PHYSIOLOGICAL EFFECTS INDUCED BY THE HYDROALCOHOLIC EXTRACT OF *VIOLAE TRICOLORIS HERBA* (WILD PANSY AERIAL PARTS) ON *TRITICUM AESTIVUM* L.

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Abstract. Wild pansy (*Viola tricolor*) hydroalcoholic extract was prepared by extraction of powdered dried – flowering aerial parts with ethanol 70% v/v (1:10), by reflux for two hours. This was diluted with distilled water to give the final concentrations of 0.5, 1.0 and 5% (v/v) (VTEx1, VTEx2 and VTEx3). These extracts were tested for their effects on seed germination and seedlings growth of wheat (*Triticum aestivum*) in a laboratory experiment. Distilled water was used as a control (C). After the 10 days of experiment, we evaluated seed germination of wheat and seedlings growth (roots and shoots lengths, their fresh and dry biomass).

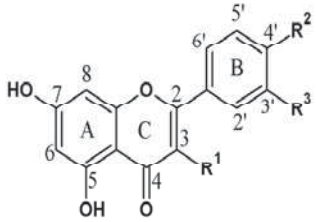
INTRODUCTION

Medicinal plants have been used in folk medicine for millennia (Akinboro and Bakare, 2007; Celik and Aslnatürk, 2010). It is known that green plants in general are a primary source of polyphenol compounds with antioxidant and free radical scavenging - properties. Among the active polyphenol compounds, flavonoids have antioxidant and anticarcinogenic properties (Okiemy Akeli *et al*., 2010). But, despite the current availability of many anticancer agents, there is a continuous search for new compounds that may be more effective and safe (Kumar and Singhal, 2009). Recent investigations have revealed that many plants used as food or in traditional medicine have mutagenic effects and cytotoxic and genotoxic effects *in vitro* and *in vivo* assays (Celik and Aslnatürk, 2010). As a consequence it is extremely important the employment of genotoxicity tests to identify their possible mutagenic potential (Celik and Aslnatürk, 2010; Saulo *et al*., 2009). Genotoxic effects of many plants have been widely evaluated using cytogenetic approaches.

Wild pansy (*Viola tricolor* L.) is very spread in the spontaneous flora of Romania and it is considered one of the most important medicinal plants within its family (Toiu *et al*., 2009). The aerial parts are used in traditional medicine to treat various skin conditions, bronchitis and rheumatism (Toiu *et al*., 2009). Their anti – inflammatory, expectorant and diuretic properties are due to the presence of: salicylic acid and its derivatives such as the methyl ester and violutoside (the glucosidoarabinoside of salicylic acid methyl ester), phenol carboxylic acids such as trans - caffeic acid, protocatechuic acid, p - coumaric acid (Fig.1b), mucilages (glucose, galactose, arabinose and rhamnose), tannins, flavonoids (rutin, violaquercitrin, violanthin, scoparin, saponaretin, orientin, vicenin, anthocyanidin glycosides) (Table 1, Fig.1a), carotenoids (violaxanthin, zeaxanthin etc.), coumarins (umbelliferone), small amounts of saponins, ascorbic acid and tocopherol (Rimkienė *et al*., 2003; Toiu *et al*., 2009; Vukics, 2009).

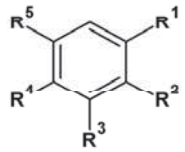
Table 1. Structures of flavonoid glycosides in *Viola tricolor* L. (Vukics, 2009).

Name	Structure
isoorientin	luteolin-6-*C*-glucoside
isovitexin	apigenin-6-*C*-glucoside
isoschaftoside	apigenin-6-*C*-arabinoside-*8*-C-glucoside
orientin	luteolin-6-*C*-glucoside
rutin	quercetin-3-*O*-rhamnosyl(1 → 6)glucoside
saponarin	apigenin-6-*C*-glucoside-7-*O*-glucoside
schaftoside	apigenin-6-*C*-glucoside-8-*C*-arabinoside
scoparin	chrysoeriol-8-β -D-*C*-glucoside
swertiajaponin	7-methoxy-luteolin-6-*C*-glucoside
vicenin-2	apigenin-6,8-di-*C*-glucoside
violanin	delphinidin-3-*O*-(p-coumaroyl-rhamnosylglucoside)
violanthin	apigenin-6-*C*-β-D-glucoside-8-*C*-α-D-rhamnoside
violarvensin	apigenin-6-*C*-β-D-glucoside-8-*C*-β-D-rhamnoside
vitexin	apigenin-8-*C*-glucoside



