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Applying Numerical Indicators of Absorbance Spectrum to Evaluating Color of Flower Petals

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Abstract

Background/Objectives: The study deals with spectral photometry of extracts from flower petals. The objective is to apply numerical indicators at turning points of absorbance spectrum contours to identify the colors. **Methods:** Absorbance spectra of ethanol extracts from red, white and yellow flowers of 15 breeds of plants belonging to 8 genera have been registered by digital spectrophotometer in ultraviolet and in visible light range. The obtained spectra were processed by the proprietary computer program. Student's t-test was applied for statistical data processing. **Findings:** Wavelength, absorbency, the values of the first-order derivatives at maximum points, at turning points, at the steps of the absorption band contour and the absorption intensity values have been identified for each registered spectrum. Absorbance spectra of the analyzed extracts from petals of the flowers belonging to the same genus and to different genera with various coloring have been found plausibly and considerably different in terms of their numerical indicators. The most significant differences have been identified in such parameters as wavelength, absorbency at turning points and the values of the first-order derivatives at the step points of the absorbance spectra in the extracts from red flower petals, and in maximum wavelengths and absorption intensity in cases with white and yellow flower petals. The sets of the numerical indicators, grouped at the turning points of the absorbance spectrum contours, are individually specific for the flower petals of each plant genus. **Applications/Improvements:** The absorbance spectrum numerical indicators of petal extracts can serve as a generalized distinguishing taxonomic attribute to be employed in floriculture for certification of the newly created flowering plants.

Keywords: Absorbance Spectrum, Applied Spectral Photometry, Flower, Petal Color

1. Introduction

The color of flower petals is the most important specific characteristic of a plant. Fast developing floriculture sets the tasks of identifying the flowers of the new cultivars of garden plants. Visual evaluation of color is of subjective character. In particular, the color of the petals depends on microscopic surface asperities[1], temperature[2] and other factors. Measuring devices applied in paint and coatings industry and in textile industry are of little use here, insofar as their readings are affected by surface texture, moisture and other external conditions[3] that could hardly be standardized in cases of flower petals. Presently, the development of objective instrumental methods[4] for evaluating the color of the flowers of the newly created plants is of current importance. Given the fact that the color of the petals depends on the light absorbed by chromophores of the pigments contained therein[5], the data obtained through absorbance spectral photometry of the extracts could be used as attributes for certifying the genera of flowering plants in floricultural practices.

Principal numerical indicators of optical Absorbance Spectra (AS) are the Wavelengths (λm) and the absorbency values (Am) of absorption maximums. Additional

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Numerical Indicators (ANI) are represented by half-breadth of a spectral band, integral value of absorbency, asymmetry factor and others, to determine which the researchers traditionally use the wavelengths at the points where the contours of the Absorption Band (AB) cross the level of half-maximum $0.5Am$[6], that is usually realized in bell curves of AS.

For example (Figure 1, curve Sp_1), AS contour of the tincture made of the petals of white flowers of common lily (*Lilium candidum* L., genus Liliaceae) crosses the half-level at two points *b* and *c* at wavelengths λ_b and λ_c.

The half-breadth of the Absorption Band (AB) is found as the difference between those waves, and the absorbency is equal to the area under AS contour within the abovementioned points.

However, the shapes of the AS band contours of the plant extracts differ considerably from the bell shaped curve, and it is not always possible to obtain two points where the contour crosses the half-level. For example (Figure 1, curve Sp_2), AS contour of the tincture made of red petals of garden rose (*Rosa* × *hybrida* (New Ton), genus *Rosaceae*), is crossed at the half-level at just one point *h*, and in such cases conventional ANI cannot be determined. Besides, the half-level points cannot be applied for the steps that are often observed in AS contours of flowers (see, for example, Figure 1, curve Sp_2 steps S_1 and S_2). Here comes the issue of developing the method for determining ANI for AS of the flower petal extracts with different shapes of AB contours. The problem can be solved by using the Turning Points (TP) of AB contour instead of the "half-level points" to determine ANI. Application of numerical indicators, obtained at the relevant TPs, to evaluate color in the course of spectral photometric analysis of flower petal extracts has not been properly studied yet. This fact initiated the present study.

