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Foliar flavonoids of Annonaceae from Brazil: taxonomic significance

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Abstract

Foliar flavonoids of 31 species of the Annonaceae native to Brazil, amounting to 76 compounds, were isolated and identified. All phenols found were glycosides of either flavones (apigenin, scutellarein, hispidulin and luteolin) or flavonols (kaempferol, rhamnocitrin, 6-hydroxyrhamnocitrin, quercetin, isorhamnetin and rhamnetin), with the latter predominating. Some members of the tribe Bocageeae are distinctive for accumulating 6-oxygenated flavones and flavonols, in addition to 7-*O*-methylated flavonols, a feature possibly linked to the assumed advanced condition of the tribe within the family. Members of *Duguetia* stand out for the apparent absence of quercetin glycosides. *Anaxagorea dolichocharpa* seemingly lacks flavones and flavonols entirely. A UPGMA analysis based on the distribution of flavonoids does not group the analyzed species according to the available tribal division of the Annonaceae. However, several taxonomically meaningful groupings emerged through the multivariate analysis. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Annonaceae; Flavonoids; Flavones; Flavonols; UPGMA; Chemotaxonomy

1. Introduction

The Annonaceae represent a large pantropical family comprising approximately 120 genera and 2000–2200 species. There is general agreement as to the phyletic position of the Annonaceae at the base of the dicotyledons. Close relationships of the family with Magnoliaceae, Eupomatiaceae, Myristicaceae and Degeneriaceae are recognized, although the links between these groups are still not clear (Cronquist, 1988; Chase et al., 1993; Doyle and Le Thomas, 1996).

Most studies of annonaceous flavonoids have dealt with stem bark and roots of *Uvaria* species, which to date have yielded mainly flavanones and chalcones. Such studies have been driven primarily by pharmacological interests (Hufford and Oguntimein, 1982; Kodpinid et al., 1985; Fleischer et al., 1998). Despite the widespread occurrence of flavanones and chalcones in vascular plants (Bohm, 1988), the *Uvaria* flavonoids so far reported have distinctive characteristics, such as association with benzylic groups and absence of B-ring substitution (Leboeuf et al., 1982; Kodpinid et al., 1985; Nkunya et al., 1993). Relatively few Annonaceae genera, such as *Annona*, *Artabotrys*, *Asimina*, *Cananga* and *Dasymaschalon*, have been investigated for foliar flavonoids; *O*- and *C*-glycosylflavones and flavonol derivatives have been reported (Leboeuf et al., 1982; Li et al., 1997; Sinz et al., 1998). So far, however, chemotaxonomic studies based on the distribution of flavonoids in Annonaceae have largely been neglected.

This work reports the foliar flavonoids of some genera and species of Annonaceae native to Brazil, with the purpose of gaining new insight into the foliar flavonoid patterns of the family and evaluating their taxonomic potential.

2. Results and discussion

A total of 76 flavonoids were isolated, with an overwhelming prevalence of flavonols (67) over flavones (9). The flavonols found are glycosides of kaempferol, rhamnocitrin, 6-hydroxyrhamnocitrin, quercetin, rhamnetin and isorhamnetin. In addition to *C*-glycoflavones, the flavones obtained are *O*-glycosides of apigenin, hispidulin, scutellarein and luteolin (Table 1). The wide diversity of structures found is due to the presence

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Table 1
Flavonoids isolated from species of Annonaceae

Flavones

1. Apigenin-7-*O*-glucoside
2. Apigenin-7-*O*-glucosylglucoside
3. Apigenin-8-*C*-glucoside (isovitexin)
4. Apigenin-6-*C*-glucoside (vitexin)
5. Scutellarein-6-*O*-galactoside
6. Scutellarein-6-*O*-glucosylglucoside
7. Hispidulin-7-*O*-glucosylglucoside
8. Luteolin-7-*O*-glucoside
9. Luteolin-6-hydroxy-7-*O*-rhamnosylglucoside