	MW	R^1	R^2	R^3
apigenin	270	H	OH	H
luteolin	286	H	OH	OH
kaempferol	286	OH	OH	H
chrysoeriol	300	H	OH	OCH_3
diosmetin	300	H	OCH_3	OH
quercetin	302	OH	OH	OH
isorhamnetin	316	OH	OH	OCH_3

a)



	R^1	R^2	R^3	R^4	R^5
p-hydroxy-benzoic acid	COOH			OH	
salicylic acid	COOH	OH			
protocatechuic acid	COOH		OH	OH	
gentisinic acid	COOH	OH			OH
vanillic aid	COOH		OCH_3	OH	
p-hydroxy-phenylacetic acid	CH_3–COOH			OH	
p-coumaric acid	CH_2=CH_2–COOH			OH	
caffeic acid	CH_2=CH_2–COOH			OH	OH

b)

Fig. 1. Structures of flavonoid aglycones (a) and phenolic acid derivatives (Vukics, 2009).

Wild pansy is used externally and internally. The therapeutic activity has been identified in treating various skin conditions, such as eczema, seborrhea, impetigo, acne, catarrh of the respiratory tract, and whooping cough. It is also helpful in cases of cradle cap in babies. The herb is employed in treating frequent and painful urination in conditions such as cystitis. Due to the high concentration of rutin in the herb, it may be employed in preventing bruising and broken capillaries, in checking the fluid build up in the tissues and to help blood pressure reduction. The drug is mildly laxative. It was formerly in much repute as a remedy for epilepsy and numerous other complaints, and the flowers were considered as cordial and helpful in treating heart diseases, from which may have arisen its popular name if heartsease (Rimkienė *et al*., 2003).

In our study, we investigated some physiological effects of a wild pansy - 70% hydroalcoholic extract on wheat (*Triticum aestivum*, *Dropia* cultivar) seeds germination, root and shoot length, and fresh and dry biomass.

MATERIALS AND METHODS

Extract preparation. The fluid hydroalcoholic extract of *Viola tricolor* (wild pansy) flowering aerial parts (VTEx) was obtained from powdered dried material by reflux with 70% ethanol (1:10), for two hours. The hydroalcoholic extract was filtered through a textile filter, and used as a stock extract. This was diluted with distilled water to give the final concentrations of 0.5, 1.0 and 5% (v/v) (VTEx1, VTEx2 and VTEx3).

Extract analysis. The stock extract was qualitative and quantitatively analyzed. The qualitative analysis consisted in phytochemical screening and spectroanalytical profile by HPTLC (Cretu *el al*., 2010) and UV/VIS absorption spectroscopy.

Phytochemical screening was done by specific chemical reactions for the identification of phytochemicals presence in the stock extract (Ciulei *et al.,* 1994).

Spectroanalytical profile was done in order to detect the presence of rutin (from flavone O- glycosides class) in wild pansy extract (VTEx).

Detection of rutin - Equipment: CAMAG LINOMAT IV, CAMAG TLC 3 Scanner, WINCATS Planar Chromatography Manager. Chromatographic conditions: Stationary phase - HPTLC plates G60F254 10 x 10 cm, 0.2 mm thickness (Merck); Wavelength - 366 nm after derivatization; Mobile phase - ethyl acetate: formic acid: glacial acetic acid: ethyl – methyl - ketone: water = 25:3.5:1.5:15:5 v/v; Derivatization - 1% diphenylboryloxyethylamine (Natural Product, NP) in methanol, followed by 5% polyethylene glycol - 4000 (PEG) in methanol; Reference - rutin.

UV/VIS absorption spectrum of wild pansy extract was done with a CARY 50 UV/VIS spectrophotometer, by reading the extract maximum absorption in the 200 - 400 nm range.

