Research Article

Photophysical chracterization of *Galanthus elwesii* hook

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Abstract

The investigation of photophysical properties of the *Galanthus elwesii* Hook different plant organs (root, leaf, flower and bulb) at different growing stages (start and after flowering, fruit ripening) has been carried out via UV-Vis spectrophotometry, fluorescence spectrometer and the cyclic voltammetry analysis. Polyphenol contents are a group of natural compounds for drug discovery. The correlation of their potential to act biological activite (depending on polyphenolic content) and their photophysical properties have been investigated. Fruit ripening and beginning of flowering stage were observed at highest emission values; beginning of the flowering bulb has highest fluorescence quantum yield. Fruit ripening and beginning of the flowering stages were observed at the highest emission values. The quantum yields were calculated and as a result, beginning of the flowering bulb stage showed the highest quantum yield value.

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1. Introduction

The giant snowdrop (*Galanthus elwesii* Hook.) is a highly valued but rare spring ornamental species from Eastern Europe and Asia Minor. The Giant snowdrop (*Galanthus elwesii* Hook.) is the most important and endangered species of wild-collected bulbs in Turkey's flora. It is also one of the most important bulbous plants exported from Turkey to the western part of Europe (Maslanka, 2013). Galanthus contains a variety of seconder metabolites, flavonoids, phenolics, terpenoids and some important alkaloids such as galanthamine and lycorine which are important for the modern pharmacological and therapeutical strategies. However, the most common scientific studies interest determination

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of alkaloid amount and reproducing (Bozkurt et al., 2013). We described recently polyphenol contents (Figure 1) and antioxidant activities of Giant Snowdrop (*Galanthus elwesii* Hook) (Ay et al., 2018).

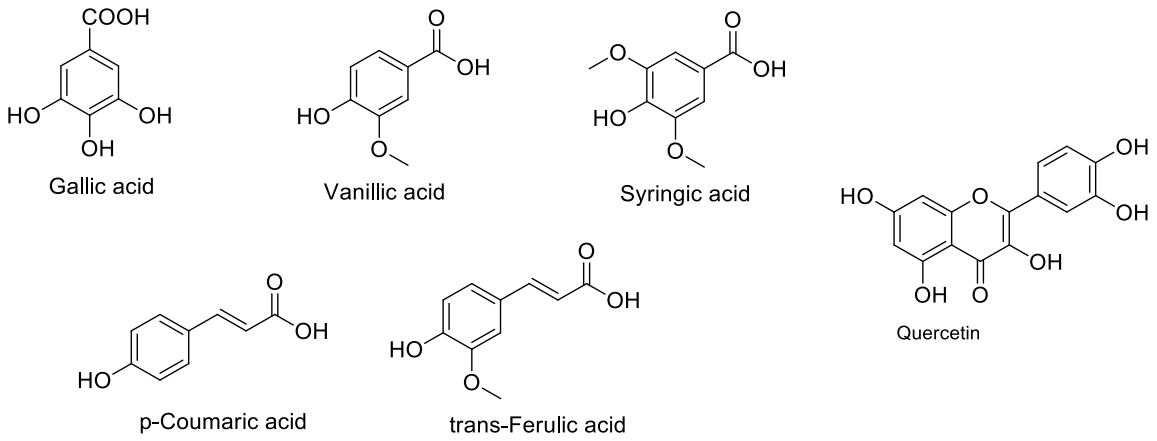

Figure 1. Some phenolic compound of determined in *Galanthus elwesii* Hook.

The main results were that detection of the higher concentrations of polyphenol compounds in the leaf samples than the other plant parts, especially after flowering stage in Table 1.

Table 1. Total phenolic contents of the *Galanthus elwesii Hook*

Plant Parts	Beginning of flowering	After flowering	Fruit ripening
Leaf	BL / 25.19	AL / 30.18	FL / 42.58
Bulb	BB / 28.52	AB / 33.75	FB / 42.63
Root	BR / 22.55	AR / 20.18	FR / 18.15
Flower	F / 19.58		

In recent years for the medical aspect of the plant has been studied on both the chemical component and amount. According from the literature, there are lots of plants have attributed to the broad biological activity in traditional medicinal treatment. For instance; aspirin has been obtained from willow bark as appeared in clay tablets from ancient Sumer, morphine has been isolated from the Opium poppy. Both aspirin and morphine are still currently commercially available drugs. Another example is the plant of Dorstenia's roots was the treatment to irritated skin with sunshine at the ancient time, today as knowing PUVA (psoralen + UVA) therapy (Lewis, 1993).

In a plant; besides the primary metabolites that carry out vital functions (such as photosynthesis, growth, reproduction), there are secondary metabolites involved in strengthening the plant and protecting it against ecological factors such as pathogens (Craney, 2013). The other important effects of secondary metabolites are that they have a special chemical structure that will protect the plant against environmental factors such as light and salt sensitivity (Hussein, 2018).