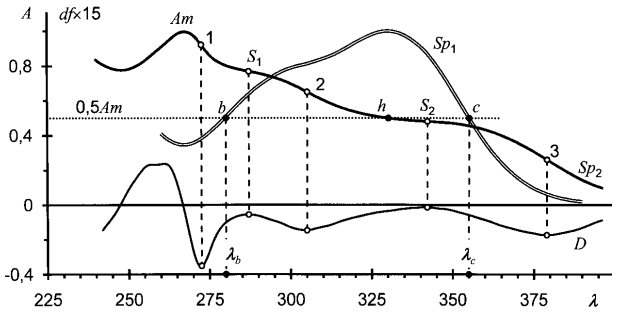

Figure 1. Absorbance spectra of extracts from white flower petals of lily (*Sp*1), red flower petals of rose (*Sp*2) and its first-order derivative (*D*). 1, 2, 3 are turning points, *S*1, *S*2 are steps, $0.5Am$ is half-level. In vertical direction: absorbency *A* and first-order derivative *df*; in horizontal direction: wavelength λ, nanometers.

2. Methods

The study analyzed the flower petals of 15 breeds of agrarian and cultivated plants belonging to 8 genera: Asteraceae, genus Leucanthemum Mill, field daisy (*L. vulgare* L.), genus Matricaria L., wild chamomile (*M. recutita* L.); Iridaceae, genus Iris L., flag-leaf of breeds as follows: Zime smothy, Blue Birds Gost, Mister Roberts; Cactaceae, genus Schlumbergera Lem., Schlumbergera (*S. truncate* (Haw.) Moran); Brassicaeae, genus Brassica L., brown mustard (*B. juncea* (L.) Czern.); Malvaceae, genus Malva L., hollyhock (*M. alcea* L., × *hybrida* (Scarlet)); Paeoniaceae, genus Paeonia L., anomalous peony (*P. anomala* L.); Rosaceae. genus Rosa L., garden roses of breeds as follows: Flame-tanz, Alein, Flame King and genus Potentilla L., silverweed cinquefoil (*P. anserine* L.); Compositae, genus Inula L., Japanese elecampane (*I. japonica* Thunb.) and genus Heliantus L., sunflower (*H. annuus* × *hybrida*). Only pseudo-semiflosculous ray florets have been studied in daisy, chamomile, elecampane and sunflower.

According to their visible color, the petals of the flowers were conventionally split in three groups: red, white and yellow. Fifteen flowers were taken from different plants of one genus. As petals were selected, the middle third of their apical part was cut out, ground in mortar box with quartz sand and 95%-ethanol, infused in dark-glass bottles during 24-36 hours, and then filtered through the paper filter. Filtrate absorbance spectra were measured by digital spectrophotometer UV-2501PC (Shimadzu, Japan) with 1 nanometer step in the range of 220 to 550 nanometers. The spectra were processed by special computer program, registered by the authors with the State Registry of Intellectual Property under register number 2009614442[7]. The program included algorithms for numerical differentiation and for calculating special points of AB contours taking into account the facts that the first-order derivative at the maximum point of the function reverses sign from "+" to "–", at the turning points it reaches the maximum, and at the step points it reaches the minimum absolute values (Figure 1, curve *D*, the scale of derivative *df* along vertical axis has been adjusted to the scale of *A* for the purposes of visualization). Applying the abovementioned program, the AS was normalized by the greatest maximum; the wavelengths λ,

absorption density values *A* and the values of derivatives *df* at TPs and at the steps of AB contours were calculated together with the absorption intensity values *S* as an integral within the limits of the lengths of the waves located next to the right and to the left of the TP maximum. The obtained data were statistically processed applying Student's t-test[8].