The total flavonoids and polyphenols of wild pansy stock extract (VTEx) were quantitatively evaluated. Total flavonoid content was determined by following colorimetric aluminium chloride method and calculated as rutin (g/100 ml). The absorbance of reaction mixture was measured at 430 nm with a CARY 50 UV/VIS spectrophotometer (Cretu *et*

al., 2011). Total polyphenol content was determined by Folin - Ciocalteu method and expressed in terms of gallic acid equivalent, which is a common reference compound. The absorbance of reaction mixture was measured at 760 nm with a CARY 50 UV/VIS spectrophotometer (g/100 ml) (Cretu *et al*., 2011). Results were presented as mean of three determinations ± SD (standard deviation).

All reagents were of purity grade.

Seeds treatment. Seeds (one hundred) of *Triticum aestivum* L. (*Dropia* cultivar, obtained from Secuieni Agricultural Research and Development Station, Neamt) were treated with different concentrations (0.5%, 1% and 5%) of the wild pansy – 70% hydroalcoholic extract, for 12 hours. Distilled water was used as a control (C). Seeds were washed with distilled water and placed on an inert material, in hydroponic system (constant level of water) and maintained under 23 ± 1^0C and natural light (day/night alternance, with a photoperiod of 12 hours), for 10 days. The experiment was performed in the laboratory of the Society for Medicinal Plant Research and Processing “PLANTAVOREL” Piatra Neamt, between 1.03- 10.03. 2010.

Seeds bioassay. After the 10 days of experiment, there were determined: germination capacity (by counting the number of germinated seeds and expressed as total percentage), the root and shoot lengths (by measuring representative seedlings), fresh and dry biomass (these were calculated by seedlings separation into root and shoot parts, and fresh samples were dried at room temperature) (Cho *et al*., 2007). The results of biometrical measurements were statistically evaluated by Student’s test.

RESULTS AND DISCUSSION

Analysis of wild pansy extract

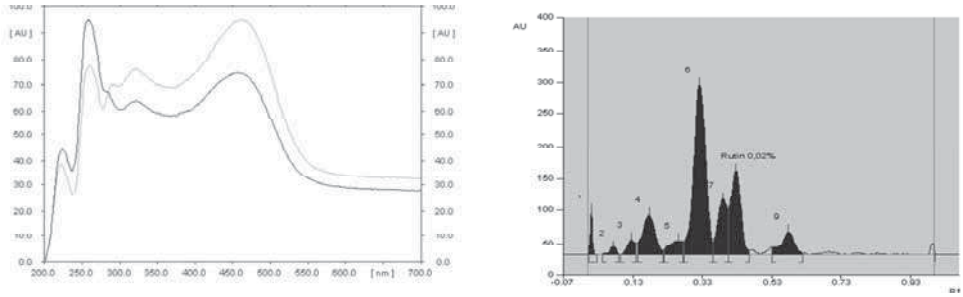
The result of the phytochemical screening (Cretu *et al*., 2011) revealed that tannins, reducing sugars, aminoacids, flavonoids, flavonoid glycosides, polyphenols, coumarins, sterolic saponins were present in the hydroalcoholic extract of *Viola tricolor* aerial parts (Table 2).

Table 2. Phytochemical screening of the 70% hydroalcoholic extract of *Viola tricolor* aerial parts

Phytochemical	Inherence
Hydroalcoholic extract	
Tannins	+ +
Reducing sugars	+
Alcaloids	-
Aminoacids	+++
Flavonoids	+ + +
Polyphenols	+++
Hydrolized hydroalcoholic extract	
Anthracyanosides	-
Coumarins	+ (?) green
Cardiotonic heterosides	-
Sterolic saponins	+ + (cherry- red ring)
Triterpens	-
Flavonoid glycosides	+
Proanthocyanidols	-
Anthocyanosides	-

“+” = present;
“-“ =absent.

The HPTLC profiles identified the presence flavonoids represented by rutin (Fig. 2a, b) in the wild pansy 70% hydroalcoholic extract.



a) **b)**

Fig. 2. Rutin detection: a) UV spectrum of rutin in wild pansy extract; b) Analogue curve of wild pansy extract at 366 nm after derivatization.

