A General Description of Apocynaceae Iridoids Chromatography

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1. Introduction

1.1. Iridoids

Iridoids are considered atypical monoterpenoid compounds, based on a methylcyclopentan-[C]-pyran skeleton, often fused to a six-membered oxygen ring consisting of ten, nine or in rare cases, eight carbon atoms (Figure 1a) [1, 2]. More than 2500 iridoid compounds have been described in nature, with structural differences related mainly to the degree and type of substitution in the cyclopentane ring skeleton [3]. Iridoids can be found in nature as secoiridoids (Figure 1b), a large group characterized by cleavage of the 7,8-bond on the cyclopentane ring, glycosides, mainly 1-*O*-glucosides, and nor-iridoids, originating from oxidative decarboxylation on C₁₀ or C₁₁ (Figure 1) [3, 4].

Figure 1. Basic skeleton a) iridoid; b) seco-iridoid (R=H or glucose)

Iridoids are derived from isoprene units, which are considered the universal building blocks of all terpenoids, formed through intermediates of the mevalonic acid (MVA) pathway in the cytosol, and the novel 2-methyl-D-erythritol 4-phosphate (MEP) pathway in the plastids of plant cells [2, 5, 6]. The participation of two pathways in iridoid biosynthesis has not yet been clarified, but recent analyses have described the major role of the MEP in the yield of the source for the iridoid isoprene units when compared with the MVA pathway [7, 8, 9]. Iridoid biosynthesis shows two pathways, called route I and II, in which secoiridoids and carboxylated or decarboxylated iridoids are formed, respectively. Route I, considered the main pathway, is responsible for yielding the precursor of the carboxylic iridoids, from iridodial which is oxidized a iridotrial and subsequently converted to a series the iridoids, as occurs in loganin, secologanin, derived secoiridoids, and complex indole alkaloids. In route II, the presence of 8-epi-iridodial, 8-epi-iridotrial and 8-epi-deoxyloganic acid have been reported, forming a source of decarboxylated carbocyclic iridoids such as aucubin and catalpol [10, 3, 11, 12].

Iridoids have shown a broad range of biological activities, such as an antibacterial, antifungal, anti-inflammatory, antitumoral, hepatoprotective, cardioprotective, antioxidative, anti-protozoal and anti-insect properties [13, 14, 15, 16, 17, 18, 19]. *In vitro* activities inhibiting the hepatitis C virus, the differentiation of the adipocyte, and PPAR α activation activities have been also described [20, 21].

The distribution of iridoids in the Eudicotyledoneae has potential usefulness in the taxonomy of the families, related to their presence in a restricted number of families. Iridoid are considered good chemotaxonomic markers of different taxonomic groups, and can be used, in combination with order, tribe and family, to establish the phylogenetic relationship [22, 23, 10, 3, 24, 25].

According to an update of the Angiosperm Phylogeny Group (APGIII) [28,29], the presence of iridoids has been reported in approximately fifty plant families, and can be considered as one of the synapomorphies of the Asterid clade. It is divided into Lamiids, which presents iridoids of the Gentianales, Garryales and Lamiales orders, and Campanulids, which presents secoiridoids of the Asterales and Dipsacales orders. The Gentianales order comprises five families: Apocynaceae, Gelsemiaceae, Gentianaceae, Loganiaceae and Rubiaceae (APG III, 2009), highlighted for the diversity of their iridoids [11, 29, 30].

1.2. Apocynaceae family

Apocynaceae is the most important family within this order, with ca 5000 species distributed worldwide. Seventy percent of the genus, and half of the species distributed in the Neotropical region, are found in the native Brazilian flora [31, 33]. Today, five subfamilies are described for the Apocynaceae family: Rauvolfioideae, Apocynoideae, Secamonoideae and Asclepiadoideae. The latter is the major subfamily of the Apocynaceae, and comprises approximately 3000 species divided into 172 genera, distributed mainly in Neotropic areas of South America, such as Brazil, where the highest diversity of the species has been found [32, 33, 34].

The iridoids class has significant distribution within Apocynaceae family, but is concentrated in just a few genera. The most representative of these are: *Plumeria*, *Himatanthus*, *Allamanda*

and *Cerbera*. According to the traditional classification, the family comprises approximately 87 iridoids, the main ones being plumeride, plumericin and isoplumericin. A review of literature comprising works published on the identification of this class of constituents within Apocynaceae showed, as major natural sources, the following species (numbers in brackets indicate species as shown in Table 1) [36]:

(1). *Plumeria rubra* L. [*P. acuminata* W. T. Aiton; *P. acutifolia* Poir.; *P. bicolor* Ruiz & Pav.]; (2). *P. lancifolia* Müll. Arg.; (3). *P. acutifolia* Poir. [*P. rubra* L.]; (4). *P. alba* L. [*P. alba* var. *jacquiniana* A. DC.; *P. hypoleuca* Gasp.; *P. hypoleuca* var. *angustifolia* Gasp.]; (5). *P. bracteata* A. DC.; (6). *P. obtusifolia* Steud.; (7). *P. obtusa* L. [*P. multiflora* Standl.]; (8). *P. rubra* var. *alba*; (9). *P. multiflora* Standl.; (10). *Allamanda neriifolia* Hook. [*A. cathartica* var. *Schottii* (Pohl) L.H. Bailey & Raffill]; (11). *Allamanda cathartica* L. [*A. grandiflora* (Aubl.) Lam.; *A. schottii* Hook.]; (12). *Alstonia boonei* De Wild. [*A. congensis* Engl.]; (13). *Cerbera manghas* L.; (14). *Alyxia reinwardtii* Blume; (15). *Alstonia scholaris* (L.) R.Br.; (16). *Himatanthus sukuuba* (Spruce ex Müll. Arg.) Woodson [*H. tarapotensis* (K. Schum. Ex Markgr.) Plumel; *Plumeria floribunda* Müll. Arg.; *P. tarapotensis* K. Schum. ex Markgr.]; (17). *Vinca* sp. L.; (18). *Thevetia peruviana* (Pers.) K. Schum. [*T. neriifolia* Juss. ex Steud.]; (19). *Himatanthus phagedaenicus* (Mart.) Woodson [*Plumeria floribunda* var. *crassipes* Müll. Arg.; *P. lancifolia* var. *major* Müll. Arg.; *P. phagedaenica* Mart.]; (20). *Plumeria bicolor* Seem.; (21). *Plumeria acuminata* W.T. Aiton [*P. rubra* L.]; (22). *Cerbera odollam* Gaertn.; (23). *Allamanda. schottii* Pohl [*A. brasiliensis* Schott ex Pohl; *A. cathartica* Schrad; *A. neriifolia* Hook.]; (24). *Himatanthus articulatus* (Vahl) Woodson [*H. rigidus* Wild. Ex Roem. & Schult.; *Plumeria articulata* Vahl; *P. drastica* Mart.; *P. microcalyx* Standl.]; (25). *Rauwolfia grandiflora* Mart. Ex A. DC.; (26). *Plumeria inodora* Jacq. [*P. alba* L.; *P. alba* var. *fragrans* Kunth; *P. alba* var. *fragrantissima* G. Don; *P. alba* var. *inodora* (Jacq.) G. Don]; (27). *Himatanthus bracteatus* (A. DC.) Woodson; (28). *Himatanthus stenophyllus* Plumel; (29). *Himatanthus fallax* (Müll. Arg.) Plumel [*Plumeria fallax* Müll. Arg.]; (30). *Himatanthus obovatus* (Müll. Arg.) Woodson; (31). *Allamanda doniana* Müll. Arg.; (32). *Catharanthus roseus* (L.) G. Don; (33). *Nerium indicum* Mill. [*N. oleander* L.]; (34). *Alstonia macrophylla* Wall. ex G. Don; (35). *Winchia calophylla* A. DC.

