

Quantitative chemobiology*

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Abstract: One of the mysteries of man's contact with nature concerns the question why shamanism and science so often lead to consistent results in the search for useful natural products. To find clues toward an answer via a coherent chemobiological language, structural and functional information is taken into account. The functional information, given by ethnobotanical codes based on traditional knowledge (via number of useful dicotyledon species), is confronted with the structural information, given by metabolic codes based on micromolecular diversification (via number of occurrences in dicotyledons). The challenge to integrate these databases implies in the development of qualitative (via dahlgrenograms) and quantitative (via Sporne indices) models into an evolutionary framework. The follow-up of ethnobotanical qualifications reveals systematic and evolutionary patterns, susceptible to juxtaposition into a phytochemical background based on gallates (GAL) and caffeates (CAF), regulators of intermediate metabolism. Comparison of the two models of information suggests that the "spectral" features of the GAL and CAF-models are coincident with the ethnobotany-guided food- and medicine-plants respectively. Hence, the complementary nature of food and medicine species can be rationalized by oscillations between GAL/CAF. Analyses of these indicators of toxicity would allow advances in the understanding of mechanisms regulating the production of bioactive products.

INTRODUCTION

The impact of the traditional healer vs. the modern physician is anticipated to detect patterns of similarity between these apparently different types of knowledge. But, how should we proceed to integrate traditional knowledge and scientific endeavour into a single framework? Only a coherent chemobiological "language" can achieve the integration of structural (chemical) and functional (biological) codes. After all, the biological functions of plants are also due to the chemical arsenal. Thus, our major challenge consists in the replacement of the traditional approach, by a quantitative methodology, one of the most potent prospects for the understanding of the functioning of nature.

DATABASE

An appropriate database was extracted from a vast ethnobotanical survey [1] elaborated during the first years of the 20th century, chiefly in Brazil, prior to the more recent, massive deforestations of South America. According to the author, this traditional dictionary constitutes an integrated report of the essential characters of each species listed: therapeutic information (having been confirmed or merely recommended for chemical and/or physiological studies), nutritional properties, associated supersti-

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internal locations of the diagram. In addition, preference for plants used as foods characterizes two clusters (SI 38–52 and 43–56) appearing at more internal locations, the Hamamelidae–Dilleniidae–Rosidae complex, displayed at the diagonal from bottom left to top right.

ETHNOBOTANY: QUANTITATIVE MODEL

To quantify the ethnobotanical patterns observed, we developed a model taking the number of useful species of each dicotyledon family into account. Next, the dicotyledon families were classified according to their evolutionary status, each SI corresponding to a percentage of food (full lines) and medicinal (dashed lines) species (Fig. 2). While food species are usually characterized by intermediate SIs ($\cong 50$), medicine species occupy a large SI range reaching the higher values (>60). An uncertain situation occurs with families of intermediate status, where we find a quasi equilibrium between types of useful plants.

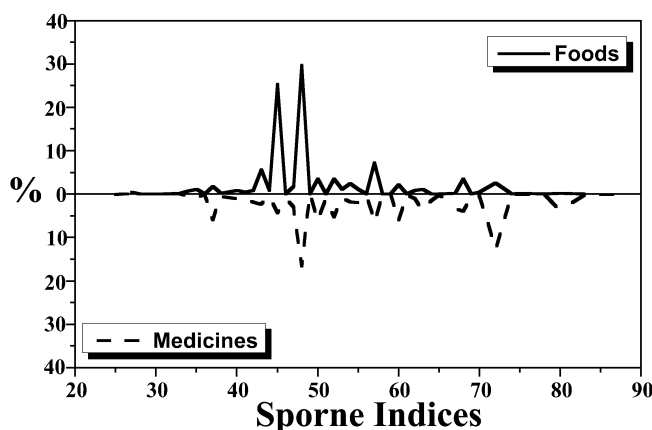


Fig. 2 Evolutionary spectrum indicating, for each evolutionary status (described by SI) [3], the number of species (in percentage values) qualified as foods (full lines, top) and as medicines (dashed lines, bottom) in an ethnobotanical survey [1]. Normalization was effected considering the total number of species cited for each ethnobotanical application (foods and medicines).

PHYTOCHEMISTRY: QUANTITATIVE MODEL

To quantify the phytochemical patterns, we developed a model taking the number of occurrences (i.e., the number of compounds per species) of selected micromolecular categories of each dicotyledon family into account. Similarly to the ethnobotanical model, the families considered were classified according to SIs.