3. Results

The obtained data (1) show that normalized absorbance spectra (NAS) of the extracts from visually red flower petals have one maximum within ultraviolet range of 270-290 nanometers; at the right slope of AB step they have one indicator for the extracts from the garden rose (Flame King) petals (Figure 1) and for hollyhock (Scarlet) petals or two indicators for the extracts from all the rest of the petals under investigation; accordingly, two or three TPs with their wavelengths, absorbency values, and band absorption intensity, that makes 12-17 numerical indicators in all. Registered AS are different in terms of their numeric indicators. Thus, among the numerical indicators of NAS of the extracts from the petals of rose breeds Flame-tanz and Alein, the greatest difference in wavelengths at the right slope of the 1st step makes 62, at 1st-3rd turning points on the right slope of the first AB contour it amounts to 22-36 nanometers. Absorbency values at the points of the 1st and the 2nd steps are different by factor of 3.35-6; at 2nd and 3rd TPs they are different by factor of 3.33-3.87, and as regards the values of the first-order derivative *df* of the steps, they differ by factor of 1.22-1.73. Thereat, absorption intensity values *S* of the bands are different by factor 2.85. Of these 17 numerical indicators, only 6 coincide statistically; all the rest are significantly different ($p < 0.05$).

Similar results have been obtained for anomalous peony: in AS of the extracts from its petals, among 17 numerical indicators, the differences were insignificant only for 5 indicators: absorbency of the maximum, lengths of the waves at the left, and at the 1st and 2nd right TPs, absorption intensity; the rest are significantly different. In AS of the extracts from hollyhock (Scarlet) just 3 indicators are insignificantly different: absorbency of maximum, the values of the first-order derivative at the first step and the absorption intensity; all the rest of the differences are significant ($p < 0.05$). Besides, it should be noted that in AS of the extract from the petals of this plant there is only one step, by contrast to AS of the extracts from the flower petals of the abovementioned plants (Table 1).

Table 1. Numerical indicators of AS of extracts from visually red flower petals of different plants

Indicators				Plants			
				Rose, breed		Anomalous peony	Hollyhock Scarlet
				Flame-tanz	Alein		
λm				270±1	277±1	279±1	290±1
*Am***				1±0.022	1±0.019	1±0.018	1±0.016
TTP	on the left		*λ*	262±1*	268±1	263±1*	279±1
			A	0.931±0.076*	0.937±0.056*	0.742±0.062	0.810±0.063
	on the right	1	*λ*	273±1	306±1*	307±1*	304±1
			A	0.985±0.058	0.534±0.032	0.659±0.048	0.725±0.068
		2	*λ*	306±1	342±1*	341±1*	348±1
			A	0.580±0.024	0.150±0.008	0.287±0.016	0.250±0.012
		3	*λ*	337±1	359±1	387±1	N/A
			A	0.323±0.014	0.097±0.005	0.083±0.006	
Step on the right	1		*λ*	277±1	339±1	325±1	327±1
			A	0.966±0.071	0.161±0.005	0.415±0.018	0.350±0.022
			df	-0.0027* ±0.00018	-0.0033 ±0.00028	-0.0069 ±0.00056	-0.0026* ±0.00017
	2		*λ*	333±1	358±1	372±1	N/A
			A	0.332±0.028	0.099±0.007	0.118±0.009	
			df	-0.0015 ±0.00012	-0.0026 ±0.00022	-0.0020 ±0.00018	
S				11.7±1.2	33.3±2.8	24.4±1.8*	25.9±2.1*

(Note: insignificant difference: * – between the pair of data, ** – between the data of the whole line at $p > 0.05$)

The obtained data show that NAS of the extracts from visually white flower petals include two ABs with maximums in UV range of 261-271 and 329-346 nanometers accordingly. AB contours are smooth without any steps (Table 2) or with one step (Figure 2, curve 2) at the right slope of the second AB. Each AB is characterized by absorption intensity *S*. 14 numerical indicators have been obtained for these spectra.

Given the obtained data, it can be maintained that the registered NAS are different in many numeric indicators. Among the numerical indicators of NAS of the extracts from the white petals of the investigated breeds of flag-leaf, the differences in the wavelengths of maximums amount to 10-17 nanometers; those at the right TPs differ by 9-12 nanometers, and absorption intensity values are different by factor of 1.37 - 1.52. Absorbency values at all TPs and those of the second maximums differ by factor of 1.03 - 1.46, and at the first maximum point they statistically coincide. Thereat, for six of fourteen numerical indicators no statistically significant differences have been discovered, the rest differ significantly ($p < 0.05$).

Similar results have been obtained for NAS of the extracts from the petals of daisy and NAS of the extracts from Schlumbergera, where the coincidence has been discovered only in the wavelengths of the first maximum, in absorption intensity of both bands and in absorbency values of the first maximum. In other words, there are only four insignificant differences among 14 obtained indicators; the rest differ significantly ($p < 0.05$).

