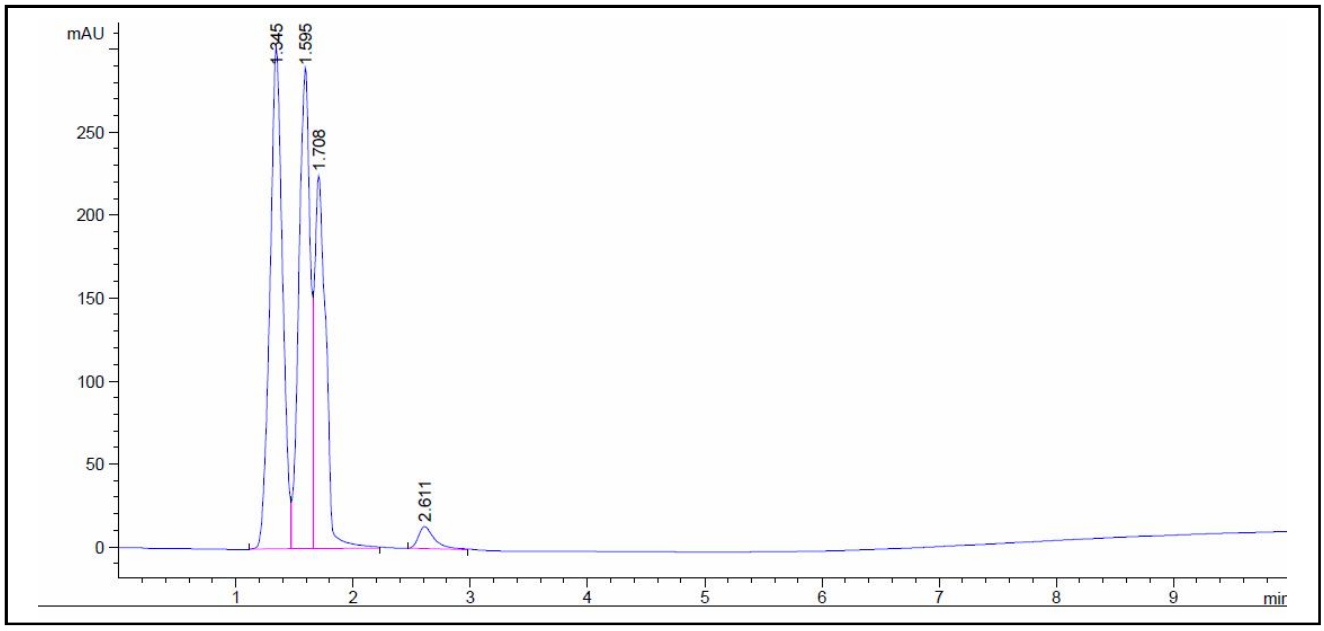


Figure 1: HPLC of rutin and quercetin obtained extract of *A. europaeum*.

In Table 1, the results of determining the composition of the alcohol extract of *A. europaeum* are presented. In the work, the treatment with solvents was carried out twice. The solvents were distilled off on a water bath, the dry residues were dissolved in 96% ethanol. The aqueous residue after extraction was also dried, dissolved in ethanol, and all alcohol solutions were used for further qualitative analysis. The selection of a solvent system for thin-layer chromatography was carried out on the basis of the literature and experimental data of trial experiments, taking into account the best separation of the sums of flavonoids, the clarity of the spots of individual substances. The chemicals isolated from the solution were studied using UV spectroscopy. At the same time, it was found that the intensity of absorption bands depends on the probability of transition from one electronic state to another, as well as on the concentration of light-absorbing particles (Puntener *et al.*, 1981).

Table 1: The composition of the alcohol extract of *A. europaeum*

No.	Substance name	Quantity	
		mg/g	%
1	Rutin	0,1344	0,0134
2	Quercetin	0,0211	0,0021

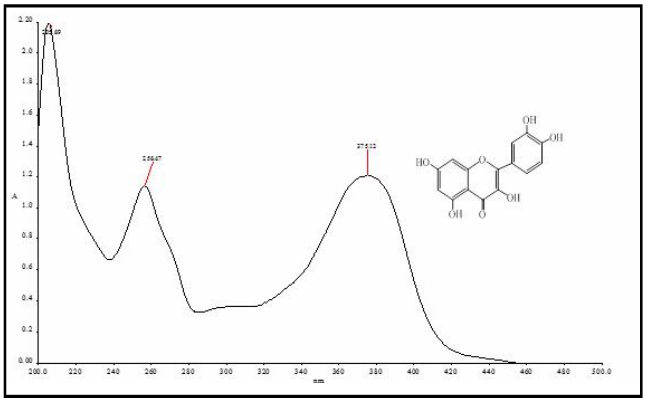


Figure 2: UV spectra of quercetin.