1.3. Iridoids of Apocynaceae family

IRIDOIDS	SPECIES	PLANT MATERIAL	REF.
6''O-acetylplumeride <i>p</i> -E-coumarate	7: <i>Plumeria obtusa</i>	7:leaves	7: [37]; 7: [38]
6''O-acetylplumeride <i>p</i> -Z-coumarate	7: <i>Plumeria obtusa</i>	7:leaves	7: [37]; 7: [38]
13-O-acetylplumeride	10: <i>Allamanda neriifolia</i>	10:stem; 10:leaves	10: [39]; 10: [40]
allamancin	10: <i>Allamanda neriifolia</i>	10:stem	10: [39]; 10: [40]
allamandicin	10: <i>Allamanda neriifolia</i>	10:stem	10: [39]; 10: [41]; 10: [42]
allamandin	1: <i>Plumeria rubra</i> ; 10: <i>A. neriifolia</i> ; 11: <i>A. cathartica</i> ;	1:stem bark; 19,23:stem; 10,11:leaves; 11,16:root	1: [43] ; 1: [44]; 1: [48]; 1: [49]; 10: [39]; 11: [41];

IRIDOIDS	SPECIES	PLANT MATERIAL	REF.
	23: <i>A. schottii</i> ; 16: <i>Himatanthus sucuuba</i> ; 19: <i>H.</i> <i>phagedaenicus</i> ;		11: [42]; 16: [50]; 16: [51]; 19: [45]; 19: [46]; 23: [47]
allamanoid	10: <i>Allamanda neriifolia</i>	10: aerial parts	10: [52]; 10: [53]
allamcidin A	1: <i>Plumeria rubra</i> ; 10: <i>Allamanda neriifolia</i>	1: bark; 10: leaves	1: [43]; 1: [44]; 10: [39]; 10: [40]
allamcidin B	1: <i>Plumeria rubra</i> ; 10: <i>Allamanda neriifolia</i>	1: bark; 10: leaves	1: [43]; 1: [44]; 10: [39]; 10: [40]
allamcidin B β -D-glucose	10: <i>Allamanda neriifolia</i>	10: stem	10: [39]; 10: [40]
allamcin	1: <i>Plumeria rubra</i> ; 10: <i>Allamanda neriifolia</i> ; 23: <i>A. schottii</i>	1: stem bark; 10: leaves; 23: stem	1: [43]; 1: [44]; 1: [48]; 10: [39]; 10: [40]; 23: [47]
allamdin	11: <i>Allamanda cathartica</i>	11: root	11: [41]; 11: [42]
allaneroside	10: <i>Allamanda neriifolia</i>	10: leaves	10: [40]; 10: [54]
3,10-bis-O-allosylcerberidol	13: <i>Cerbera manghas</i> ; 22: <i>C.</i> <i>odollam</i>	13,22: leaves	13: [40]; 13: [56]; 13,22: [55]
3-O-allosylcerberidol	13: <i>Cerbera manghas</i> ; 22: <i>C.</i> <i>odollam</i>	13,22: leaves	13: [40]; 13: [56]; 13,22: [55]
3-O-allosylcyclocerberidol	13: <i>Cerbera manghas</i> ; 22: <i>C.</i> <i>odollam</i>	13,22: leaves	13: [40]; 13: [56]; 13,22: [55]
3-O-allosylepoxycerberidol	13: <i>Cerbera manghas</i> ; 22: <i>C.</i> <i>odollam</i>	13,22: leaves	13: [40]; 13: [56]; 13,22: [55]
alstonoside	15: <i>Alstonia scholaris</i>	15: stem	15: [57]; 15: [58]
alyxialactone	14: <i>Alyxia reinwardtii</i> ; 15: <i>Alstonia scholaris</i>	14: leaves; 15: bark	14: [59]; 15: [60]
10-O-benzoyltheveside	13: <i>Cerbera manghas</i>	13: leaves	13: [56]; 13: [61]
10-O-benzoyltheviridoside	13: <i>Cerbera manghas</i> ; 18: <i>Thevetia peruviana</i>	13,18: leaves	13: [56]; 13,18: [62]
boonein	12: <i>Alstonia boonei</i> ; 25: <i>Rauwolfia grandiflora</i>	12: root; 25: bark	12: [40]; 12: [63]; 12: [64]; 25: [1]
13-O-caffeoylplumieride	1: <i>Plumeria rubra</i> ; 3: <i>P.</i> <i>acutifolia</i>	1,3: root	1: [65]; 3: [38]; 3: [40]; 3: [43]; 3: [66]
10-carboxyloganin	13: <i>Cerbera manghas</i>	13: leaves	13: [56]; 13: [62]
cerberic acid	13: <i>Cerbera manghas</i>	13: bark	13: [40]; 13: [56]; 13: [67]
cerberidol	13: <i>Cerbera manghas</i> ; 22: <i>C.</i> <i>odollam</i>	13,22: leaves	13: [40]; 13: [56]; 13,22 [55]
cerberinic acid	13: <i>Cerbera manghas</i>	13: bark	13: [40]; 13: [56]; 13: [67]

IRIDOIDS	SPECIES	PLANT MATERIAL	REF.
cerbinal	13: <i>Cerbera manghas</i>	13:bark; 13:cortex	13: [56]; 13: [67]; 13: [68]
champalinin ([(E)-p-methoxycinnamoyloxy] plumieride)	7: <i>Plumeria obtusa</i>	7:leaves; 7:stem bark	7: [43]; 7: [69]
cyclocerberidol	13: <i>Cerbera manghas</i> ; 22: <i>C. odollam</i>	13,22:leaves	13: [40]; 13: [56]; 13,22: [55]
cyclocerberidol-3-O-β-D-glucoside	13: <i>Cerbera manghas</i>	13:leaves	13: [56]; 13: [62]
deglycosylplumieride	10: <i>Allamanda neriifolia</i>	10:stem	10: [39]
dehydrogardenoside-10	10: <i>Allamanda neriifolia</i>	10: leaves	10: [39]
10-dehydrogeniposide	13: <i>Cerbera manghas</i>	13:leaves	13: [56]; 13: [61]
15-demethylisoplumieride	1: <i>Plumeria rubra</i> ; 16: <i>Himatanthus sucuuba</i>	1,16:bark; 16:latex	1: [43]; 1,16: [53]; 1,16: [70]; 16: [51]
15-demethylplumieride	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i> ; 4: <i>P. alba</i> ; 7: <i>P. obtusa</i> ; 16: <i>Himatanthus sucuuba</i>	1,16:bark; 3,4:leaves; 4:root; 7:aerial parts; 16:latex	1: [44]; 1,4: [43]; 3: [71]; 7: [72]; 16: [51]; 16: [73]
13-deoxyplumieride	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i>	1,3:root	1: [65]; 3: [38]; 3: [40]; 3: [43]; 3: [66]
β-dihydroplumericin acid	1: <i>Plumeria rubra</i>	1:root	1: [38]; 1: [42]; 1: [43]; 1: [74]
β-dihydroplumericin	1: <i>Plumeria rubra</i> ; 16: <i>Himatanthus sucuuba</i>	1,16:root; 1:stem bark	1: [38]; 1: [42]; 1: [43]; 1: [48]; 1: [74]; 16: [51]
epiplumeridoid C	1: <i>Plumeria rubra</i>	1: stem bark	1: [48]
epoxycerberidol	13: <i>Cerbera manghas</i> ; 22: <i>C. odollam</i>	13,22:leaves	13: [40]; 13: [56]; 13,22: [55]
epoxycerberidol-3-O-β-D-glucoside	13: <i>Cerbera manghas</i>	13:leaves	13: [56]; 13: [62]
fulvoplumierin	1: <i>Plumeria rubra</i> L., 3: <i>P. acutifolia</i> , 5: <i>P. bracteata</i> , 8: <i>P. rubra</i> var. <i>alba</i> ; 21: <i>P. acuminata</i> ; 16: <i>Himatanthus sucuuba</i>	1,3,8,21:stem bark; 1,16:bark; 1:root, 1:descorticated stem, 3:rind, 5:root cortex	1: [43]; 1: [48]; 1: [65]; 1: [74]; 1: [75]; 1: [76]; 1: [77]; 1,3,5,8: [38]; 1,3,21: [44]; 3: [78]; 3: [79]; 3: [80]; 3,8: [42]; 3,8: [83]; 5: [81]; 8: [82]; 16: [51]
gardenoside	10: <i>Allamanda neriifolia</i>	10:stem;10:leaves	10: [39]
isoallamandicin	10: <i>Allamanda neriifolia</i>	10:stem	10: [39]; 10: [40]