The obtained data show that NAS contours of the extracts from visually yellow petals have a representation that includes five (Table 3) or more ABs (Figure 2, curve 3) without steps, smooth or with one or two steps.

The first maximums are within UV range of 265-360 nanometers, and the last three maximums are in "blue" area of the visible range of 413-461 nanometers and they

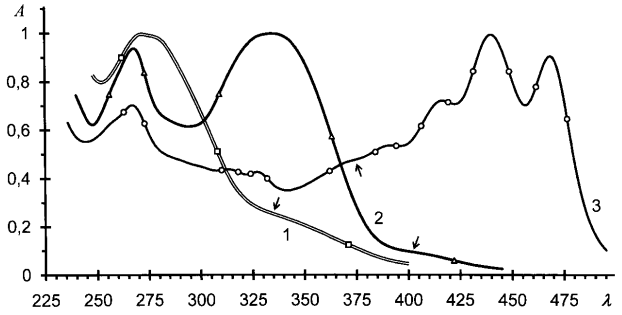

Figure 2. Absorbance spectra of extracts from red flower petals of garden rose (Flame King) (1), from white ray florets of wild chamomile (2) and from yellow flowers of silverweed cinquefoil (3). Tags identify turning points, arrows identify steps; in vertical direction: absorbency *A* per unit; in horizontal direction: wavelength λ, nanometers.

Table 2. Numerical indicators of AS of extracts from visually white flower petals of different plants

No. of band	Indicators			Plants			
				Field daisy	Flag-leaf, breed		Schlumbergera
					Zime smothy	Blue Birds Gost	
1	λm			269±1*	261±1	271±1*	268±1*
	Am			0.745±0.061	1±0.021*	1±0.024*	1±0.023*
	TP	left	λ	252±1	255±1*	256±1*	263±1
			A	0.540±0.048	0.962±0.082*	0.934±0.056	0.961±0.078*
		right	λ	273±1	262±1	277±1	285±1
			A	0.673±0.045	0.998±0.076	0.906±0.084	0.752±0.066
	S			7.9±0.5*	11.9±1.2	8.7±0.4*	21.2±1.3
2	λm			334±1	346±1*	329±1	344±1*
	Am			1±0.022	0.893±0.079	0.934±0.071	0.577±0.042
	TP	left	λ	312±1*	315±1	311±1*	324±1
			A	0.742±0.066	0.647±0.063	0.790±0.053	0.498±0.034
		right	λ	361±1	378±1	387±1	372±1
			A	0.559±0.072	0.567±0.044	0.388±0.029	0.363±0.031
	S			33.1±1.8*	50.4±4.3	33.1±2.1*	25.9±1.2

(Note: * – insignificant difference between data at $p > 0.05$)

Table 3. Numerical indicators of AS of extracts from visually yellow flower petals of different plants

