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Research Article

Using the Principles of Fractal Analysis for Description of Plant Flavonids Metabolism

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ABSTRACT.

Flavonoids are physiologically active substances that regulate many functions in plants and animals [28, 50]. Despite the long history of the study, interest in this group of substances is not weakening [14, 35], the market of flavonoids in the health industry is also growing [16].

In the Southern Trans-Urals, we found considerable fluctuations in the accumulation of the amount of flavonoids in many plants of local flora [1, 4, 30]. Later significant differences were shown in the accumulation of flavonoids and organic compounds with similar physicochemical properties at the level of individual compounds within local co-deliminations, as well as between spatially isolated populations [38]. At the same time, it is known that the correlation of the accumulation of flavonoids and the content in the soils of copper, zinc, and other metal salts are unstable [38, 44]. In other words it is not possible to identify by standard statistical methods environmental factors that uniquely regulate the accumulation of flavonoids in the field.

In recent years, numerous information is accumulated that in ecologically equivalent environmental conditions alternative biological and ecological processes can be realized. Thus, there are prerequisites to consider the biosynthesis of flavonoids in real environmental-cenotic conditions both from the standpoint of traditional biochemistry [16, 25, 50], and from the standpoint of neutralist concepts.

The general principles of the formation of a variety of flavonoid molecules were investigated. It is proved that the ways and mechanisms of flavonoid biosynthesis may have properties of a stochastic fractal. It is precisely the fractal properties that can explain the relatively weak relationship between environmental conditions and the spectrum of flavonoids synthesized by plants.

Keywords: stochastic fractals, flavonoid biosynthesis, fractal analysis, metabolism, Akaike information criterion.

INTRODUCTION.

In various modifications of unified neutral theory, a weak (statistically insignificant) dependence of various biological processes on environmental conditions is approved [18, 21, 37]. This lead to our attempt to apply neutralistic approaches to the analysis of the arrays of our data justified. One of the most generalized concept of the stochastic appearance of self-similar structures is fractal analysis [13, 27, 42, 43].

The synthesis of flavonoids is also subject to strict rules of addition / substitution of radicals to the original structure. The main question is: how does the self-similarity feature manifest in the metabolic system formed by rigidly deterministic elementary metabolic cells?

In this connection, the question arises: can the flavonoid biosynthesis system ensure the emergence of numerous ecologically equivalent sets of flavonoids that are not ecologically equivalent but which do not coincide in physico-chemical properties?

Let us consider the properties of flavonoids as a system of classes of chemical compounds from the viewpoint of fractal analysis.

METHODS AND MATERIALS.

The source literature data on a common variety of really described compounds and their derivatives can also be represented as a matrix. To construct this matrix, the works of the following authors were used: Korulkin (2007), Internet resource Biocyc. org (https://bsubcyc.org/), Petrussa, et.al. (2013), Harborne (2013). This data is shown in Table 1.

Table 1: The distribution matrix of the described flavonoids by classes and their derivatives

Flavonoid classes	Variants of the modification of molecules and examples									
	Single metabolites	monoglicosydes	2-3 glicosydes	Isopren derivates	Mono and digallates	Methyl derivates	Sulfur derivates	dimers	Galogen derivates.	**Total**
Chalcons	1 Naringenin-chalcon	-	1	4	-	1	4	-	-	**10**
Flavons	10 Eriodictiol, Naringenin, Pentahidroxy-flavanon Baikalein	17	-	-	-	15	12	-	2	**36**
Flavanons	5 Hesperitin, Naringenin Liquiritigenin	7	5 Naringin	3	-	4	2	-	-	**26**
Flavanonols	8 Dehydro-quercetin	2		3			2			**15**
Flavan-3-ols, catechins	9 Gallokatechin	10	-	-	8	-	-	-	8	**35**
6Flavan-4-ols	2	-	-	-	-	-	-	-	-	**2**
Flavonols	13 Fisetin, Qiercetin Morin	11 Isoquercetin	7 Rutin	5	-	11	8	-	-	**55**
Flavan-3,4 diols	7	-	-	1	-	-	-	8	8	**16**
Anthocyans	6	12	90	-	-	-	-	1	-	**109**
Flavans	-	-	-	2	-	1	-	-	-	**3**
Isoflavans	-	-	-	2	-	4	-	-	-	**6**
Isoflava-nons	4	8	1	6	-	13	-	-	1	**33**
Neo-flavans	12	-	-	-	-	-	-	-	-	**12**
Total	**77**	**67**	**104**	**26**	**8**	**49**	**28**	**9**	**19**	**387**

Hierarchical structure of flavonoid metabolism

The advantage of objects of fractal nature lies in their hierarchy, which allows to judge about the array as a whole based on the analysis of a random sample.