IRIDOIDS	SPECIES	PLANT MATERIAL	REF.
isoboonein	15: <i>Alstonia scholaris</i> ; 25: <i>Rauwolfia grandiflora</i>	15,25:bark	15: [60]; 25: [1]
isoplumericin	1: <i>Plumeria rubra</i> , 3: <i>P. acutifolia</i> ; 4: <i>P. alba</i> , 7: <i>P. obtusa</i> ; 20: <i>P. bicolor</i> ; 10: <i>Allamanda neriifolia</i> ; 11: <i>A. cathartica</i> ; 23: <i>A. schottii</i> ; 31: <i>A. doniana</i> ; 16: <i>Himatanthus sucuuba</i> ; 19: <i>H. phagedaenicus</i> ; 24: <i>H. articulatus</i> ; 29: <i>H. fallax</i> ; 30: <i>H. obovatus</i> ;	1,16,20: stem bark; 1,4,7,16,30,31:root; 1,3: wood; 10,19,23,29:stem; 11,16:leaves; 16,24:bark; 16:latex	1: [42]; 1: [48]; 1: [58]; 1: [74]; 1: [75]; 1: [84]; 1,3: [85]; 1,4,7: [38]; 1,4,7: [43]; 4,7: [84]; 10: [39]; 11: [49]; 11,29,30,31: [89]; 16: [51]; 16: [88], 16: [90]; 16: [91]; 16: [92]; 19: [45]; 19: [46]; 20: [86]; 23: [47]; 24: [87]; 30,31: [93]; 30,31: [94]
isoplumieride	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i> ; 16: <i>H. sucuuba</i> ; 27: <i>H. bracteatus</i> ; 28: <i>H. stenophyllus</i>	1,3:root; 16,27,28:leaves; 16,27,28:bark; 16,27,28:latex	1: [65]; 3: [38]; 3: [40]; 3: [43]; 3: [66]; 16: [51]; 16: [73]; 16,27,28: [95]
loganic acid	25: <i>Rauwolfia grandiflora</i> ; 32: <i>Catharanthus roseus</i>	25: bark; 32:seed	25: [1]; 32: [4]
loganin	12: <i>Alstonia boonei</i> ; 15: <i>A. scholaris</i> ; 13: <i>Cerbera manghas</i> ; 17: <i>Vinca</i> sp.; 25: <i>Rauwolfia grandiflora</i> ; 32: <i>Catharanthus roseus</i> ; 35: <i>Winchia calophylla</i>	12,13,17:leaves; 15,25:bark; 32:seed; 35:stem bark	12: [64]; 13: [46]; 13: [56]; 13: [61]; 13: [62]; 15: [60]; 17: [42]; 17: [96]; 25: [1]; 32: [4]; 35: [129]
3-O-methylallamancin	10: <i>Allamanda neriifolia</i>	10:leaves	10: [39]; 10: [40]
3-O-methylallamcim	10: <i>Allamanda neriifolia</i>	10:leaves	10: [39]; 10: [40]
naresuanoside	34: <i>Alstonia macrophylla</i>	34:stem	34: [128]
obtusadoid A	7: <i>Plumeria obtusa</i>	7:aerial parts	7: [72]
obtusadoid B	7: <i>Plumeria obtusa</i>	7:aerial parts	7: [72]
plumenoside (β -dihydroplumericinic acid glucosylester)	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i>	1,3:root	1: [65]; 3: [38]; 3: [40]; 3: [43]; 3: [66]
plumeric acid 1- β -O- β -D-glucopyranosyl	24: <i>Himatanthus articulata</i>	24:bark	24: [87]
plumericidine	1: <i>Plumeria rubra</i>	1: flowers	1: [97]
plumericin	1: <i>Plumeria. rubra</i> , 3: <i>P. acutifolia</i> ; 4: <i>P. alba</i> , 5: <i>P. bracteata</i> , 7: <i>P. obtusa</i> ,	1,16,20:stem bark; 1,4,7,8,9,16,33:root; 1,3:wood; 5,8,16:root cortex; 1,19,23,29:stem;	1: [42]; 1: [48]; 1: [58]; 1: [74]; 1: [75]; 1: [82]; 1: [98]; 1,3: [85]; 1, 4, 7: [43]; 1,4,5,7,8,9: [38];

IRIDIDS	SPECIES	PLANT MATERIAL	REF.
	8: <i>P. rubra</i> var. <i>alba</i> ; 9: <i>P. multiflora</i> ; 20: <i>P. bicolor</i> ; 21: <i>P. acuminata</i> ; 10: <i>Allamanda neriifolia</i> ; 11: <i>A. cathartica</i> ; 23: <i>A. schottii</i> ; 31: <i>A. doniana</i> ; 16: <i>Himatanthus</i> <i>sucuuba</i> ; 19: <i>H. phagedaenicus</i> ; 24: <i>H. articulatus</i> ; 29: <i>H. fallax</i> ; 30: <i>H. obovatus</i> ; 33: <i>Nerium indicum</i>	1,10,11,16,23:leaves; 1,16,20,24:bark; 1:fruits; 16:latex	1,21: [44]; 4,7: [84]; 5: [81]; 8: [82]; 9: [99]; 10: [39]; 11: [49]; 11,29,30,31: [89]; 16: [50]; 16: [51]; 16: [88]; 16: [90]; 16: [91]; 16: [92]; 16: [100]; 19: [45]; 19: [46]; 20: [86]; 20: [101]; 23: [47]; 23: [102]; 24: [87]; 33: [84]; 33: [130]
plumeridoid A	1: <i>Plumeria rubra</i>	1: stem bark	1: [48]
plumeridoid B	1: <i>Plumeria rubra</i>	1: stem bark	1: [48]
plumeridoid C	1: <i>Plumeria rubra</i> ; 16: <i>Himatanthus sucuuba</i>	1,16: stem bark	1: [48]; 16: [103]
plumiepoide	10: <i>Allamanda neriifolia</i>	10:stem; 10:leaves	10: [39]; 10: [40]
plumieride	1: <i>Plumeria rubra</i> L.; 2: <i>P. lancifolia</i> ; 3: <i>P. acutifolia</i> ; 4: <i>P. alba</i> ; 5: <i>P. bracteata</i> ; 6: <i>P. obtusifolia</i> ; 7: <i>P. obtusa</i> ; 8: <i>P. rubra</i> var. <i>alba</i> ; 20: <i>P. bicolor</i> ; 26: <i>P. inodora</i> ; 10: <i>Allamanda neriifolia</i> ; 11: <i>A. cathartica</i> ; 23: <i>A. schottii</i> ; 16: <i>Himatanthus sucuuba</i> ; 24: <i>H. articulatus</i> ; 27: <i>H. bracteatus</i> ; 28: <i>H. stenophyllus</i> ; 29: <i>H. fallax</i>	1,2,7,8:stem bark, 1,3,4,8:root, 1,2,3,4,5,7,8,10,11,16,27 ,28:leaves, 1,8:stem wood, 1,2,3,5:wood, 1,4,7:seed, 1,2,3,4,5,7:flowers, 1:descorticated stem, 2,3,5:root cortex, 2,3,5:pith, 2,3,4,5:cortex, 3:rind; 10,20,23,26,29: stem; 16,20,24,27,28: bark; 16,23,27,28: latex	1: [42]; 1: [48]; 1: [65]; 1: [75]; 1: [76]; 1: [77]; 1: [82]; 1: [84]; 1: [105]; 1: [111]; 1,2,3,4,5,6,7,8,; [38]; 1,4,7,20: [86]; 1,3: [85]; 1,8: [104]; 2: [81]; 3: [66]; 3: [80]; 3: [81]; 3: [106]; 3: [107]; 4: [84]; 4: [108]; 4: [109]; 5: [81]; 6: [110]; 7: [84]; 8: [105]; 10: [39]; 11: [49]; 11: [112]; 16: [51]; 16: [73]; 16,27,28: [95]; 20: [114]; 23: [102]; 24: [87]; 24: [115]; 26: [113]; 29: [89]
plumieride coumarate (13-O-coumaroylplumieride; plumieride <i>p</i> -E-coumarate)	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i> ; 4: <i>P. alba</i> ; 7: <i>P. obtusa</i> ; 10: <i>Allamanda neriifolia</i> ; 11: <i>A. cathartica</i>	1:bark; 1,3,4,7,11:root; 1,4,7:stem; 1,4,7,10: leaves; 1,7: flowers; 1,3: wood	1: [44]; 1,3: [85]; 1,3,4,7: [38]; 1,3,4,7: [43]; 1,4,7: [84]; 3: [66]; 7: [37]; 10: [39]; 11: [89]; 11: [116]
plumieride coumarate glucoside (protoplumericin A; 13-O- <i>p</i> -O-glucosylcoumaroyl plumieride)	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i> ; 4: <i>P. alba</i> ; 7: <i>P. obtusa</i> ; 10: <i>Allamanda neriifolia</i> ; 11: <i>A. cathartica</i>	1,3,4,11:root; 1,3:wood, 4,7,10:stem; 4,7,10:leaves; 4,7:flowers; 1:bark	1,3: [85]; 1,3,4,7: [38]; 1,4,7: [43]; 1,4,7: [84]; 1,7,11: [118]; 3: [66]; 10: [39]; 10: [40]; 10: [116]; 10: [117]; 11: [89]
plumieride <i>p</i> -Z-coumarate	7: <i>Plumeria obtusa</i>	7:leaves	7: [37]; 7: [38]