Today the interactions between light and biological molecules are commonly used at the especially fluorescence diagnostic techniques and photodynamic therapy. Photochemistry is used to generate redox reactions by plants for photosynthetic energy. Redox is caused not only leads to the photosynthesis mechanism but also fundamental to the development role of the plant immune response (Frederickson, 2014). To achieve this, the plant is needed energy transferred via on the secondary metabolites. The properties of plant extracts can be determined relative to the interaction of secondary metabolites with light. Polyphenol moieties are conjugated systems as have chromophore and fluorophore properties. The energy has emerged from the chromophore and fluorophore groups in the seconder metabolite of the plant. Knowing, polyphenol content amount of plant is not more important than the polyphenol compound structure for the biological activity (Krol, 2014). On the shed light of these studies, the determination of photophysical properties depends on the chemical structure of secondary metabolites. As known when photosynthetic process are inhibited the toxic molecules derived from oxygen is generated (Apel, 2004). Reactive oxygen derivatives are eliminated by antioxidant molecules for instance flavonoid, phenolic compounds (Procházková et al., 2011). As with antioxidant mechanisms, the energy is inhibited by electron or hydrogen transfer (Mayer, 2002). Both transfer mechanisms realized to the frontier orbitals. Uv-vis spectrometer, fluorescence luminescence methods detailed HOMO-LUMO energy gap and emitting energy. The oxidation and reduction potential is determined using cyclic voltammetry, and these values allow us to calculate HOMO (highest occupied molecular orbital) and LUMO (low unoccupied molecular orbital) energy levels. Depending on these energy levels, the molecules emit absorption or emissions due to external energy.

As a result, as the antioxidant mechanism is one of the most important mechanisms affecting healthy plant growth, physical analyses and plant extracts are compared. By obtained values, we can have an idea about which species obtained from the extract contains antioxidant molecules which cause the plant to be healthier. We will outline which Galanthus

part and growing stage are generated high quantum yield and how polyphenol content sensed the changes band gap value in the extracts. Regarded as, flavonoid (quercetin) and phenolic (gallic, trans-ferrulic) compounds have been selected the photophysical standards since they are found commonly and highly content in extracts.

2. Materials and Methods

2.1. Experimental

Plant material was grown in Amasya province of Turkey, in autumn-winter growing period of 2016-2017. Samples were taken from different plant organs (root, leaf, flower and bulb) at different growing stages (start and after flowering, fruit ripening).

2.2. Preparation of Galanthus elwesii Hook Extracts

After each plant material was dried at room temperature, extracted with 70% aqueous ethanol using a magnetic mixer. Evaporated on a rotary evaporator, and plant extracts kept on 4°C.

2.3. Optical Characterization

Absorption spectra were recorded on a PerkinElmer-Lambda35 and emission spectra were carried out with an Agilent-Cary Eclipse G9800A. Each of extracts was dissolved polar protic EtOH and polar aprotic DMSO (Sigma-HPLC grade). For the UV-Vis absorption spectra were recorded six different concentrations which were dissolved between 1.10^{-1} M and 1.10^{-7} M. All experiments were operated to the temperature value as 25°C. Fluorescence spectrum was performed in DMSO, EtOH and 10^{-4} and 10^{-7} M concentration.

2.4. Electrochemical Characterization

The cyclic voltammetry experiment was carried out on Gamry-Reference 3000 model and using working electrode-glassy carbon, the counter electrode- platinum, reference electrode-silver-silver chloride (Ag/AgCl/4M KCl). All analysis were recorded $[(nBu)_4N]ClO_4$ 0.1 M in

acetonitrile: DMSO (1:1) solvent system. Ferrocene was used as the reference standard for the cyclic voltammetry measurement of all potentials.

3. Results and Discussion

To investigate the optical properties of the *Galanthus elwesii* Hook. extracts, which is named beginning of flowering stage of leaf (BL), bulb (BB), root (BR); after flowering stage leaf (AL), bulb (AB), root (AR); Fruit ripening stage of leaf (FL), bulb (FB), root (FR), UV-vis absorption and fluorescence spectra were studied (Table 2 and 3). For the UV-vis typical absorption peak was assigned to the π–π* transition above 255 nm (at EtOH solvent) and also above 290 nm (at DMSO solvent). Another absorption peak was based on n–π* transition which can be observed, and the strong absorption below 670 nm in EtOH solvent and above 498 nm in DMDO solvent. Fluorescence behaviour was dependent on the excitation wavelength. The fluorescence excitation spectrum was obtained with the highest value in EtOH showed 703 nm. Although 5 different exciting values changed between 255 and 674, generally only two emission values recorded.