If, the probability of an electron transition from the lower to the upper level is small, then the intensity of the corresponding band in the spectrum will also have a small exponent, even at a high concentration of light-absorbing particles.

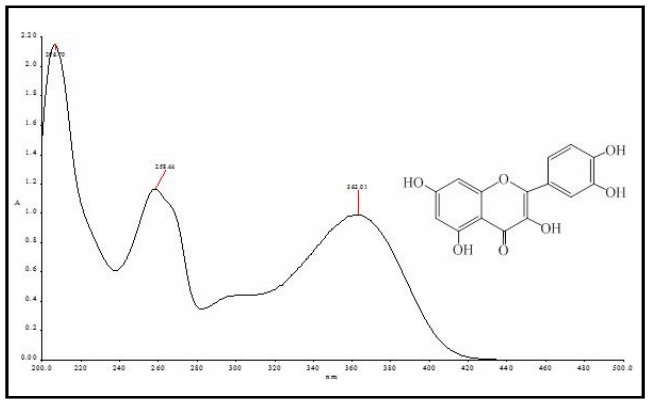


Figure 3: UV spectra of rutin.

As shown in Figures 2 and 3, the maximum absorption spectra correspond to the standard value of the wavelengths characteristic of rutin and quercetin.

When quantitatively determining the presence of micro and macro-elements in the composition of the selected eluent by the method of inductively coupled plasma mass spectrometry (ICP-MS), the results presented were obtained in Table 2.

Table 2: Indicators of micro and macro-elemental composition of the extract of *A. europaeum*

Element	Content, mcg/g	Element	Content, mcg/g	Element	Content, mcg/g
Macroelements		Selenium (Se)	0.302	Rubidium (Rb)	5.088
Potassium (K)	41526,479	Titanium (Ti)	23.083	Strontium (Sr)	141.038
Calcium (Ca)	28936,645	Vanadium (V)	2.049	Zirconium (Zr)	1.280
Silicon (Si)	259,275	Chromium (Cr)	4.909	Niobium (Nb)	0.079
Magnesium (Mg)	4647,475	Manganese (Mn)	35.618	Molybdenum (Mo)	0.801
Sodium (Na)	593,325	Iron (Fe)	1593.820	Silver (Ag)	3.369
Phosphorus (P)	1439,850	Cobalt (Co)	0.631	Cadmium (Cd)	0.108
Micro and ultramicroelements		Nickel (Ni)	2.690	Tin (Sn)	0.574
Lithium (Li)	3,176	Copper (Cu)	8.636	Indium (In)	0.003
Beryllium (Be)	0,108	Zinc (Zn)	38.112	Antimony (Sb)	0.167
Boron (B)	16,901	Gallium (Ga)	3.931	Cesium (Cs)	0.123
Aluminum (Al)	917,141	Germanium (Ge)	0.016	Barium (Ba)	155.165
Sulfur (S)	10.373	Arsenic (As)	0.693	Tantalum (Ta)	0.024
Tungsten (W)	0.013	Rhenium (Re)	0.013	Mercury (Hg)	0.263
Thallium (Tl)	0.024	Lead (Pb)	5.726	Bismuth (Bi)	0.058
Uranium (U)	0.052				

4. Discussion

4.1 Growth-stimulating activity of the plant extract under the code KR-1 (Rakitina and Rudnik 1966)

Tests for growth-stimulating activity were carried out in the Department of Organic Synthesis and Plant Protection of the IHRV of the Academy of Sciences of the Russian Federation. The range of tested concentrations for growth-stimulating activity was in the range of 0.1-0.001%. The Uchkun growth regulator was used as a reference. In the experiments, wheat seeds (Tatiana variety), cucumbers (Orzu variety), and melons (Mirzachulskaya variety) were used, which corresponded to a number of necessary requirements. During the tests, the method of Rakitin and Rudnik was used. "Primary biological evaluation of chemical compounds as a plant growth regulator and herbicides" (Rakitin and Rudnik, 1966).

The growth-stimulating activity of the plant extract of *A. europaeum* (European ungulate) was studied. The tests were carried out in the Department of Organic Synthesis and Plant Protection. Based on obtained data, it can be concluded that in the case of wheat (monocotyledons), the best growth-stimulating activity was shown by the extract "KR-1" in all three concentrations. The root growth of the extract "KR-1" in 0.1% concentration was 1.49 cm, in 0.01% - 3.37 cm and in 0.001% -1.39 cm. In the percentage ratio in 0.1% of the concentration-77.4%, in 0.01% -52.4% and in 0.001% - 300.2% relative to the control. In stems in 0.1% concentration-0.86 %, in 0.01% - by % and in 0.001% concentration-by 12.5% relative to the control. In the Uchkun standard, the root length exceeded the control variant by 34.8% and the stem height by 20.4%, respectively (Table 3).