IRIDOIDS	SPECIES	PLANT MATERIAL	REF.
plumieride-1a	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i> ; 7: <i>P. obtusa</i>	1,3:root; 7:leaves; 7:aerial parts	1: [65]; 3: [38]; 3: [40]; 3: [66]; 7: [72]; 7: [119]
plumieride-1 β -O- β -D-glucopyranosyl	24: <i>Himatanthus articulatus</i>	24:bark	24: [87]
plumieridin A	1: <i>Plumeria rubra</i> ; 7: <i>Plumeria obtusa</i>	1:flowers; 7:aerial parts	1: [43]; 1: [118]; 1: [120]; 7: [72]
plumieridin B	1: <i>Plumeria rubra</i>	1: flowers	1: [43]; 1: [120]
protoplumericin A-1a	1: <i>Plumeria rubra</i> ; 3: <i>P. acutifolia</i> ; 10: <i>Allamanda neriifolia</i>	1, 3:root; 10:stem; 10:leaves	1: [65]; 3: [38]; 3: [40]; 3: [66]; 10: [39]
protoplumericin B (13-O-p-O-glucosylcaffeoyl-plumieride)	10: <i>Allamanda neriifolia</i>	10:stem; 10:leaves	10: [39]; 10: [40]
pulosarioside	14: <i>Alyxia reinuardtii</i>	14:bark	14: [40]; 14: [121]
scholarein A	15: <i>Alstonia scholaris</i>	15:bark	15: [60]
scholarein B	15: <i>Alstonia scholaris</i>	15:bark	15: [60]
scholarein C	15: <i>Alstonia scholaris</i>	15:bark	15: [60]
scholarein D	15: <i>Alstonia scholaris</i>	15:bark	15: [60]
secologanin	32: <i>Catharanthus roseus</i>	32:leaves	32: [4]
sweroside	34: <i>Alstonia macrophylla</i>	34:stem	34: [128]
theveside	13: <i>Cerbera manghas</i> ; 18: <i>Thevetia peruviana</i>	13:fruit; 13,18:leaves; 18:root	13: [46]; 13: [56]; 13: [61]; 13: [124]; 13,18: [42]; 13,18: [62]; 18: [122]; 18: [123]; 18: [125]
theviridoside	13: <i>Cerbera manghas</i> ; 18: <i>Thevetia peruviana</i>	13:fruit; 13:cortex; 13,18:leaves; 18:root	13: [46]; 13: [56]; 13: [61]; 13: [68]; 13: [124]; 13,18: [42]; 13,18: [62]; 18: [125]; 18: [126]
theviridoside-10-O- β -D-fructofuranosyl	18: <i>Thevetia peruviana</i>	18:leaves; 18:root	18: [125]; 18: [127]
theviridoside-10-O- β -D-glucopyranosyl	18: <i>Thevetia peruviana</i>	18:root	18: [125]
theviridoside-3'-O- β -D-glucopyranosyl	18: <i>Thevetia peruviana</i>	18:root	18: [125]
theviridoside-6'-O- β -D-glucopyranosyl	18: <i>Thevetia peruviana</i>	18:leaves; 18:root	18: [125]; 18: [127]

Table 1. Iridoids found in Apocynaceae family.

2. Extracts preparation and iridoids isolated

Table 2 illustrates the extraction methods and the iridoids isolated of some genera of Apocynaceae.

SPECIES / PLANT MATERIAL	SOLVENT/ EXTRACTION METHOD	ISOLATED IRIDOIDS	REF.
<i>Allamanda neriifolia</i> Hook./ stem and leaves	MeOH/ Percolation	Isoallamandicin, allamcin, 3-O-methylallamcin, allamancin, 3-O-methylallamancin, allamcidin, plumericin, isoplumericin, allamandin, allamandicin, plumieride 13-O-acetate, deglucosylplumieride, 13-O-coumaroylplumieride, allamcidin B β -D-glucoside, plumiepoixide, protoplumericin B, plumieride, protoplumericin A, gardenoside, 10-dehydrogardenoside	[39]
<i>A. neriifolia</i> Hook./ aerial parts	EtOH/ Maceration	Allamanoid, plumieride, protoplumericin, nicotiflorin	[52]
<i>A. neriifolia</i> Hook./ stem	MeOH/ Percolation	Plumericin, plumieride, protoplumericin	[117]
<i>A. schottii</i> Pohl/ stem	EtOH/ Maceration	Allamandin, allamcin, plumericin, isoplumericin, scoparone, scopoletin, pinosresinol	[47]
<i>A. cathartica</i> L. / root bark	EtOH/ Hot extraction	Allamandin, allamandicin, allamdin, plumericin, isoplumericin	[41]
<i>A. cathartica</i> L. /root	MeOH/ Hot extraction	Isoplumericin, plumericin, plumieride, plumieride coumarate, plumieride coumarate glucoside	[116]
<i>A. cathartica</i> L. / root bark	MeOH and CHCl ₃ / Not described	Plumericin, isoplumericin, plumieride, plumieride coumarate, plumieride coumarate glucoside	[84]
<i>A. cathartica</i> L./ root (inner part)	MeOH and CHCl ₃ / Soxhlet apparatus	Plumieride, plumieride coumarate, plumieride coumarate glucoside	[84]
<i>A. cathartica</i> L. / leaves	EtOAc/ Not described	Isoplumericin, plumericin	[89]
<i>Alstonia macrophylla</i> Wall. ex G. Don / stem	EtOH/ Maceration	Sweroside, naresuanoside	[128]
<i>A. scholaris</i> (L.) R. Br./ bark	EtOH/ Maceration	Scholarein A, scholarcin B, scholarcin C, scholarcin D, isoboonein, alyxialactone, loganin	[60]

SPECIES / PLANT MATERIAL	SOLVENT/ EXTRACTION METHOD	ISOLATED IRIDOIDS	REF.
<i>Alyxia reinwardtii</i> Blume/ leaves	MeOH/ Maceration	Alyxialactone, 4-epi-alyxialactone	[59]
<i>Cerbera manghas</i> L./ leaves	MeOH/ Not described	Theviridoside, theveside	[124]
<i>C. manghas</i> L./ stem bark and root bark	MeOH/ Percolation	Cerbinal, cerberic acid, cerberinic acid	[67]
<i>C. manghas</i> L./ leaves	MeOH/ Percolation	Cerberidol, epoxycerberidol, cyclocerberidol, cerberidol-3-O- β -D-allopyranoside, cerberidol-3,10-bis-O- β -D- allopyranoside, epoxycerberidol-3-O- β -D-allopyranoside, cyclocerberidol-3-O- β -D-allopyranoside	[55]
<i>C. manghas</i> L./ leaves	MeOH/ Percolation	Theviridoside, 10-carboxyloganin, loganin, cyclocerberidol-3-O- β -D-glucoside, epoxycerberidol-3-O- β - D-glucoside	[62]
<i>C. manghas</i> L./ leaves	MeOH/ Percolation	10-O-benzoyltheveside, 10-dehydrogeniposide, loganin, theviridoside, theveside	[61]
<i>C. odollam</i> Gaertn./ leaves	MeOH/ Percolation	Cerberidol, cyclocerberidol, cerberidol-3-O- β -D- allopyranoside, cyclocerberidol-3-O- β -D-allopyranoside	[55]
<i>Himatanthus articulatus</i> (Vahl) Woodson/ bark	MeOH/ Maceration	1 β -O- β -D-glucopyranosylplumeric acid, plumeride-1 β -O- β - D-glucopyranosyl, plumericin, isoplumericin	[87]
<i>H. fallax</i> (Müll. Arg.) Plumel/ stem	EtOAc/ Maceration	Isoplumericin, plumericin, plumeride	[89]
<i>H. sucuuba</i> (Spruce ex Müll. Arg.) Woodson / latex	H ₂ O/ Not described	15-demethylisoplumeride, 15- demethylplumeride, plumeride, isoplumeride	[70]
<i>H. sucuuba</i> (Spruce ex Müll. Arg.) Woodson/ bark	EtOH/ Maceration	Isoplumericin, plumericin	[88]
<i>H. sucuuba</i> (Spruce ex Müll. Arg.) Woodson / latex	H ₂ O/ Not described	Plumericin, isoplumericin	[92]
<i>H. sucuuba</i> (Spruce ex Müll. Arg.) Woodson /	BuOH/ Partition	Plumeride, isoplumeride, 15-demethylplumeride	[73]