Table 2. Excitation and emission maxima and Stoke's shifts of *Galanthus elwesii* Hook. in the DMSO solvent system

Compound/DMSO	λ_{abs}^{max} **(nm)**	λ_{em}^{max} **(nm)**	**Stoke's shift (*λ*, nm; *E*,** cm^{-1}**)**
BL	411, 508, 538, 610, 667	453, 702	35 (748 cm^{-1})
BB	413, 545, 603, 672	461 and 709	37 (777 cm^{-1})
BR	411, 498, 526, 624	458 and 652	28 (688 $cm^{-1)}$
AL	330, 375, 411, 505, 538, 607, 666	448 and 456	118 and 81 (2667 cm^{-1})
AB	335, 411, 522, 634	456 and 598	121 and 76 (2005 cm^{-1})
AR	298, 410, 523, 627	450	40 (2168 cm^{-1})
FL	285, 292, 583, 647	326	34 (3571 cm^{-1})
FB	294, 370, 636, 668	327 and 725	33 and 57 (1085 cm^{-1})
FR	290, 345, 625	327 and 676	37 and 51 (1207 cm^{-1})
F	280, 411, 508, 535, 610, 668	328 and 712	48 and 44 (926 cm^{-1})

Table 3. Excitation and emission maxima and Stoke's shifts of *Galanthus elwesii* Hook. in the EtOH solvent system

Compound/EtOH	λ_{abs} (nm)	λ_{em}^{max} (nm)	Stoke's shift (nm)
BL	257, 408, 505, 535, 666	261 and 695	29 (627 cm^{-1})
BB	259, 411, 546, 674	272 and 703	29 (612 cm^{-1})
BR	255, 349, 538, 612	267 and 644	32 (812 cm^{-1})
AL	256, 322, 520, 622	313, 426, and 643	23 (575 cm^{-1})
AB	258, 370, 461, 566	333 and 664	98 (612 cm^{-1})
AR	259, 345, 510	338 and 660	150 (4456 cm^{-1})
FL	260, 404, 506, 536, 666	325, 448	44 (2431 cm^{-1})
FB	272, 411, 546, 603	334, 449	38 (2059 cm^{-1})
FR	255, 403, 539, 612	305, 440	37 (2087 cm^{-1})
F	406, 673	710	37 (774 cm^{-1})

The ethanolic solution of extracts corresponded to the 1.3 absorbance when the DMSO corresponding absorption band was below 1.0 absorbance. These results are shown for the solvatochromic effects, which depended on hydrogen bonding properties of extracts.

Beginning of flowering stage absorption spectra was recorded higher than after of flowering stage and fruit ripening stage. The highest absorption value was the beginning of the flowering stage on the bulb (BB) 672 nm in DMSO and 674 nm in EtOH, which is red-shifted 2 nm approximately. Compared to the results of after flowering stage experiments seem to be quiet high although flower extracts. These extracts, especially the AL, AB, and AR, exhibit large Stokes shifts (around 100-190 nm) in polar protic/aprotic solvents. The excitation of an electron from the HOMO to the LUMO of the chromophore was observed Stokes shifts are differences between base and excited state of dipol moment (Horvath, 2014). The highest shifted accompanied due to the protic solvent as ethanol. Fluorescence quantum yield was calculated based on standard under the same optical concentration of quercetin. High quantum yield in the extracts indicates that the energy transfer is stronger.

Table 4 shows that beginning of flowering bulb extract has the highest optical quantum yield. In the beginning flowering period, we observed that the energy transfer was higher and the lowest value was after-flowering-leaf.

The Quantum Yield Formula:

$$\Phi_f^i = \frac{F^i f_s n_i^2}{F^s f_i n_s^2} \Phi_f^s$$

Φ^i_f - Φ^s_f : photoluminescence quantum yield of the sample and that of the standard,

f :used because in most cases one is dealing with fluorescence.

F^i and F^s : integrated intensities (areas) of sample and standard spectra,

f_x is the absorption factor (also known under the obsolute term “absorptance”)

the fraction of the light impinging on the sample that is absorbed ($fx = 1 – 10^{–Ax}$, where A = absorbance); n_i and n_s : refractive indices of the sample and reference solution

Compared to the cyclic voltammetry results of beginning flowering stage HOMO level, seem to be very close with the fruit ripening stage but isn't compared after the flowering stage. Figure 2 is the cyclic voltammograms of some extracts. Ferrocene is used as a standard to calculate the energy of the HOMO and LUMO levels, including the ferrocene value of -4.4 eV. (Bredas et al., 1983) equations was used HOMO level calculation:

E (HOMO) =-e [Eoxonset + 4.4]