Flavonols

10. Kaempferol-3-*O*-arabinoside
11. Kaempferol-3-*O*-galactoside
12. Kaempferol-3-*O*-glucoside
13. Kaempferol-3-*O*-rhamnoside
14. Kaempferol-3-*O*-arabinosylarabinoside
15. Kaempferol-3-*O*-(arabinose-glucose)[a]
16. Kaempferol-3-*O*-arabinosylrhamnoside
17. Kaempferol-3-*O*-(galactose-glucuronic acid)[a]
18. Kaempferol-3-*O*-galactosylgalactoside
19. Kaempferol-3-*O*-galactosylglucoside
20. Kaempferol-3-*O*-galactosylrhamnoside
21. Kaempferol-3-*O*-glucosylglucoside
22. Kaempferol-3-*O*-glucosylrhamnoside
23. Kaempferol-3-*O*-rhamnosylarabinoside
24. Kaempferol-3-*O*-rhamnosylgalactoside
25. Kaempferol-3-*O*-rhamnosylglucoside
26. Kaempferol-3-*O*-rhamnosylrhamnoside
27. Kaempferol-3-*O*-(xylose-glucuronic acid)[a]
28. Kaempferol-3-*O*-glucosylglucosilglucoside
29. Kaempferol-3-*O*-(glucose-glucose-rhamnose)[a]
30. Kaempferol-3-*O*-(glucose-rhamnose-rhamnose)[a]
31. Kaempferol-3-*O*-(rhamnose-galactose)[a]glucoside
32. Kaempferol-3-*O*-glucoside-7-*O*-rhamnoside
33. Kaempferol-3-*O*-rhamnoside-7-*O*-arabinoside
34. Kaempferol-3-*O*-glucoside-7-*O*-glucosylrhamnoside
35. Kaempferol-3-*O*-rhamnoside-7-*O*-glucosylglucoside
36. Rhamnocitrin-3-*O*-glucoside (7-O-methylKaempferol-3-*O*-glucoside)
37. Rhamnocitrin-3-*O*-rhamnosylglucoside
38. 6-Hydroxyrhamnocitrin-3-*O*-glucoside
39. 6-Hydroxyrhamnocitrin-3-*O*-(glucose-rhamnose)[a]
40. Quercetin-3-*O*-arabinoside
41. Quercetin-3-*O*-galactoside
42. Quercetin-3-*O*-glucoside
43. Quercetin-3-*O*-rhamnoside
44. Quercetin-3-*O*-arabinosylarabinoside
45. Quercetin-3-*O*-arabinosylgalactoside
46. Quercetin-3-*O*-arabinosylglucoside
47. Quercetin-3-*O*-arabinosylglucuronide
48. Quercetin-3-*O*-arabinosylrhamnoside
49. Quercetin-3-*O*-arabinosylxyloside
50. Quercetin-3-*O*-galactosylglucoside
51. Quercetin-3-*O*-galactosylrhamnoside
52. Quercetin-3-*O*-glucosylglucoside
53. Quercetin-3-*O*-glucosylrhamnoside
54. Quercetin-3-*O*-rhamnosylgalactoside
55. Quercetin-3-*O*-rhamnosylglucoside
56. Quercetin-3-*O*-rhamnosylrhamnoside
57. Quercetin-3-*O*-(xylose-glucuronic acid)[a]
58. Quercetin-3-*O*-arabinoside-7-*O*-arabinoside
59. Quercetin-3-*O*-galactoside-7-*O*-galactoside
60. Quercetin-3-*O*-glucoside-7-*O*-glucoside
61. Quercetin-3-*O*-glucoside-7-*O*-rhamnoside
62. Quercetin-3-*O*-rhamnoside-7-*O*-arabinoside
63. Quercetin-3-*O*-rhamnoside-7-*O*-glucoside
64. Quercetin-3-*O*-rhamnoside-7-*O*-rhamnoside
65. Quercetin-3-7-*O*-(arabinose-glucose)[a]
66. Quercetin-3-7-*O*-(arabinose-xylose)[a]
67. Quercetin-3-*O*-rhamnoside-7-*O*-rhamnoside-3′-*O*- rhamnoside
68. Isorhamnetin-3-*O*-galactoside
69. Isorhamnetin-3-*O*-glucoside
70. Isorhamnetin-3-*O*-galactosylgalactoside
71. Isorhamnetin-3-*O*-galactosylrhamnoside
72. Isorhamnetin-3-*O*-glucosylglucoside
73. Isorhamnetin-3-*O*-rhamnosylglucoside
74. Rhamnetin-3-*O*-glucosylglucoside
75. Rhamnetin-3-*O*-glucosylrhamnoside
76. Rhamnetin-3-*O*-rhamnosylglucoside

[a] Relative position of sugars not determined.

of mono-, di- and triglycosides of different sugars and the possibility of glycosylation at the 3, 3/7, or 3/7/3′ positions. Oxygenation at the 6-position and *O*-methylation of A or B rings were also observed in some of the studied samples (Table 1).