The UV/VIS absorption spectrum evidenced maximum peaks at 202, 205, 210, 213, 214.9, 219, 222.9, 270 and 336 nm (Fig. 3). According to literature, the peaks in the 210 - 310 nm range are due to the phenolic group, and those in the 255 - 280 nm range are specific to the flavonoids. Also, the 255 - 270 nm range are due to the aromatic structures and the chromopherous groups >C=O (Manole, 2008).

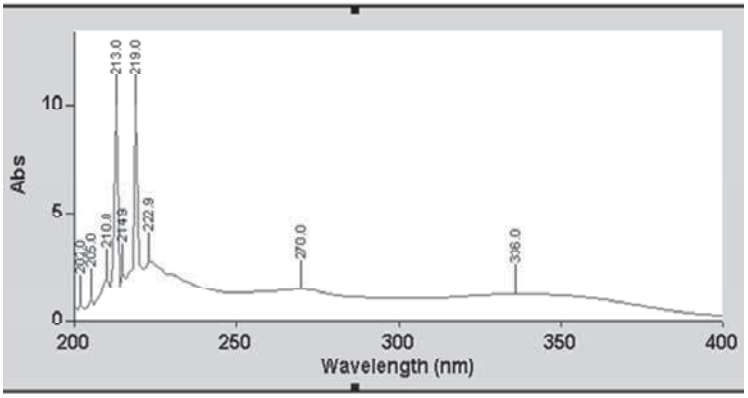


Fig. 3. UV/VIS spectrum of the 70% hydroalcoholic extract of *Viola tricolor* aerial parts

Total content of flavonoid compounds (as rutin) and polyphenols (as gallic acid) in wild pansy stock extract is presented in Table 3.

Table 3. Total flavonoid content in wild pansy 70% hydroalcoholic extract

Phytochemical content	Wild pansy extract (VTEx)
Total flavonoids as rutin (g/100 ml)	0.1570 ± 0.012*
Total polyphenols as gallic acid (g/100 ml)	0.1379 ± 0.0146*

*= mean of three determinations ± SD (standard deviation)

Qualitative (phytochemical screening, HPTLC profile and UV/VIS absorption spectrum) and quantitative (total flavonoid and polyphenol content) analyses revealed the complex composition of the 70% hydroalcoholic extract of *Viola tricolor* aerial parts (VTEx).

Seed bioassay

The effect of wild pansy extract - different concentrations on the wheat seed germination is shown in Table 4. The control treatment produced a germination rate of 96%. Compared to control (C), we can observe an inhibition of seed germination proportional to the increasing of extract concentration, ranging between 1% and 7%. According to these results, the tested hydroalcoholic extract of *Viola tricolor* aerial parts did not significantly influence seed germination at concentrations of 0.5% and 1.0%, compared to control.

Table 4. Effect of wild pansy extract on germination (G %) of *Triticum aestivum* seeds

Variants	G %
Control (C)	96
VTEx1 (0.5%)	95
VTEx2 (1%)	93
VTEx3 (5%)	89

The values presented in Table 5 revealed that wild pansy extract stimulated root length, compared to control, with a maximum stimulation of 20% at VTEx2 treatment. The other concentrations of wild pansy extract had a stimulating effect on root growth of wheat seedlings with 14% and 17%, respectively. We observed a slightly stimulation of shoot length only at VTEx2 and VTEx3 treatments.

Table 5. Effect of wild pansy extract on seedling growth of *Triticum aestivum*

Variants	Root				Shoot			
	Length (mm)				Length (mm)			
	x ± sx		s%	C=100	x ± sx		s%	C=100
Control (C)	121.35	2.87	19.64	100	98.81	2.35	19.79	100
VTEx1 (0.5%)	138.10	2.91	18.74	114	98.95	2.21	19.88	100
VTEx2 (1%)	145.21	2.91	17.03	120	100.24	2.35	19.86	101
VTEx3 (5%)	142.57	2.06	17.31	117	99.32	2.48	20.10	101

The effects of the tested concentrations (0.5, 1.0 and 5%) on root and shoot length of wheat seedlings may be related to the presence of allelochemicals including tannins, flavonoids and phenolic acids. We found some literature results according to the alleopathic plant extracts which generally have more pronounced effects on radicle, rather than hypocotyl growth. This may be explained by the fact that radicles are the first to come in contact with allelochemicals (Ashrafi *et al*., 2008). Also, the alleopathic effect may be attributed to the synergistic action rather than single one (Siddiqui *et al.,* 2009). Other studies showed that the response to allelochemicals may be dependent on concentration (Ashrafi *et al*., 2009).