SPECIES / PLANT MATERIAL	SOLVENT/ EXTRACTION METHOD	ISOLATED IRIDOIDS	REF.
latex			
<i>H. succuba</i> , (Spruce ex Müll. Arg.) Woodson / bark	EtOH/ Maceration	Plumericin	[100]
<i>Nerium indicum</i> Mill. / root	Petrol/ Soxhlet apparatus	Plumericin	[130]
<i>Plumeria acutifolia</i> Poir. / root	MeOH/ Percolation	Plumericin, 13-O-coumaroylplumieride, 13-O-caffeoylplumieride, 13-deoxyplumieride, plumenoside, 1 α -plumieride, 1 α -protoplumericin A, plumieride, protoplumericin A, 8-isoplumieride	[66]
<i>P. acutifolia</i> Poir. / leaves	MeOH/ Exhaustive Maceration	15-demethylplumieride	[71]
<i>P. bicolor</i> Seem. / bark	MeOH/ Exhaustive Maceration	Plumericin, isoplumericin	[86]
<i>P. rubra</i> L. var. <i>acutifolia</i> (Poir.) Woodson / bark	MeOH/ Maceration	15-demethylisoplumieride, 15-demetilplumieride, plumieride, isoplumieride	[70]
<i>P. rubra</i> L. / steam bark	CH ₂ Cl ₂ :MeOH (1:1) and MeOH/ Maceration	Plumericin, isoplumericin, plumieride, fulvoplumierin	[75]
<i>P. rubra</i> L. / heartwood	EtOAc/ Percolation	Plumericin, isoplumericin, plumieride, 13-O-coumaroylplumieride, protoplumericine A	[85]
<i>P. rubra</i> L. / stem bark	MeOH/ Maceration	Fulvoplumierin, allamandin, allamcin, plumericin, 15-demethylplumieride, plumieride, α -allamcin, β -allamcin, 13-O- <i>trans</i> -p-coumaroylplumieride	[44]
<i>P. rubra</i> L. / stem bark	MeOH/ Maceration	Fulvoplumerin, dihydroplumericin, plumieride, plumeridoids A, B and C, isoplumericin, plumericin, allamcin, allamandin, mixture of plumeridoid C and epiplumeridoid C	[48]
<i>P. rubra</i> L. var. <i>acutifolia</i> (Poir.) Woodson /	EtOH/ Maceration	Plumieridin A, plumieridin B, plumericin, plumieride	[120]

SPECIES / PLANT MATERIAL	SOLVENT/ EXTRACTION METHOD	ISOLATED IRIDOIDS	REF.
flowers			
<i>P. rubra</i> L. var. <i>acutifolia</i> (Poir.) Woodson/ flowers	EtOH/ Maceration	Plumericidine	[97]
<i>P. obtusa</i> L. / flower	EtOH/ Maceration	Plumieride coumarate glucoside	[118]
<i>P. obtusa</i> L. / aerial parts	MeOH/ Maceration	Obtusadoids A and B, plumieridin A, plumieridine, 1 α -plumieride, 15-demethylplumieride	[72]
<i>P. obtusa</i> L. / leaves	MeOH/ Maceration	6''-O-acetylplumieride <i>p</i> -E-coumarate, 6''-O-acetylplumieride, <i>p</i> -Z-coumarate, plumieride, plumieride <i>p</i> -Z-coumarate, plumieride <i>p</i> -E-coumarate	[37]
<i>P. obtusa</i> L. / leaves	MeOH/ Maceration	1 α -plumieride	[119]
<i>P. inodora</i> Jacq. / stems	H ₂ O/ Maceration	Plumieride	[113]
<i>P. bicolor</i> Seem. / bark	MeOH/ Maceration	Plumieride	[114]
<i>Rauwolfia grandiflora</i> Mart. Ex A. DC. / bark	EtOH/ Maceration	Loganic acid, loganin, boonein, isoboonein	[1]
<i>Thevetia peruviana</i> (Pers.) K. Schum. / Leaves	MeOH/ Percolation	10-O- β -D-fructofuranosyltheviridoside, 6'O- β -D-glucopyranosyltheviridoside	[125]
<i>T. peruviana</i> (Pers.) K. Schum. / root	MeOH/ Percolation	Theveside, theviridoside, 10-O- β -D-fructofuranosyltheviridoside, 6'O- β -D-glucopyranosyltheviridoside, 10-O- β -D-glucopyranosyltheviridoside, 3'O- β -D-glucopyranosyltheviridoside	[127]
<i>Winchia calophylla</i> A. DC. / stem bark	EtOH/ Hot extraction	Loganin	[129]

Table 2. Main extraction methods to obtain iridoids of Apocynaceae family.

3. Chemical structures

1 *Plumericin* R = H; R¹ = CH₃

3 *Allamcin*

4 *Allamandin*

5 *Fulvoplumerin*

2 *Isoplumericin* R = CH₃; R¹ = H

6 *Plumieride* R = H; R¹ = CH₃

8 *Isoplumieride*

9 *Loganin*

7 *15-demethylplumieride* R=R¹= H

10 *Plumieride coumarate*

11 *Plumieride coumarate glucoside*

Figure 2. The most isolated iridoids of Apocynaceae family

4. Chromatographic separation

4.1. Thin layer chromatography

Preparative chromatography was performed with thin layer chromatography (TLC) aluminum sheets and 8:2 chloroform/ methanol as mobile phase of a fraction from the 95% EtOH extract of stems of *Alstonia macrophylla*. This procedure led to the isolation of the compounds sweroside (2 mg) and naresuanoside (3 mg) [128]

Another interesting application of preparative chromatography is described in [47] with ethanol extract of ground stems of *A. schottii*. This extract was fractionated by bioassay monitoring and after successive liquid-liquid partition and flash column chromatography; the authors obtained the iridoids allamandin; allamcin and a mixture of plumericin and isoplumericin. This separation was performed with a Chromatotron rotor (silica gel (Si gel), 2-mm) with 1 % methanol in chloroform as solvent system.

The bioassay-guided fractionation of the extracts of *Plumeria rubra* barks also proved to be a successful strategy, leading to the isolation of eleven substances, of which eight were iridoids. In this context, the aqueous extract of *Plumeria rubra* bark has iridoids such as the epimers, α -allamcidin and β -allamcidin, which were resolved by preparative TLC on Si-gel G plates (20 × 20 cm, 250 μ m, Merck®), using chloroform/ethyl acetate/methanol (3:3:1) as solvent system [44].

Ferreira and coworkers [95] describe the HPTLC analysis of the bark, latex and leaf extracts and substances of *Himatanthus sucuuba*. The solutions of the extracts in methanol (10 mg mL⁻¹) and the isolated iridoids (3 mg mL⁻¹ - plumeride; 1 mg mL⁻¹ - isoplumeride) were applied (5 μ L) using Linomat IV, a Camag semi-automatic spotter. The analysis was carried out on a precoated silica gel 60 F254 (Merck) HPTLC plate (0.2 mm of layer thickness and 10 × 10 cm size) using chloroform/ methanol (8:2) as developing system. The resulting chromatogram was dried and the spots were visualized by spraying with vanillin – sulfuric acid solution, followed by heating at 100 °C. Table 3 shows other studies using TLC analysis of Apocynaceae iridoids.

SPECIES	PLANT MATERIAL	IRIDIDS	STATIONARY PHASE	MOBILE PHASE	REF.
<i>Plumeria obtusa</i>	Flowers	Plumeride coumarate glucoside	Si gel 60G F ₂₅₄	CHCl ₃ -MeOH (3:1)	[118]
<i>Plumeria obtusa</i>	Leaves	6"-O-acetylplumeride <i>p</i> -E-coumarate	1- Si gel GF ₂₅₄	1- CHCl ₃ -MeOH (8.5:1.5)	[37]
		6"-O-acetylplumeride <i>p</i> -Z-coumarate	2- Si gel	2- Gradients of Petrol-EtOAc	
<i>Plumeria rubra</i>	Stem bark	Plumeridoid A	1- Si gel GF ₂₅₄	1- Hexane-EtOAc (20%)	[48]
		Plumeridoid B	2- Si gel GF ₂₅₄	2- Hexane-EtOAc (4%)	
<i>Himatanthus fallax</i>	Stems	Plumericin	Si gel	10% Me ₂ CO in CHCl ₃	[89]
		Isoplumericin			
		Plumeride		CHCl ₃ -MeOH (85:15)	

SPECIES	PLANT MATERIAL	IRIDOIDS	STATIONARY PHASE	MOBILE PHASE	REF.
<i>Himatanthus sucuuba</i>	Latex	Plumericin Isoplumericin	Si gel GF ₂₅₄	Hexane-EtOAc (6:4)	[92]
<i>Alyxia reinwardti</i>	Leaves	Alyxialactone 4- <i>epi</i> -alyxialactone	Si gel GF ₂₅₄	CHCl ₃ -MeOH (95:5)	[59]
<i>Allamanda cathartica</i>	Roots	Plumericin Isoplumericin Allamandicin Allamadin	1- Si gel 2- Si gel 3- Si gel	1- CHCl ₃ 2- CHCl ₃ -MeOH (95:5) 3- ether-hexane (1:1)	[41]

Table 3. TLC analyses of Apocynaceae iridoids.

4.2. Open column chromatography

The methanol extract of the leaves of *Cerbera manghas* and its fruit contain the iridoids theveside and theviridoside, as described in [124]. The methanol extract of the leaves, after the addition of water, was sequentially partitioned with chloroform, acetic acid and butanol. This extract and the aqueous phase were submitted to column chromatography with charcoal and water/methanol as eluent. Theveside was isolated from the aqueous phase. Fractions of the butanol extract, which turn blue after heating with mineral acid, were chromatographed over a silica gel column with a gradient of increasing polarity of chloroform/methanol to afford theviridoside.