Table 3: Growth-stimulating activity of KR-1 extract in relation to wheat seeds of the Tatiana variety

Variants	Length, cm	
	L-root wheat	L-wheat stalk
B/o control	1,84 ± 1,60	1,34 ± 1,23
Uchkun + 0.0001%	1,49 ± 0,12	1,04 ± 0,4
KR-1 0,1%	1,28 ± 0,16	0,86 ± 0,17
KR-1 0,01%	3,37 ± 0,45	2,68 ± 0,31
KR-1 0,001%	1,39 ± 0,16	0,95 ± 0,2

According to the results of studies on cucumber seeds (dicotyledonous), the extract under the ciphers: KR-1 in 0.01% - 0.001% concentration showed good growth-stimulating activity. KR-1 extract in 0.01% -0.001% concentrations contributed to root growth by 71.0 % - 30.3% and stem height by 81.7% - 30.3% relative to the control, whereas in the reference version these data were 20.9% - root length and 5.6% - stem height, respectively. In the KR-1 variant at a 0.1% dose, the root length was 2.08 cm and, the stem height was 1.42 cm, this means less control. KR-1 in 0.1% concentration did not show growth-stimulating activity against cucumber (Mironova *et al*., 2012).

Table 4: Growth-stimulating activity of KR-1 extract in relation to cucumber seeds of the Orzu variety

Variants	Length, cm	
	L-root cucumber	L-stem cucumber
B/o control	2,24 ± 1,63	1,42 ± 1,23
Uchkun plus 0.0001%	2,41 ± 0,66	1,20 ± 0,39
KR-1 0,1%	2,08 ± 0,48	1,42 ± 0,23
KR-1 0,01%	3,83 ± 0,82	2,58 ± 0,18
KR-1 0,001%	2,92 ± 0,85	1,85 ± 0,78

In next stage of our research, we have concluded the investigation of the growth-stimulating activity on melon seeds (dicotyledonous). In the screening, KR-1 extract showed good growth-stimulating activity in concentration 0.01-0.001%. The growth of melon roots in the extract KR-1 in 0.01% concentration was 3.44 cm, in 0.001% - 2.11 cm and in the stems in 0.01% concentration-0.53 cm, in 0.001% - 0.37 cm relative to the control.

Table 5: Growth-stimulating activity of KR-1 extract in relation to melon seeds of the Mirzachulskaya variety

Variants	Length, cm	
	L-melon root	L-stalk melon
B/o control	1,15 ± 1,14	0,41± 0,5
Uchkun plus 0.0001%	1,63 ± 0,33	0,48 ± 0,14
KR-1 0,1%	1,38 ± 0,18	0,22 ± 0,02
KR-1 0,01%	3,44 ± 0,49	0,43 ± 0,04
KR-1 0,001%	2,11± 0,4	0,37± 0,06

The field of phytomedicine is actually a multifaceted arena including botany, biochemistry, pharmacology, ethnobotany, molecular biology, biostatistics, *etc*. Considering this, amid thousands of journals, researchers always search a platform where they can think to publish their findings after their innovative and strenuous hard work. Phytomedicines related research need a dedicated platform where a team of experts is available to help them in lucid publication with critical analysis of their findings. In this perspective, “Annals of Phytomedicine: An International Journal” with both online and high quality, glossy print versions, is an excellent channel to publish quality research outcomes in the area of medicinal plants.This journal has an excellent team of committed scientists and academicians who are serving altruistically to provide timely publication of progressive and contemporary research in this area. It is highly difficult task to perform but this journal is doing so highly successfully since many years. I am delighted to appreciate that the journal, “Annals of Phytomedicine: An International Journal” is holding its flag high and gaining well deserved popularity and is highly acclaimed by the peers (Tamanna Malik *et al*., 2020; Warrier, 2021).

5. Conclusion

As a result of the conducted research, the qualitative analysis of flavonoids obtained from the initial extracts of extracts was established. *A. europaeum* using UV spectroscopy. It was experimentally established that the intensity of the absorption bands

depends on the probability of transition from one electronic state to another, as well as on the concentration of light-absorbing particles.

The totality of the data obtained indicates that the study of raw materials, *A. europaeum* (leaves) revealed the main classes of compounds studied (flavonols and flavonol-3-glycosides, catechin derivatives) and individual substances - quercetin and rutin.

It is shown that the isolated extract from *A. europaeum* under the code KR-1 exhibits growth-stimulating activity against the vegetative organs of representatives of monocotyledonous and dicotyledonous classes of the plant.

Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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