In this case, when fractal analysis was performed, the minimal self-similar structure was a single molecule of flavonoid with possible variants of its modification and addition of individual radicals (Tab. 1). Schematically, the process of biosynthesis of flavonoids, described in Table 1, is presented in Figure 1.

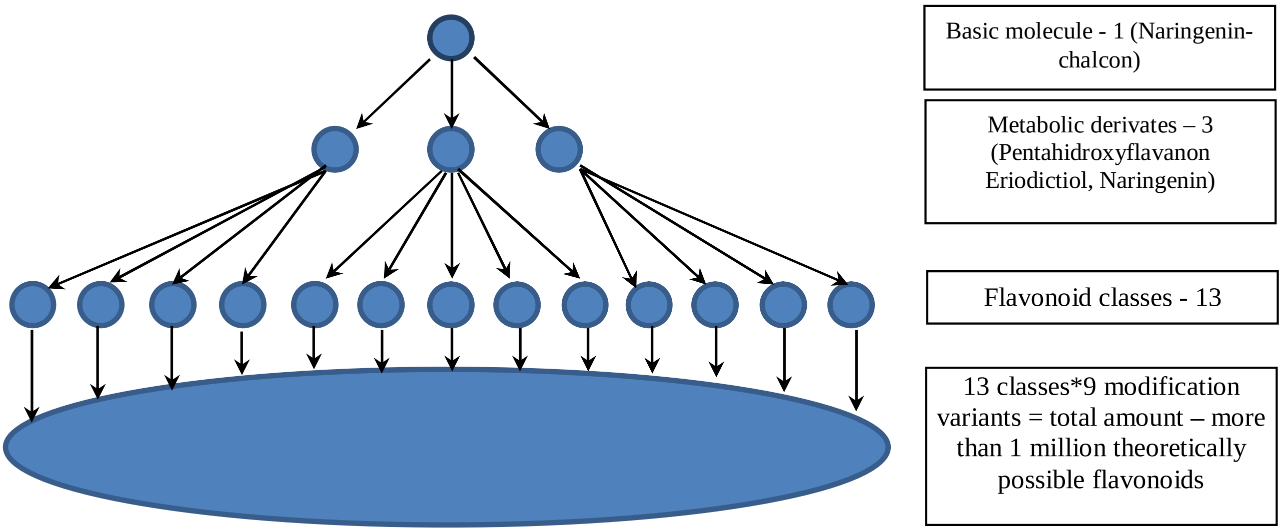

Figure 1. Hierarchy of radical substitutions in the formation of a variety of flavonoids

As follows from the table, different classes of compounds and groups of their derivatives are represented by a different number of compounds. Various authors give the following reasons for such an uneven distribution of substances by class:

1. Insufficient number of studied plant species. It is believed that by now the chemical composition of no more than 10 percent of all known plant species is described.
2. Specificity of accumulation in the studied plant species. It is generally accepted that, along with the ubiquitous classes of flavonoids (flavonols), there are classes specific only for certain plant taxa. For example, it is believed that halogen derivatives are mainly characteristic of gymnosperms, gallates for representatives of the families Fabaceae and Theaceae, etc.
3. Unequal physiological significance and ecological role of representatives of individual classes of flavonoids and their derivatives. For example, free forms of flavonoids can not be used by plants as cryoprotectants, and catechins cause a variety of coloring of petals of flowers.
4. Difficulty of extraction and identification. Not all compounds can be isolated from organs and tissues in the native state, not to mention the adequate determination of their intravital quantitative content in plants. This applies to the greatest extent to glycosylated forms, for the decomposition of which often a slight acidification of vacuolar juice.