Cerberidol, epoxycerberidol, cyclocerberidol, cerberidol-3-*O*- β -D-allopyranoside, cerberidol-3,10-bis-*O*- β -D-allopyranoside, epoxycerberidol-3-*O*- β -D-allopyranoside and cyclocerberidol-3-*O*- β -D-allopyranoside are present in the leaves of *C. manghas* [55]. The methanol extract from 1.45 kg of dried leaves was concentrated, re-suspended in water, and sequentially partitioned with benzene and chloroform. The aqueous phase was chromatographed using MCI-gel column (elution with gradients of water/methanol) and the fraction eluted with 20% methanol was chromatographed in two steps: using RQ-1 (Fuji-gel phase) column (elution with water/acetonitrile) and using a silica gel column (elution with gradient of chloroform/methanol/water 7:3:1 to 7:3:1.2). Fractionation afforded cerberidol (75 mg), epoxycerberidol (10 mg), cyclocerberidol (160 mg), cerberidol-3-*O*- β -D-allopyranoside (135 mg), cerberidol-3,10-bis-*O*- β -D-allopyranoside (20 mg), epoxycerberidol-3-*O*- β -D-allopyranoside (27 mg) and cyclocerberidol-3-*O*- β -D-allopyranoside (250 mg). Some of these iridoids are also found in dried leaves of *Cerbera odollam* Gaertn [55]. The same methodology was used to isolate cerberidol (15 mg), cyclocerberidol (48 mg), cerberidol-3-*O*- β -D-allopyranoside (28 mg) and cyclocerberidol-3-*O*- β -D-allopyranoside (14 mg).

The iridoids cerbinal, cerberic acid and cerberinic acid are found in the methanol extract of the bark of *C. manghas* [67]. The crude extract obtained by percolation from 4 kg of stem bark and 1.9 kg of root bark were diluted with water to 50% water:methanol. The mixture was washed with hexane and partitioned with benzene. Benzene fractions were re-suspended in methanol and cerbinal (120 mg from stem bark extract and 300 mg from root bark extract) precipitated.

The supernatant was chromatographed on a silica gel column and cerbinal, cerberic acid and cerberinic acid were eluted with benzene/acetone.

The iridoids isoplumericin, plumericin, plumieride, plumieride coumarate and plumieride coumarate glucoside can be detected and quantified in several species of *Plumeria* and *Allamanda* by TLC using silica gel 60 and the following mobile phases: benzene/ethyl acetate 4:1, chloroform/methanol 4:1, chloroform/methanol 7:3, propanol/ethyl acetate/water 7:2:1 [84]. Visualization of the chromatograms is achieved by spraying with 50% sulfuric acid/ethanol solution and heating. For the analyses, it is necessary to use iridoids as standards that can be isolated from the roots of *Allamanda cathartica*. For the isolation of iridoids during the development of the method, chloroform and methanol extracts, sequentially obtained in a Soxhlet apparatus from the root bark and inner roots of *Allamanda cathartica* (15.0 g for each extract) were successively fractionated on column chromatography. In the first chromatography step, the chloroform extract from the bark (1.7 g) was applied to a silica gel column (80 g) and eluted with gradient of petrol, ethyl ether, chloroform and methanol. This procedure yielded a mixture containing isoplumericin and plumericin (160 mg). Fractions eluted with chloroform/ methanol (3:2) were mixed with the methanol extract of the bark (total mass: 2.8 g) and chromatographed on a silica gel column (150 g deactivated with water) with gradient of chloroform and methanol as mobile phase to furnish plumieride coumarate (150 mg), plumieride coumarate glucoside (480 mg) and two mixtures: one containing plumieride and plumieride coumarate, and the other containing plumieride and plumieride coumarate glucoside. The first mixture was further resolved on partition between water and ethyl acetate and afforded 180 mg of plumieride and 150 mg of plumieride coumarate. The other mixture was rechromatographed to afford plumieride (300 mg) and plumieride coumarate glucoside (410 mg). Neither the chloroform (810 mg) nor the methanol (920 mg) extracts from the inner part of the roots contained isoplumericin and plumericin. These extracts were purified on silica gel column (75 g) deactivated with water and eluted with chloroform and methanol gradient, to give plumieride (70 mg), plumieride coumarate (80 mg) and plumieride coumarate glucoside (200 mg).

The fresh leaves of *Cerbera manghas* contain the iridoids 10-*O*-benzoyltheveside, 10-dehydrogeniposide, loganin, theviridoside and theveside [124]. For the isolation, the methanol extract obtained by percolation using 2.6 kg of fresh leaves was extracted with butanol, and this extract was partitioned with benzene. After partition, the remaining butanol fraction (22.8 g) was subjected to column chromatography using MCI-gel (CHP-20) as stationary phase and a gradient of methanol/water as eluent. The fraction eluted with 20% methanol was subjected to C-18 column (elution with acetonitrile/water) to afford 20 mg of 10-dehydrogeniposide and 20 mg of loganin. The fraction eluted with pure water (2.6g) in the first chromatographic step was also subjected to C-18 column (elution with acetonitrile/ water) to furnish 23 mg of 10-*O*-benzoyltheveside and 270 mg of theveside.

According to [44] the iridoids fulvoplumierin, allamandin, α - and β -allamcidin, plumieride, 15-demethylplumieride, 13-*O*-*trans*-*p*-coumaroylplumieride and plumericin are present in extracts of *Plumeria rubra*. For isolation of these iridoids, the stem bark (2.5 kg) was successively extracted with petroleum ether and methanol followed by bioguided fractionation to investi-

gate the cytotoxic activity against various cancer cells, particularly murine lymphocytic leukemia cells (P-388). Petroleum ether extract (30 g) was submitted to flash column chromatography over silica gel (750 g, 230-400 mesh) with chloroform/petroleum ether (1:1) as eluent. Fraction 002 (200 mg) was rechromatographed using silica gel (120 g) and chloroform/methanol (99:1) to isolate fulvoplumierin (25 mg after recrystallization from petroleum ether/chloroform 1:1). The methanol extract (295 g) was partitioned between chloroform and water. The chloroform extract (60 g) was successively chromatographed in a silica gel column (1.5 kg) with gradients of increasing polarity, with chloroform and methanol, and then in a silica gel (600 g) with petroleum ether/chloroform/ethyl acetate (1:3:1) as eluent to furnish allamandin (12 mg after recrystallization from chloroform). Another fraction from the first column chromatography of the methanol extract (1.2 g) was purified in a silica gel column (400 g) and ethyl acetate/methanol (97:3) and recrystallized from chloroform to furnish 9 mg of allamcin. The aqueous extract (200 g) was also subjected to column chromatography using silica gel column (2 kg) and gradient of chloroform and methanol. Plumieride (55 g) was obtained directly from fraction F 022 after recrystallization from methanol. Fraction F022 (29 g) also furnished 15-demethylplumieride and 13-*O-trans-p*-coumaroylplumieride (3 g) by column chromatography over silica gel and gradient of chloroform and methanol. Fraction F018 (800 mg) was purified over silica gel (400 g) with gradient of ethyl acetate and methanol to afford plumericin (18 mg after recrystallization with ethyl acetate). Another fraction (F019) was purified over silica gel (250 g) with ethyl acetate/chloroform/methanol (6:6:1) solvent system to furnish an unstable iridoid aldehyde and a mixture of α - and β -allamcin (12 mg and 16 mg, respectively) further isolated by TLC on silica gel plates (20 cm x 20 cm, 250 μ m) with ethyl acetate/chloroform/methanol (3:3:1).

Plumericin, isoplumericin, plumieride, 13-*O*-coumaroylplumieride and protoplumericine A were isolated from the ethyl acetate extract of *P. rubra* heartwood [85]. The extract (44 g) obtained by percolation was submitted to column chromatography on silica gel with light petroleum ether/ethyl acetate (1:1) and methanol as eluent to furnish four fractions (A-D). Fraction C directly afforded plumericin (1.22 g) after recrystallization from toluene/ethyl acetate. Fraction B was rechromatographed on a silica gel with toluene/ethyl acetate (9:1), and isoplumericin (140 mg) was isolated from fraction 2. Fraction D was also rechromatographed on a silica gel column, but with chloroform/methanol/water (90:10:0.5 to 70:30:10) as solvent system. Fraction 3' was 13-*O*-coumaroylplumieride (2.0 g) and fraction 7' was protoplumericine (1.6 g). Fraction 5' was subjected to Sephadex LH20 column eluted with methanol, followed by silica gel column eluted with chloroform/methanol/water (85:15:0.7) to furnish plumieride (120 mg).

The iridoids, plumericin, isoplumericin, plumieride and fulvoplumierin, were present in the extracts of *Plumeria rubra* bark. After maceration of the powdered bark (3.5 kg) with dichloromethane/methanol (1:1) and pure methanol, the combined extracts were partitioned between water and ethyl acetate. To isolate the four iridoids, the organic layer was chromatographed twice in a column using silica gel and gradient of increasing polarity with hexane and ethyl acetate, ethyl acetate and methanol, and then pure methanol. The amounts of the compounds isolated were not reported [75].