This procedure revealed a common evolutionary pattern: the diversification of compounds increases gradually, reaching maxima prior to fast decrease and substitution of one category by another one, but not by clear-cut exclusions (Fig. 3A) [5–8]. Substitutions among these categories demonstrate that this evolutionary process operates in countercurrent to the biosynthetic sequence, i.e., by successive step-by-step shortening of the shikimate pathway toward replacement by acetate derivatives (“evolutionary channeling”) [9]. This process leads to the accumulation of gallates (GAL). An activator of the enzyme phenylalanine ammonia-lyase (PAL) [10], GAL opposes, via “negative feedback”, the trend of evolutionary channeling and plays a decisive regulatory role in this history. In contradistinction, caffeates (CAF), inhibitors of PAL [10], supply “positive feedback”, revigorating the pathway in its original evolutionary direction. Thus, upon juxtaposition of the GAL and the CAF models on the SI

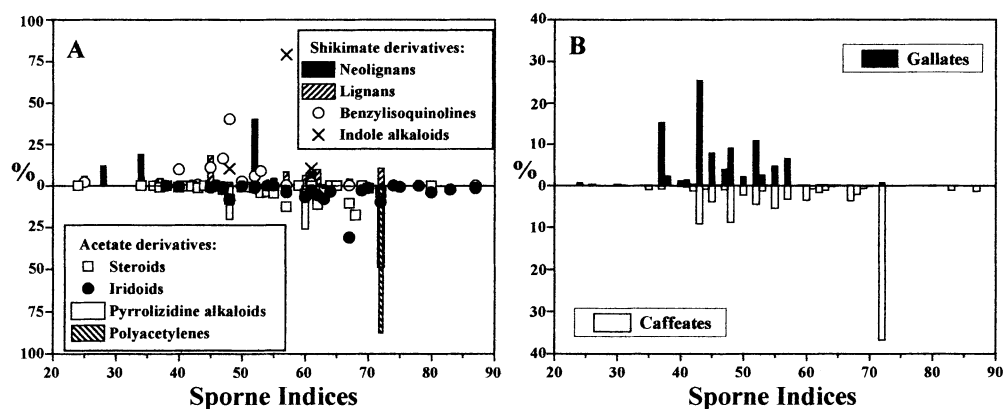


Fig. 3 Number of occurrences, in percentage values, of selected micromolecular categories (each normalized to 100) per morphological advancement indices (Sporne indices) [3] of chemically better known dicotyledon families. A: shikimate (top) and acetate (bottom) derivatives; B: gallate (top) and caffeate (bottom) derivatives. For database see [11,12].

scale, at first sight a very different feature with two complementary parts appears (Fig. 3B). It is easy to distinguish a central part (SI \cong 50) with about equally balanced GAL/CAF portions, flanked by much more intense and practically unbalanced GAL and CAF terminals situated respectively at the low (<45) and the high (>60) SI terminals.

Hence, comparison between these micromolecular evolutionary patterns reveals at the SIs between 35 and 45 decrease of metabolic diversification (Fig. 3A) to coincide with the great diversification of GAL (Fig. 3B). On the other hand, at high evolutionary status (SI 72) increase of metabolic diversification (Fig. 3A) coincides with the great diversification of CAF (Fig. 3B).

These observations suggest the dualistic nature of the GAL/CAF-model, a neat antagonistic pattern [5], possibly a valuable model for bioactive compounds.

ETHNOBOTANY VS. PHYTOCHEMISTRY: QUANTITATIVE MODEL

Comparison of the quantitative model based on ethnobotany (Fig. 2) with the universal phytochemical background (Fig. 3B), suggests that the “spectral” features of the GAL model and of the ethnobotany-guided food plants are coincident (Fig. 4). The analogous features of the CAF model and of the ethnobotany-guided medicine plants are also coincident.

Statistical validation of the degree of association between the quantitative models based on ethnobotany and phytochemistry, was obtained via coefficients of linear regression (r). Analysis of the coefficients for medicinal species revealed a stronger degree of association with diversity of CAF ($r = 0.66$; $P = 0.0001$) than of GAL ($r = 0.36$; $P = 0.0203$). In opposition, degree of association between food plants and GAL is rather low ($r = 0.42$; $P = 0.0086$) even in comparison with CAF diversification ($r = 0.24$; $P = 0.1322$). This is probably due to the fact that plants with higher concentration of GAL, at SIs 37 and 43 (Figs. 3B and 4), are poor in nutritional value [13]. However, at SI 45 the antagonistic effect of CAF diminishes the diversity of GAL, decreasing the astringency, thus increasing their value as food (Fig. 4). This occurs because in spite of decrease in astringency, the GAL concentration is still sufficiently high to cause the inhibitory effect on micromolecular diversity [8,12,14–17] and hence, toxicity remains.