Among the classes of compounds, the most common are anthocyanins, flavonols, flavones and isoflavones. Moreover, among the compounds there are also classes represented by a small number of compounds and their derivatives - flavans, neoflavans and flavan-4-ol.

Among the variants of modification of compounds, the most common are free substances, glycosides of various composition, methyl, sulfo and prenyl derivatives. The smallest number are the esters of gallic acid and the dimers of the compounds. However, no variant of the modification of the structure of compounds is found in all classes of compounds [16, 23, 32].

Thus, the variety of flavonoids is realized in response to the action of two fundamentally different processes. The first process concerns the formation of a diversity of the base molecule (the addition of hydroxyl groups, the formation of double bonds, the use of oxygen in the gerocycles, etc.) and the formation of derivatives of different nature (glycosides, prenyl and acyl derivatives, gallates, etc [23, 40].

Fractal analysis

Calculations were carried out according to the methodology described [13]. At the first stage of the analysis, individual samples and the general logic of their subsequent grouping into more and more large samples are selected. This determines the further procedure for compiling a hierarchical matrix for fractal analysis.

Thus, the number of flavonoids (397) is described as part of a theoretically possible (more than 1 million) sample. Therefore, it was this sample of compounds (Tab. 2) that was evaluated for a fractal nature. To do this, these tables were previously converted into a hierarchical matrix according to the following algorithm (Tab. 2) [13].

Table 2: General results of the fractal analysis of flavonoids diversity

Stages of analysis	**Procedure**	**Results**
Sampling	Non-formalized selection of an assumed self-similar structure	minimal self-similar structure was defined as a separate single flavonoid molecule
Skaling	Estimation of the range of variation scales of a set of self-similar structures	The identified range (N) was from one molecule to all theoretically possible diversity (Fig. 1)
The calculation of the indicator ni - the share of each value in the formation of the overall picture for each of the parameters	The process is a classic ranking by variables. For each parameter, the value of each cell is divided by the total amount indicated at the bottom. As a result, relative values appear in the matrix	The hierarchical matrix was compiled
The choice of the values of the index of distribution "q"	A fractal object must have self-similarity properties in a wide range of scales. This range is given by "q" values	In practice, it is enough to apply a series of "q" values from - 3 to 3 in increments of 0,1-0,5
The calculation of the indicator of the set of moments of distribution "Mq"	1. For each q value, the matrix content is raised to a power equal to "q" 2. In each of the resulting matrices, the sum of the values of each horizontal data series is calculated in each row	The result will be a measure of the order of the moments of the distribution "Mq"
Estimation of self-simulation through the assessment of the relationship between indicators "N" and "Mq"	$M_q(N)= \sum_{i=1}^{(N)} p_i^q$	The entire chosen set of flavonoid molecules possess the property of self-similarity, since all the correlations between the logarithms of Mq and N are significant (p> 0.05), and the values of the correlation coefficients tend to 1 (Tab. 3)
Estimation of stochasticity by applying linear and quadratic models and evaluating their applicability by Akaike information criterion (AIC)	$AIC = ln\frac{RSS}{n} + \frac{n+k}{n-k-2},$	In all cases, the nonlinear model is better applicable to the observed pattern than the linear one (Fig 2)
Estimation fractal properties of total flavonoids variety	Flavonoid biosynthesis is stochastic organized self-similar structure, forming fractal stochastic system	

RESULTS.

The results of fractal analysis of the compounds described in the literature

As a result of the analysis, it was found that the data array has the properties of a fractal object.

Table 3: Results of the correlation analysis of the dependence of indicators log N and log Mq for matrix of actually described substances

q	q=0,2	q=0,3	q=0,5	q= 1,2	q=1,5	q=2
correlation coefficient	0,75	0,76	0,74	0,66	0,65	0,65
p-level	0.01	0.00	0.00	0.00	0.01	0.01

Thus, the self-similarity of the observed pattern can also be considered proven.