Several authors have characterized the basal groups of angiosperms by the prevalence of flavonols over flavones and the absence of myricetin derivatives (Harborne, 1988; Gottlieb et al., 1993). The results of the present work support the position of the Annonaceae among the basal angiosperms.

Among the Magnoliidae, chemical similarities between Annonaceae and Myristicaceae had already been pointed by Gottlieb et al. (1993), based on the common presence of apigenin, luteolin, kaempferol and quercetin. However, the flavonoids from stems and roots of the Myristicaceae have substituted B-rings and are never apparently associated with benzylic groups (Romoff and Yoshida, 1997), in contrast to the Annonaceae (see Introduction). The Magnoliaceae may be distinguished from the Annonaceae by the absence of flavones in the former (Gottlieb et al., 1993), although the two groups are similar in the common presence of *O*-methylated flavonols. The latter compounds take apart both Amonaceae and Magnoliaceae from Myristicaceae. Although Young (1983) indicated the predominance of flavones in the Eupomatiaceae, more recent works have reported the presence of kaempferol and quercetin derivatives in the latter family (Gottlieb et al., 1993). Thus the exact nature of the distinction

between Annonaceae and Eupomatiaceae remains unresolved.

Results of the present work and those of other investigators clearly show that the chemical characterization of the Annonaceae by the absence of B-ring substitution (Leboeuf et al., 1982) is misleading. In fact, among the 36 samples analyzed, apparently none exhibited the presence of flavonoids with that of characteristic or detectable flavanones and chalcones (Table 1), which are commonly found in stems and roots of *Uvaria*. Unfortunately, no information is available about foliar flavonoids of the latter genus, which could permit an evaluation of whether those flavonoid features in Annonaceae characterize plant parts or botanical groups.

The prevalence of flavones and flavonols in leaves, in lieu of the chalcones and flavanones often found in stem barks and roots, suggests different functions for these flavonoid classes. Flavones and flavonols have been assigned a role as UV-B barriers (Wollenweber, 1986), perhaps explaining their wide occurrence in leaves, where they presumably shield constituents of the organelles involved in photosynthesis (Harborne, 1993). On the other hand, flavanones have been shown to possess activity against insects and pathogens (Simmonds, 1998), and flavanones isolated from *Uvaria* possess activity towards microbial agents (Fleischer et al., 1998).

Table 2 presents the distribution of flavonoids in the investigated species, grouped according to Fries (1959). Intraspecific variation is visible in *Guatteria*; only one of the two specimens of *G. notabilis* studied is devoid of kaempferol glycosides. In addition, although quercetin-3-*O*-glucoside is shared, no quercetin diglycosides are common to both specimens (Table 2). Among the three specimens of *G. villosissima* studied, two of them (CFSC 10328 and CFCR 11818) have profiles more complex than that of specimen CFSC 11314, with the inclusion of 3,7-di-*O*-glycosides.

Flavones are virtually restricted to the tribe Bocageeae. Among the six genera studied, only *Cardiopetalum* and *Porcelia* did not yield detectable amounts of flavones (Table 2). Outside Bocageeae, only *Annona tomentosa* accumulated flavones. Another important feature of some members of Bocageeae (genera *Cardiopetalum* and *Cymbopetalum*) is the 6-oxygenation of flavones and flavonols (Table 2). Among the Magnoliidae, only species of the Lauraceae were noted by Gottlieb et al. (1993) to possess both flavones and flavonols 6-oxygenated, a characteristic they regard as a strengthening point for the advanced condition of the family in the subclass. In the present work, three species of the Bocageeae yielded 6-hydroxy-7-*O*-methyl derivatives of kaempferol; other species of the same tribe accumulated 6-hydroxy flavones (Table 2). Considering the views of Gottlieb and co-workers, the tribe Bocageeae would be viewed as chemically advanced within the studied Annonaceae, in agreement with the cladistic analyses of Doyle and Le Thomas (1996). Glycosides of kaempferol and quercetin are ubiquitous among the samples studied. In addition to flavones and 6-oxygenation, 7-methoxylation of flavonols is also noted in the Bocageeae, but apparently not in the species of *Hornschuchia* and *Trigynaea* (Table 2). Derivatives of kaempferol prevail in Bocageeae, and those of quercetin prevail in the Unoneae and Uvarieae. In the tribe Unoneae, neither flavonols nor flavones were detected in samples of *Anaxagorea dolichocarpa*. Most of the compounds found in the three other genera of Unoneae are glycosides of kaempferol and quercetin. Only *Annona monticola* and *Rollinia bahiensis* yielded derivatives of isorhamnetin (Table 2). In Uvarieae, the absence of quercetin glycosides characterizes *Duguetia*; kaempferol and isorhamnetin derivatives predominate in samples of the genus (Table 2). *Guatteria* is characterized by the 3,7-di-*O*-glycosylation, mostly of quercetin, but occasionally also of kaempferol (Table 2).