No. of band	Indicators			Plants			
				Flag-leaf, Mister Roberts	Japanese elecampane	Brown mustard	Sunflower, Oreshek (Nut)
1	λm			273±1	297±1	265±1*	267±1*
	Am			1±0.012*	0.937±0.076	1±0.014*	0.645±0.048
	TP	left	λ	266±1	277±1	263±1*	263±1*
			A	0.922±0.078	0.736±0.056	0.991±0.082	0.608±0.048
		right	λ	277±1	301±1	273±1	282±1
			A	0.952±0.076	0.932±0.074	0.803±0.063	0.505±0.042
	S			11.7±1.1*	21.8±1.8	10.3±0.8	11.6±0.9*
2	λm			332±1	323±1	360±1	326±1
	Am			0.911±0.082	1±0,014	0.520±0.046	0.364±0.024
	TP	left	λ	312±1*	314±1*	345±1	324±1
			A	0.864±0.072	0.960±0.081	0.479±0.036	0.359±0.028
		right	λ	362±1	347±1	0.386±1	331±1
			A	0.653±0.054	0.628±0.051	0.433±0.038	0.334±0.026
	S			45.4±3.9	30.5±2.8	20.6±1.6	2.8±0.1
3	λm			413±1	424±1	416±1*	416±1*
	Am			0.301±0.018	0.181±0.012	0.561±0.042	0.693±0.054
	TP	left	λ	405±1	414±1	407±1	384±1
			A	0.294±0.018	0.173±0.009	0.473±0.032	0.314±0.022
		right	λ	419±1*	425±1	420±1*	419±1*
			A	0.291±0.018	0.181±0.011	0.547±0.042	0.682±0.051
	S			3.8±0.2*	2.1±0.1*	7.5±0.6	9.7±0.8
4	λm**			437±1	439±1	439±1	438±1
	Am			0.306±0.028	0.193±0.008	0.781±0.062	1±0.012
	TP	left	λ**	431±1	433±1	431±1	430±1
			A	0.290±0.018	0.187±0.012	0.628±0.052	0.816±0.074
		right	λ**	447±1	449±1	449±1	447±1
			A	0.260±0.018	0.168±0.011	0.632±0.054	0.819±0.074
	S			5.0±0.4	3.2±0.2	13.7±1.1	16.7±1.4
5	λm**			467±1	468±1	469±1	467±1
	Am			0.244±0.021	0.147±0.011	0.729±0.062	0.912±0.081
	TP	left	λ	461±1*	453±1	463±1*	461±1*
			A	0.222±0.018	0.140±0.009	0.611±0.056	0.774±0.064
		right	λ	476±1*	480±1	477±1*	475±1*
			A	0.173±0.008	0.097±0.007	0.490±0.032	0.610±0.056
	S			3.6±0.2*	2.4±0.1*	9.8±0.8	12.3±0.9

(Note: insignificant difference: * – between the pair of data, ** – between the data of the whole line at $p > 0.05$)

form the so-called "blue triad". Average maximums of the "triad" have almost similar wavelengths (437-439 nanometers) and the highest absorbency values that are by 9-27% higher than the neighboring values. The wavelengths of the first maximums of the "triad" differ from each other by just 3-8 nanometers, and those of the third maximums coincide. The values of other numerical indicators of AB contours of the "triad" approximate each other. These data show that the maximums of the "triad" are stipulated by the chromophores of the substances of carotenoid nature. Obviously, these substances are the derivatives of lutein that mostly give yellow color to the petals of the flowers under investigation. Carotenoids (as well as flavonoids) are characteristic for the majority of yellow flowers of the plants of various genera and breeds: marigolds[9], saffron crocus[10], tomatoes[11], canoe-wood[12], artificially bred Prairie gentian[13], orchids[14] and others[15]. Due to the abovementioned fact, the maximums of the "triad" can hardly serve as distinguishing attributes in analyzing AS of the extracts from the flowers of this group and, therefore, they are not considered any further.

To identify NAS of the extracts from visually yellow petals, the numerical indicators of the contours of the first ultraviolet AB can be used. Here, 14 numerical indicators have been obtained out of which only 5 are statistically insignificant, namely, the wavelengths and absorbency values of the first maximum, wavelengths of the left TP contours at first and second ABs, and also the absorption intensity of the first AB (Table 3); the rest of the numerical indicators differ significantly ($p < 0.05$).

NAS of the extracts from various flower petals of the plans of the same genus (Iris L., Rosa L.), and of the plants of different genera differ significantly. The aggregates of the numerical indicators at AS contour turning points are specific and individual for the colors of the flower petals of each particular plant genera.

It should also be noted that spectral photometric identification of petal color applying AS numerical indicators is quite simple as compared to other methods (for example, thin-layer chromatography), and it does not require any additional reagents, except the extracting agent. It takes just 8 minutes to obtain numerical indicators for one AS; thereat, the accuracy of digital spectrophotometer, is known to be within the range of ±0.004 *A*.

4. Conclusion

1. The suggested method for evaluating the color of flower petals applying numerical indicators of NAS of the relevant extracts represents comparatively less labor-intensive and quite precise technique.
2. The aggregate of numerical indicators of NAS creates a spectral photometry "picture" or passport of the extracts from the flower petals that is unique for the flowers of one genus of plants and that can serve as a generalized taxonomic attribute.
3. The developed method of spectral photometry evaluation based on conventional and non-conventional (turning points and steps) numerical indicators can be advantageously applied in floriculture to describe and to certify newly created breeds of flowering plants.

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