The flowers of *Plumeria rubra* L. cv. *acutifolia* can provide plumericidine, as described by [97]. The ethanol (95%) extract, obtained from 2.9 kg of flowers, was successively partitioned with petroleum ether, ethyl acetate and butanol. The ethyl acetate fraction was sequentially submitted twice to column chromatography using silica gel and gradient of chloroform/methanol as mobile phase. Chromatography on a Sephadex LH20 column yielded 20 mg of plumericidine.

According to [72], several iridoids can be isolated from the aerial parts of *Plumeria obtusa*: obtusadoid A, obtusadoid B, plumieridin A, 1 α -plumieride, 15-demethylplumieride and plumieridine. The methanol extract (400 g) was obtained from 10 kg of the plant material and sequentially partitioned with hexane and ethyl acetate. The ethyl acetate extract was chromatographed using a silica gel column and gradients of hexane, ethyl acetate and methanol. The less polar fractions were rechromatographed in the same stationary phase, and eluted with hexane/dichloromethane (1:1) to afford obtusadoid A (6 mg), obtusadoid B (11.5 mg), plumieridin A (8 mg) and plumieridine (12 mg). The more polar fraction obtained in the first chromatography was filtered on a Sephadex LH20 column using methanol, and further submitted to RP-8 flash column chromatography. Elution with 50% methanol afforded 1 α -plumieride (22 mg) and 15-demethylplumieride (13 mg).

Plumieride also can be isolated from the bark of *Plumeria bicolor* [114]. Powdered bark (4 kg) was extracted in methanol, and the crude extract was washed with acetonitrile. The material was re-extracted with chloroform, and this extract was fractionated in column chromatography using silica gel (900 g) and different solvents of increasing polarity. Plumieride was eluted with chloroform/ethyl acetate (1:1) and recrystallized from methanol.

The bark of *Plumeria bicolor* also contains plumericin and isoplumericin, as described in [86]. The methanol extract (100 g), after washing with acetonitrile, was extracted with chloroform and chromatographed on a column containing 800 g of silica gel G (60-120 mesh). Elution was carried out using gradients of increasing polarities with benzene, chloroform and methanol. Plumericin and isoplumericin was recrystallized from methanol.

Isoplumericin and plumericin are present in the bark of *Himatanthus sucuuba* [88]. For the isolation of these iridoids, 95% ethanol extract (2 g), obtained from 50 g of plant material was submitted to column chromatography using silica gel and gradients of increasing polarities with hexane, ethyl acetate and methanol. After recrystallization, isoplumericin (18 mg) was obtained from ethyl acetate and plumericin (70 mg) from methanol.

The stem bark of *Winchia calophylla* contains loganin (1.25 g) [129]. The 95% ethanol extract (600 g) from the dried stem bark (10.5 kg) was partitioned between petroleum ether and water. The petroleum ether extract was submitted to acid-base extraction and after adjustment to pH 9-10 with ammonium hydroxide; the aqueous layer was extracted with petroleum ether, chloroform and butanol. The chromatography of the butanol fraction using silica gel H column led to the isolation of loganin.

The iridoids, scholarein A, B, C and D, can be obtained by the fractionation of the ethanol extract from bark of *Alstonia scholaris* [60]. The crude extract, obtained from 15 kg of the plant material, was partitioned between ethyl acetate and water. The organic layer (190 g) was

sequentially chromatographed on a column. The first chromatography, over silica gel (2.1 kg) and using gradient of chloroform and ethyl ether, furnished five fractions (1-5). From fraction 2, 8 mg of scholarein B and 7 mg of scholarein D were isolated after silica gel chromatography and elution with petroleum ether/ethyl ether (3:1). From fraction 3, 25 mg of scholarein A and 60 mg of scholarein C were obtained after successive columns using silica gel and chloroform/ethyl ether as stationary and mobile phases, respectively.

4.3. Gas Chromatography (GC)

In the study on iridoids, the technique of gas chromatography is generally used for analytical purposes. Gas chromatography represents an advantage over thin layer chromatography, particularly for detecting substances in small amounts, and mass spectrometry can be used to distinguish most iridoid and secoiridoid glucosides by fragmentation patterns [112].

Methods have been developed by [112] for the detection of 33 iridoids and secoiridoid glucosides in mixtures and plant extracts using gas chromatography sometimes coupled to mass spectrometry. For the gas chromatography analyses, a Shimadzu Model GC-1C gas chromatograph with hydrogen FID-1B flame ionization detector was used. Columns were packed with 1.5% OV-1, 1.5% OV-17, 2% OV-210 and 2% OV-225 on 80-100-mesh Shimalite W AW/DMCS. In all, 33 iridoids and secoiridoids glucosides are analyzed as TMS-derivatives. Using the 1.5% OV-17 column with 1.8 m in length and 4 mm in I.D. at 270 °C, the elution order was: aucubin, 7-deoxyloganic acid and catalpol (retention time = 1.37 min), 7-deoxyloganin, monotropein, gardenoside, secologanin, loganin, scandoside, theviridoside, geniposide, scandoside methyl ester, 7-dehydrologanin, morroniside, hastatoside and forsythide (retention time = 2.55 min), forsythide 10-methyl ester, verbenalin, sweroside, gentiopicroside and swertiamarin (retention time = 3.08 min), bankakosin, kingside, amaroswerin, amarogentin and asperuloside. The 1.5% OV-17 column, 0.5 m in length and 3 mm in I.D., at 230°C, furnished the same elution order as above, but forsythide 10-methyl ester, verbenalin and sweroside eluted together (retention time = 3.20 min), while the separation of hastatoside and forsythide, gentiopicroside and swertiamarin were better. When the non-polar 1.5% OV-1 column with 1.8 m in length and 4 mm in I.D. was used at 270 °C, better separation between loganin and secologanin occurred. Better results were achieved for the 7-deoxyloganic, 7-deoxyloganic acid and catalpol. However, verbenalin and sweroside eluted together, and amaroswerin and amarogentin were not detected. The OV-17 column was slightly polar and, in general, it influenced the larger range of the retention times. The more polar columns with 2% OV-210 at 215 °C, and 2% OV-225 at 230 °C, both with 0.5 m in length, showed important differences from keto compounds, such as 7-dehydrologanin and verbenalin, and lactonic compounds, such as sweroside, gentiopicroside and gentiopicroside, reflected in their longer retention times. Amaroswerin and amarogentin were not detected. Sweroside and gentiopicroside were well-separated on OV-210 column, which was not observed using other columns. Paederoside, ligustroside, catalposide, oleuropein, 10-acetoxyligustroside and 10-acetoxyleuropein were only detected and well-separated in OV-17 and OV-1 columns with 0.5m in length at 270 °C.

For the gas chromatography-mass spectrometry studies, a Hitachi K-53 gas chromatograph and a Hitachi RMU-6 E mass spectrometer were used. The glass columns, 0.5 m x 3 mm in I.D.,

were packed with 1.5% OV-17 on 80-100-mesh Shimalite W AW/DMCS and 1.5 % OV-1 on 80-100-mesh Shimalite W AW/DMCS, and were used to the oleuropein-type glucosides detection. The authors considered the GC-MS identification of some iridoids was not satisfactory: any important peak different from the sugar moiety was detected in asperuloside and paederoside TMS-derivatives of amaroswerin, amarogaentin asperuloside and paederoside exhibited different retention times, but the same fragmentation pattern; separation using this technique was unsuccessful [112].

Aqueous extracts of different plant species with known presence of iridoid and secoridoid glucosides were analyzed by GC-FID and GC-MS [112]. One of these species was *Allamanda cathartica* var. *schottii* (Pohl) Rafill (Apocynaceae) cultivated in a greenhouse. Aqueous extracts obtained from 3-5 g of fresh plant material and hot water were treated in a column of charcoal (active carbon for column chromatography) for removal of sugars by elution with water. The sample was eluted with methanol and concentrated under reduced pressure. TMS-derivatives were prepared. For the analyses, a 1.5% OV-17 column with 1.8 m in length at 280 °C was used. GC-FID and GC-MS (70 eV) showed the presence of one iridoid glycoside. The fragmentation pattern indicated that the original glucoside was plumieride, and that relative retention time was the same as that of asperuloside.