UPGMA analysis failed to cluster species according to genera, and genera according to tribes, although some taxonomically meaningful groupings emerged. The outcome were five major clusters at the similarity level 0.5 (Fig. 1). Cluster I gathers species which invariably present kaempferol and/or quercetin-3-*O*-glycosides. Within this major group, cluster IA joins species bearing quercetin and sometimes also kaempferol-3,7-di-*O*-glycosides. Five of the seven species of *Guatteria* studied belong to this cluster, emphasizing the importance of 3,7-di-*O*-glycosylation as a chemical characteristic of the genus. In spite of its location outside cluster IA, *G. pogonopus* also presented the 3,7-di-*O*-glycosylation pattern, with additional glycosylation at the 3′ position (Table 2). Among the species of *Guatteria* studied, only *G. notabilis* is seemingly devoid of quercetin-3,7-di-*O*-glycosides. Cluster II comprises the three species of *Duguetia*, characterized by glycosides of kaempferol and isorhamnetin, but seemingly lacking glycosides of quercetin. The apparent absence of quercetin represents a support for the monophyly of the genus, as inferred from evidence raised by other workers (van Zuilen et al., 1995, 1996). The species in cluster III (genera *Cardiopetalum* and *Cymbopetalum*) are gathered on the basis of the exclusive occurrence of glycosides of 6-hydroxy-rhamnocitrin. Clusters IV and V characterize species bearing isorhamnetin and 6-*O*-apigenin glycosides, respectively. *Anaxagorea dolichocarpa* holds an isolated position in the dendrogram due to an apparent lack of flavones and flavonols (Table 2). Cladistic treatments by Doyle and Le Thomas (1996) and van Zuilen et al. (1996) suggest a basal position for *Anaxagorea* among the Annonaceae.

Despite these observations, the present analysis of 12 genera and 31 species is not conclusive for the Annonaceae. Further studies are needed to completely elucidate the proposed relationships for this large family.

Table 2
Flavonoids distribution in Annonaceae species. See Table 1 for list and codes of compounds. Numbers of voucher specimens are given in parentheses[a]

Tribe[b]/Species	Flavonoids																	
	Apigenin			Luteolin		Kaempferol						Quercetin				Isorhamn.		Rham.
	7R	C	6R	7R	6OH	3Rm	3Rd	3Rt	3R 7R	3R 7Me	3R 7Me 6OH	3Rm	3Rd	3R 7R	3R7R3′R	3Rm	3Rd	3Rd
Tribe Bocageeae																		
Bocagea sp. nov. (Benko-Iseppon 1)						12	21, 25	28, 29, 30				42	52, 55					
B. longipedunculata (Mello-Silva 1181)							(22, 25)						(53), 55					
B. viridis (Mello-Silva 1226)			5															
Cardiopetalum calophyllum (Guilherme 143)						12	25			36, 37	38, 39		55					76
Cymbopetalum brasiliense (Folli 1434)					9		(22/25)		34	(37)	39							
C. euneurum (Forzza 305)							(22), 25		35	(37)	39							
Hornschuchia bryothrophe (Mello-Silva 1174)			6															
H. citriodora (Pirani 2435)	1, 2	3, 4											52					
H. myrtillus (Mello-Silva 1171)							20, 24, 25	(29/30)										
Porcelia macrocarpa (Ivanaukas 57)							25	29				42	53, 55	61				74, 75, 76
Trigynaea oblongifolia (Leoni 943)			7			25												
Tribe Unoneae																		
Anaxagorea dolichocharpa (Mello-Silva 96)																		
A. dolichocharpa (Mello-Silva 1232)																		
Annona crassiflora (CFCR 11044)						11, 12	15, 19, 21					40, 41, 42	44, 45, 51, 55					
A. monticola (CFCR 11817)												42	54, (53/55)			69	70, 73	
A. warmingiana (CFSC 11047)							25					42, 43	52, 53, 55, 56	60				
A. tomentosa (CFSC 11046)				8			25					42	55					
Rollinia bahiensis (Pirani 2365)																69	70, 73	
R. dolabripetala (Mello-Silva 102)						12	(19), 21					42	50, 52, 55					
R. sylvatica (Mello-Silva 97)							21						52					