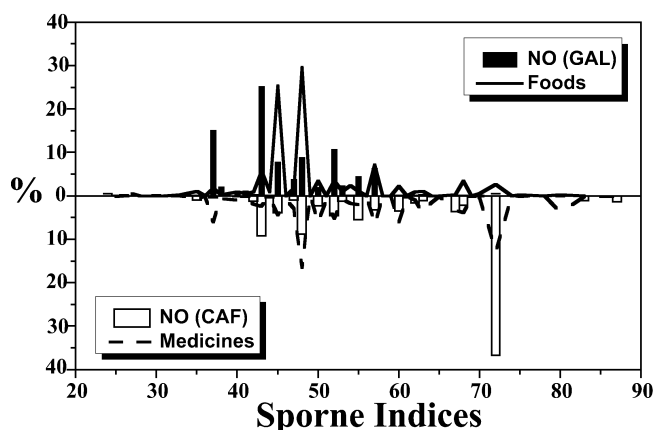


Fig. 4 Superposition of evolutionary spectra based on ethnobotanical [1] (Fig. 2) and phytochemical [11,12] (Fig. 3B) data. Top: Number of species qualified as foods (full lines) and number of occurrence of galls (dark bars). Bottom: Number of species qualified as medicines (dashed lines) and number of occurrence of caffeates (light bars).

CONCLUSION

In angiosperms, the complementary nature of food and medicine species can be rationalized by oscillations between GAL/CAF antagonists. Analyses of these indicators of toxicity would allow advances in the understanding of mechanisms regulating the production of bioactive products. An immediate consequence is the possibility to alleviate the heavy burden of random selection of plant samples, and to enhance the rate of discoveries of natural products.

Thus, now we are apt to answer the challenge question announced during the introduction of this lecture: How should we proceed to integrate traditional knowledge and scientific endeavour into a single framework? Incorporation of new codes, expressing functions in the chemobiological language, becomes possible through evolutionary concepts. Remember Dobzhansky: “nothing in biology makes sense”, and from our point of view not even integration of science and ethnobotany makes sense, “except in the light of evolution” [18].

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REFERENCES

1. M. Pio Corrêa. *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*, Imprensa Nacional, Rio de Janeiro (1984).
2. R. M. T. Dahlgren. *Bot. J. Linn. Soc.* **80**, 91–124 (1980).
3. K. R. Sporne. *New Phytol.* **85**, 419–499 (1980).
4. A. Cronquist. *The Evolution and Classification of Flowering Plants*, 2nd ed. The New York Botanical Garden, New York (1988).
5. O. R. Gottlieb and M. R. de M. B. Borin. *Phytochemistry* **49**, 1–15 (1998).
6. O. R. Gottlieb and M. R. de M. B. Borin. *An. Acad. Bras. Ci.* **70**, 719–726 (1998).

7. O. R. Gottlieb and M. R. de M. B. Borin. *Pure Appl. Chem.* **71**, 1635–1642 (1999).
8. O. R. Gottlieb and M. R. de M. B. Borin. *Mem. Inst. Oswaldo Cruz* **95**, 115–120 (2000).
9. K. Kubitzki and O. R. Gottlieb. *Taxon* **33**, 375–391 (1984).
10. A. Boudet, R. Ranjeva, P. Gadel. *Phytochemistry* **10**, 997–1005 (1971).
11. O. R. Gottlieb, M. A. C. Kaplan, M. R. de M. B. Borin. *Biodiversidade. Um Enfoque Químico-Biológico*, Editora UFRJ, Rio de Janeiro (1996).
12. O. R. Gottlieb, M. R. de M. B. Borin, M. A. C. Kaplan. *Phytochemistry* **40**, 99–113 (1995).
13. L. G. Butler. In *Plant Polyphenols: Synthesis, Properties, Significance*, R. W. Hemingway and P. E. Laks (Eds.), pp. 693–698. Plenum Press, New York (1992).
14. O. R. Gottlieb. In *Plant Polyphenols: Synthesis, Properties, Significance*, R. W. Hemingway and P. E. Laks (Eds.), pp. 523–538. Plenum Press, New York (1992).
15. O. R. Gottlieb and M. A. C. Kaplan. *Natural Product Letters* **2**, 171–176 (1993).
16. O. R. Gottlieb and M. R. de M. B. Borin. *Pure Appl. Chem.* **70**, 299–302 (1998).
17. D. H. T. Zocher, M. R. de M. B. Borin, O. R. Gottlieb. *An. Acad. Bras. Ci.* **70**, 711–717 (1998).
18. T. Dobzhansky, F. J. Ayala, G. L. Stebbins, J. W. Valentine. *Evolution*, Freeman, San Francisco (1977).