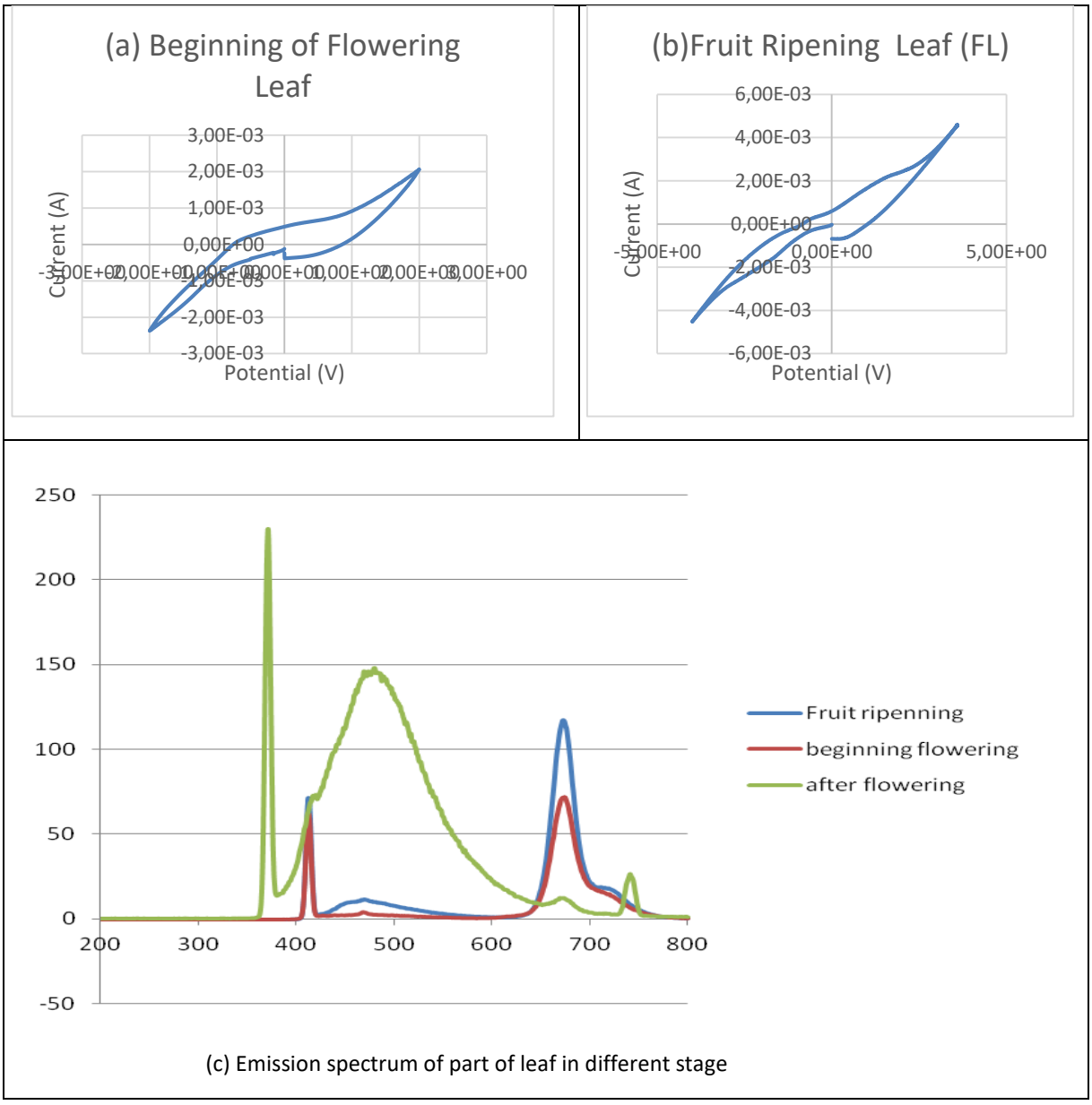

Figure 2. Photophysical properties of *Galanthus elwesii* Hook extracts

Table 4. Quantum yield and HOMO energy level of the extracts

Compound/EtOH	*Quantum Yield*	HOMO (eV)
BL	0.57	-4,38
BB	0.72	-4.22
BR	0.19	-4.19
AL	0.092	-3.57
AB	0.49	-3.96
AR	0.31	-3.65
FL	0.59	-4.12
FB	0.53	-4.75
FR	0.28	-4.36
F	0.09	-3.15

4. Conclusion

Galanthus elwesii Hook. extracts were precipitated in %70 ethanol solvent using general maceration methods. Uv-absorption band and fluorescence emission value confirmed that the different periods and different organs of the plant the light and each extracts interaction differently. By changing the solvent, absorption bands of extracts were obtained from higher value. Upon 255 and 674 nm light excitation, photoluminescence was observed and the photoluminescence bands can be tuned from 328 to 725 nm. Since the highest HOMO level was showed easily electron changeability, electron transfer period are intensively active in the fruit ripening-bulb and beginning of flowering–leaf.

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