(*continued on next page*)

Table 2 (*continued*)

Tribe[b]/Species	Flavonoids																	
	Apigenin			Luteolin		Kaempferol						Quercetin				Isorhamn.		Rham.
	7R	C	6R	7R	6OH	3Rm	3Rd	3Rt	3R 7R	3R 7Me	3R 7Me 6OH	3Rm	3Rd	3R 7R	3R7R3′R	3Rm	3Rd	3Rd
Xylopia aromatica (Mello-Silva 133)						12	25					40, 42	48, 55, 56, (53/55)					
X. emarginata (CFSC 9098)							16, 17, 20, 21, 27						46,(48), 52, 57, (53/55)					
Tribe Uvarieae																		
Duguetia bahiensis (Thomas 6009)						12*	25*	31*									72	
D. chrysocarpa (Mello-Silva 98)						12, 12*	21											
D. furfuracea (Mello-Silva 94)							18, (22/25)									68	71, 73	
Guatteria sp. 1 (Mello-Silva 105)							14		33			42		58, 62				
Guatteria sp. 2 (Mello-Silva 110)							16					40						
G. australis (Mello-Silva 992)							19, 21	31				42	(50), 52	60				
G. notabilis (Pirani 2216)												42	47, 48, 52					
G. notabilis (CFCR 11117)							18, 21, (22/25)					42	49, (53/55)					
G. pogonopus (Arbo 5541)												43		58, 64, (61/63)	67			
G. rupestris (CFCR 11752)						10, 12, 13	22, 25, 26					40, 42	53, 55	62, 63				
G. sellowiana (CFCR 11227)							(22/25)	(29/30)					53, 55	65, 66			72, 73	
G. villosissima (CFSC 11314)							23					42	47, 55					
G. villosissima (CFSC 10328)						10, 12	25		33			40, 41, 42, 43	55, 56	59				
G. villosissima (CFCR 11818)									32, 33			40, 42, 43	52, 55, 56	62				

7R: 7-*O*-glycoside; 6R: 6 substituted; C: *C*-glycoflavones; 3R: 3-*O*-glycoside; 7Me: 7-*O*-methyl; 3′R: 3′-*O*-glycoside; m: monoglycoside; d: diglycoside; t: triglycoside; ISORHAMN.: Isorhamnetin; RHAM.: Rhamnetin; *: probably acylated compounds; numbers in parentheses show compounds not confirmed.

[a] CFSC: Collection Flora of Serra do Cipó; CFCR: Collection Flora of Campos Rupestres.

[b] By Fries (1959).