Wild pansy extract had a stimulating effect on total root fresh biomass (ranging between 22 and 32%) depending of tested concentration, compared to control (Table 6). This effect was evident at extract concentration of 1.0% and decreased with extract concentration increasing.

Root dry biomass was also higher in treated plants compared to the control ones. The treatments also determined stimulation of total fresh and dry shoot biomass compared to the control seedlings (with 17% and 40%, and 1 and 18%, respectively) (Table 6). The maximum increasing of fresh shoot biomass was registered at the concentration of 0.5% wild pansy extract, and for the dry shoot biomass at concentration of 1.0% wild pansy extract. The stimulating effect on dry shoot biomass was reduced at 5% extract treatment.

Table 6. Effect of wild pansy extract on total fresh and dry biomass of *Triticum aestivum* seedlings

Variants	Root				Shoot			
	Fresh biomass (g)		Dry biomass (g)		Fresh biomass (g)		Dry biomass (g)	
	T	C=100	T	C=100	T	C=100	T	C=100
Control (C)	4.1	100	0.511	100	3.5	100	0.636	100
VTEx1 (0.5%)	5.0	122	0.549	107	4.9	140	0.737	116
VTEx2 (1%)	5.4	132	0.610	119	4.8	137	0.751	118
VTEx3 (5%)	5.1	124	0.559	104	4.1	117	0.640	101

T = total fresh and dry biomass (g)

The results regarding fresh and dry biomass of roots are correlated with those obtained for root length. In both cases, we registered the stimulation of these parameters under the effect of wild pansy extract treatments, compared to control. The situation is different for wheat shoots. Thus, we found an evident stimulation of fresh and dry biomass of shoots, but their length is comparable with control seedlings. The accumulation of fresh and dry matters in treated wheat seedlings may reflect a thickening of shoot cells rather than shoot cells elongation, compared to control.

Because of variable number of seedlings in each treatment variant, we determined the mean value of fresh and dry biomass of seedlings roots and shoots (Table 7).

Table 7. Effect of wild pansy extract on individual fresh and dry biomass of *Triticum aestivum* seedlings

Variants	Root				Shoot			
	Fresh biomass (mg)		Dry biomass (mg)		Fresh biomass (mg)		Dry biomass (mg)	
	M	C=100	M	C=100	M	C=100	M	C=100
Control (C)	59.42	100	7.41	100	50.72	100	9.22	100
VTEx1 (0.5%)	63.29	107	6.95	94	62.03	122	9.33	101
VTEx2 (1%)	75.00	126	8.47	114	66.67	131	10.43	113
VTEx3 (5%)	78.46	132	8.14	110	63.08	124	9.85	107

M = mean value of individual fresh and dry biomass (mg)

Table 7 data show that individual fresh biomass of roots increased with the increase of wild pansy extract concentration (ranging between 7 – 32%, compared to control). The individual dry matter of roots was stimulated only at the treatments with extracts of 1.0 and 5.0%. Our treatments determined an increase of individual fresh biomass of shoots (with a

maximum stimulation of 31% at VTEx2 variant). The same variant of treatment also induced the highest level of individual dry matter of shoots (Table 7).

CONCLUSIONS

Different concentrations of a wild pansy - 70% hydroalcoholic extract were tested for their physiological effects on wheat: seeds germination, root and shoot length, and fresh and dry biomass. Out tests on wheat were carried out in a laboratory experiment.
These treatments inhibited seed germination proportional to the increasing of extract concentration; alteration of seed germination capacity was significant at maximum concentration. Our treatments stimulated root length (significantly) and also shoot length (slighlty), and determined the stimulation of total and individual fresh and dry biomass of wheat seedlings, with one exception, in case of individual root dry biomass, at minimum concentration.

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