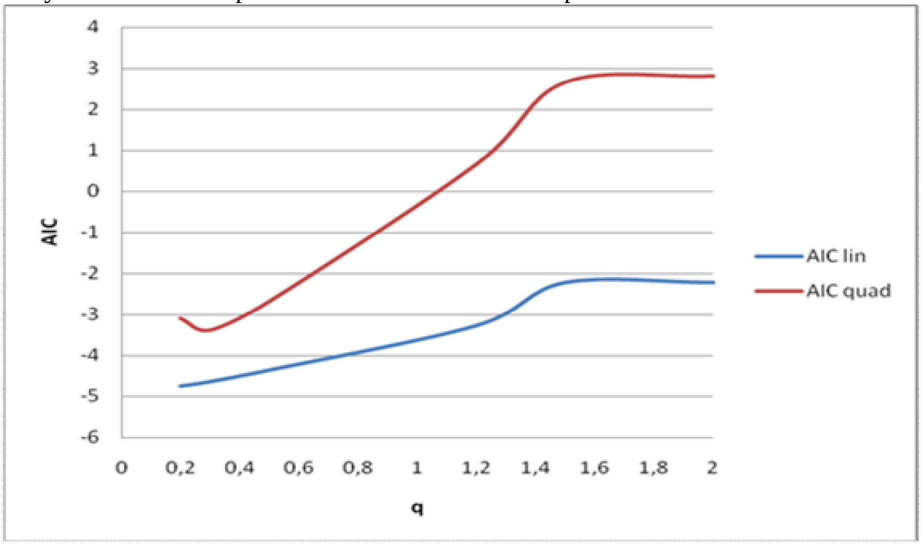

Figure 2. The value of the criterion for linear and quadratic models describing the the pattern of the distribution of substances by classes for the compounds described in the literature.

The second condition is fulfilled, therefore, we can assume that the biosynthesis system for flavonoids and substances with similar physicochemical properties, described in the literature, can be fractal in nature.

DISCUSSIONS.

The biosynthesis of flavonoids is a classical tree originating from a single predecessor (naringenin-chalcon) and forming numerous branching metabolic chains. Transitions between substances in Table 1 are determined by the general laws of addition or substitution of radicals. In any case, any substance must have a continuous chain of successive biosynthesis from the original naringenine-chalcon molecule to this substance. In addition, in the flavonoid biosynthesis system, there are numerous alternative routes (shunts) that allow you to link neighboring chains. At the same time, the total number of such shunts is currently unknown [16, 23, 31, 32, 33].

Each new substance of the metabolic tree is formed if there is an elementary conductive metabolic cell: "substrate" enzyme (gene)> product ". Only on the networks of such elementary cells can be synthesized any substance described in Table 1. The principal property of metabolic chains is their continuity: the appearance of an "end" substance is possible only under the condition of continuity of the previous links of the chain. However, such a conducting metabolic cell itself is the object of numerous regulatory influences on the genetic, substrate, physiological and ecological levels [12, 15, 17, 19, 22, 34, 35, 36, 38, 39, 52].

We can assume that the flavonoid biosynthesis system forms a network where from anywhere you can go to any point along several routes, including alternative routes (shunts), including those that are not yet described in the literature. There are numerous reports that each specific biosynthesis of flavonoids has a pronounced system of activation and inhibition [24, 45, 47, 51]. And it is the presence of branched networks and alternative biosynthetic pathways that allows one or another combination of activators and inhibitors to be bypassed through other metabolic cells for which this combination is not critical.

Apparently, the application of the approaches of one of the applications of fractal ideology to the theory of percolation, which considers the propagation conditions, the "flow" of any substrate (substance, signal) through a randomly inhomogeneous medium [13], suggests here. The appearance of a particular compound is the result of the "flow" of precursors through different metabolic cells conducting the synthesis. For different metabolic cells, there may be different threshold values that inhibit this or that conductive cell, but do not inhibit cells with other physico-chemical and physiological-biochemical regulators. The principal property of percolation systems is the continuity of the flow (signals, processes) from the reference point to the final recorded point (flux, substance, signal or process). Possessing such properties, the flavonoid biosynthesis system can be a fractal system where the self-similarity principle is observed for metabolic networks of any sizes within the hierarchy formed by permissible radical substitutions in classes of flavonoids. As noted by Gelashvili et al. (2013), fractal analysis has "a unique property that allows you to make a statistically correct conclusion about an object as a whole on the basis of available information about a part of the object ". Thus, the analysis of literature data allowed to build a model for the appearance of individual substances as a result of the fractal organization of the biosynthesis system of flavonoids and compounds with similar physicochemical properties.

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