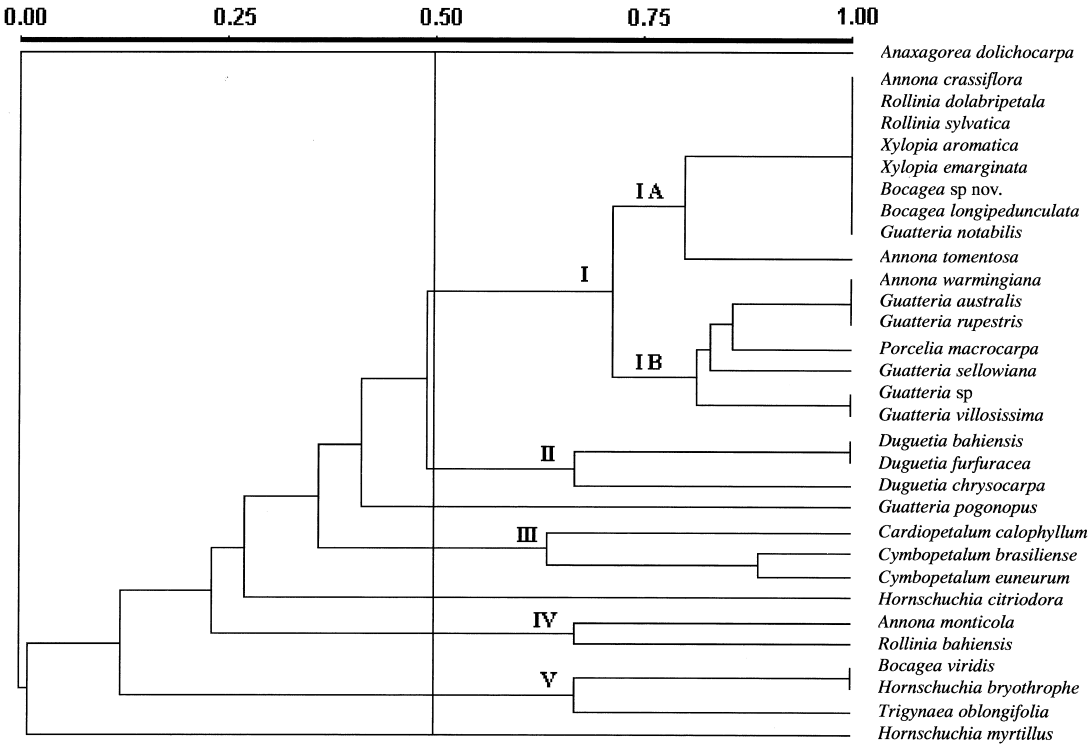

Fig. 1. Affinity relationships among samples of Annonaceae obtained with Dice Indice and UPGMA method of clustering, based on the distribution of flavonoids (see Experimental).

3. Experimental

Leaves of 31 species were collected in different states of Brazil, ranging from Amazonas (far North) to São Paulo (Southeast). Voucher specimens were deposited in the Herbaria of the Universities of São Paulo (SPF) and Uberlândia (HUFU). Dried and powdered leaves were extracted three times with 80%MeOH under reflux conditions for 1 h. The compounds in the extract were subsequently isolated by PC with Butanol:acetic acid: water (BAW) (6:1:2, v/v) and 15% AcOH in H_2O (v/v) and by CC with polyvinylpolypyrrolidone, MeOH and MeOH–H_2O (4:1) as eluents. The identification of the compounds utilised standard UV/Vis spectrometry procedures (Mabry et al., 1970; Markham, 1982) and analysis by HPLC–ESMS (HP 1090 series II chromatograph and HP 5989B mass spectrometer). A reverse phase column (Spherosil ODS II, 125×4 mm i.d., 5 μm) and a photodiode array detector at 360 nm were used. A gradient of increasing concentration of CH_3CN was used, starting with a rate of 20:80 acetonitrile: 1% AcOH for 10 min, then to 40:60 for 8 min, 75:25 for 10 min and finally to 100% acetonitrile for 2 min (Constant et al., 1997), at a flow rate of 0.5 ml min^{-1}. The mass spectrometer was adjusted to detect positive ions with CAPex of 100; the temperatures of the quadrupole and source were 150 and 200°C, respectively. The glycosides were tentatively characterized as mono-, di- and triglycosides by TLC using rutin and quercetin-3-*O*-glucoside as references (Santos et al., 1995). Acid (1 N HCl) and enzymatic hydrolysis of the glycosides were carried out for identification of sugars and establishment of their relative positions (Mabry et al., 1970; Markham, 1982). The sugars were identified by cellulose TLC using pyridine–EtOAc–AcOH–H_2O (36:36:7:21) as the solvent system (Markham, 1982) and the visualization was achieved by spraying a solution of acetone:aniline phosphate (4:2) and heating at 105°C for 5 min.

Multivariate analysis was carried out using the NTSYS-pc version 1.80 (Rolf, 1994), UPGMA method and Dice Index (Crisci and Armengol, 1983). The characters used in the UPGMA analysis were 7-*O*-glycosylapigenin, *C*-glycosylapigenin, apigenin-6-*O*R (R = Me or glycosyl), 7-*O*-glycosylluteolin, 7-*O*-glycosyl-6-hydroxyluteolin, 3-*O*-glycosylkaempferol, 3,7-di-*O*-glycosylkaempferol, 3-*O*-glycosylrhamnocitrin, 3-*O*-glycosyl-6-hydroxyrhamnocitrin, 3-*O*-glycosylquercetin, 3,7-di-*O*-glycosylquercetin, 3,7,3′-tri-*O*-glycosylquercetin, 3-*O*-glycosylisorhamnetin and 3-*O*-glycosylrhamnetin. The species of the Annonaceae chemically analyzed were used as operational taxonomic units (OTUs).

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