Isoplumericin, plumericin, plumieride, plumieride coumarate and plumieride coumarate glucoside can be detected by GC-FID [116]. For the development of the method, it was necessary to isolate these iridoids for use as standard. The methanol extract of *Allamanda cathartica* L. roots, obtained with 500 g of the dried plant material and boiling methanol, was submitted to silica gel 60 column (2.5 kg) with the eluents: petrol, petrol/ethyl ether, ethyl ether, ethyl ether/chloroform, chloroform/ methanol and pure methanol [116]. Fractionation was monitored by TLC [84]. The fractions were eluted with petrol/ethyl ether until chloroform/ methanol contained isoplumericin and plumericin (4.2 g). This mixture (1.0 g) was suspended in ethyl ether and rechromatographed on silica gel (40 g). Elution with petrol and a gradient of increasing polarity with petrol/ethyl ether furnished isoplumericin (250 mg), plumericin (140 mg) and a mixture of both (290 mg). A second fraction eluted with chloroform/methanol in the first chromatographic step (8.0 g) was resubmitted to column chromatography on silica gel (300 g). Elution with a gradient of chloroform and methanol led to the isolation of plumieride coumarate (5.1 g). The third fraction of the first chromatograph step (10.0 g), eluted with chloroform/methanol, was partitioned between water and ethyl acetate to give plumieride coumarate in the organic phase (3.8 g) and plumieride in the aqueous phase (1.8 g). Finally, 30.0 g of the forth fraction of the first chromatograph step, eluted with chloroform/methanol to methanol, was submitted to column chromatography on silica gel (1.0 kg) deactivated with water. The elution was carried out with a gradient of chloroform and methanol and furnished plumieride coumarate glucoside (6.2 g). GC-FID analyses were used to evaluate the pure grade of fractions and isolated substances. For these analyses, trimethylsilylation of iridoids using HMDS-TMCS and pyridine, and acetylations with acetic anidride and pyridine, were necessary. Plumieride coumarate (isomer mixture) and plumieride coumarate glucoside were acetylated and their products were purified on chromatography with silica gel column and ethyl ether as eluents, to afford pure penta-acetylplumieride coumarate and octa-acetylplu-

mieride coumarate glucoside, respectively. Furthermore, plumieride, plumieride coumarate and plumieride glucoside were hydrolyzed under heating and acid conditions (sulfuric acid, 1 N for 2-3 h), extracted with ethyl acetate and both organic phase and aqueous phase (after neutralization with Amberlite) were analyzed by TLC and GC. Analyses were performed on a 1.5% OV-17 glass column with 0.4 m length and 4 mm I.D. Other analytical conditions were: nitrogen as carrier gas at 50 mL/min; detector temperature, 320 °C; column temperatures: 190 °C for isoplumericin and plumericin, 240 °C for TMS-plumieride, 300 °C TMS-plumieride coumarate. Glucose was detected in aqueous phases from acid hydrolyses of plumieride, plumieride coumarate and plumieride coumarate glucoside, while *p*-coumaric acid was detected in the organic phases of plumieride coumarate and plumieride coumarate glucoside. GC analyses also showed that plumieride coumarate was isolated as an isomer mixture (20% cis and 80% trans isomers) [116].

4.4. High, Medium and Low Performance Liquid Chromatography (HPLC, MPLC and LPLC)

Studies on HPLC with iridoids of Apocynaceae focus mainly on the separation of components from extracts or fraction. The chromatography profile, the identification and quantification of these terpenes in the extracts are described. Table 4 shows the principal references on iridoids isolated from Apocynaceae by HPLC, MPLC and LPLC.

TECHNIQUE	MOBILE PHASE	COLUMN	SPECIES/ PLANT MATERIAL	SAMPLE	IRIDOID	REF
HPLC	50% methanol in water	µBondapak C-18	<i>Rauwolfia grandiflora</i> / bark	ethanol extract	boonein and isoboonein	[1]
HPLC	10% acetonitrile	Octadecylsilane (ODS)	<i>Thevetia peruviana</i> / root	methanol extract	theviridoside, 10-O-β-D-fructofuranosyltheviridoside, 6'-O-β-D-glucopyranosyltheviridoside, 10-O-β-D-glucopyranosyltheviridoside and 3'-O-β-D-glucopyranosyltheviridoside	[125]
HPLC	10% acetonitrile	Octadecylsilane (ODS)	<i>Thevetia peruviana</i> / leaves	methanol extract	10-O-β-D-fructofuranosyltheviridoside and 6'-O-β-D-glucopyranosyltheviridoside	[127]
HPLC	20 mM KH ₂ PO ₄ and acetonitrile Flow: 0.8 mL/min	Cosmosil 5 C ₁₈ -AR (5 µm, 25 cm x 4.6 mm I.D.)	<i>Rauwolfia</i> / barks	ethanol extract	loganin, loganic acid and gardenoside	[131]

TECHNIQUE	MOBILE PHASE	COLUMN	SPECIES/ PLANT MATERIAL	SAMPLE	IRIDOID	REF
HPLC	water / acetonitrile	Octadecyl-silane (ODS) (250×10mm)	<i>Plumeria acutifolia</i> / leaves	methanol extract	15-demethylplumieride	[71]
HPLC	not informed	Zorbax SB-C18	<i>Plumeria rubra</i> L. cv. <i>acutifolia</i> / flowers	ethyl acetate fraction	plumieridin A and plumieridin B	[120]
HPLC	not informed	not informed	<i>Allamanda neriifolia</i> / aerial parts	ethanol extract	allamanoid, plumieride and protoplumericin	[52]
LPLC	5% ethyl acetate in chloroform	Michel-Miller column (200 g Silica gel)	<i>Allamanda schottii</i> / stem	ethanol extract	allamandin and allamcin	[47]
MPLC	water to 50% methanol in water	RP-18 (25–40 mm particle size, 460 × 36 mm I.D., 460 × 15 mm I.D.)	<i>Himatanthus sucuuba</i> / latex	methanol/ water (1:1)	plumieride	[73]
TECHNIQUE	MOBILE PHASE	COLUMN	SPECIES/ PLANT MATERIAL	SAMPLE	IRIDOID	REF.
HPLC	Acetonitrile and water containing 0.05% trifluoro-acetic acid Flow: 1 mL/min	Lichrospher C18 (5mm, 250 mm × 4.6 mm I.D.)	<i>Himatanthus sucuuba</i> / bark and latex	methanol/ water (1:1)	plumieride, isoplumieride and demethylplumieride	[73]
MPLC and HPLC	water to 50% methanol; 30% acetonitrile in water to 50% acetonitrile MPLC Flow: 6 mL/min	LiChroprep C-18 (45 × 3.5 cm) and Shimpack C-18 (10 µm, 45 cm × 250 mm I.D.)	<i>Himatanthus sucuuba</i> / latex	aqueous fraction and fractions from this procedure	15-demethylisoplumieride, 15-demethylplumieride, plumieride and isoplumieride	[70]

Table 4. Separation by Pressure Liquid Chromatography

4.5. Counterflow

Protoplumericin and plumieride can be extracted from the methanol extract of *Allamanda neriifolia* stems [117] by droplet counter-current chromatography. Crude extract, obtained from 1.6 kg of plant material, was successively partitioned with benzene, chloro-

form and butanol. Plumericin (420 mg) was directly obtained from the benzene fraction (0.02% yield). The butanol fraction was subjected to sequential chromatographic steps, using XAD-2 column and gradient of water/methanol (mobile phase), silica gel column and solvent system containing chloroform/methanol/water or chloroform/methanol, followed by droplet current chromatography with chloroform/methanol/ water. Plumieride (1.3 g) and protoplumericin (13.2 g) were obtained with yields of 0.08% and 0.83%, respectively.

The iridoids allamcidin B β -D-glucoside, plumiepoixide and protoplumericin B were isolated from *Allamanda neriifolia* extract obtained by percolation with methanol, also by droplet counter-current chromatography [39]. The crude methanol extracts from 2.6 kg of stem and 6.7 kg of leaves were sequentially fractionated with benzene, chloroform and butanol. Previous chromatographic treatment with MCI gel (elution with water/methanol), silica gel column (mobile phases: chloroform/methanol/water; benzene/acetone; ethyl acetate/methanol/ water and ethyl acetate/hexane) and Sephadex LH20 column (mobile phase: chloroform/methanol) led to the isolation of isoallamandicin (10 mg from the stem), allamcin (230 mg from the leaves), 3-O-methylallamcin (30 mg from the leaves), allamancin (102 mg from the stem), 3-O-methylallamancin (41 mg from the leaves), allamcidin (125 mg from the leaves), plumieride 13-O-acetate (760 mg from the stem and *ca.* 2 g from the leaves). Fractions of butanol extracts from the stem and leaves were subjected to droplet counter-current chromatography using chloroform/methanol/water (5:6:4, ascending mode) to obtain allamcidin B β -D-glucoside (17 mg from the stem), plumiepoixide (7 mg from the stem and 374 mg from the leaves) and protoplumericin B (70 mg from the leaves).

Iridoids can be isolated from *Plumeria acutifolia* roots following a similar methodology [66]. Successive liquid-liquid partitions of the crude methanol extract (obtained from 6 kg of plant material) with benzene, chloroform and butanol, followed by several chromatographic steps for fractionation of the butanol fraction (using polystyrene, silica gel and octadecyl silica columns) led to the isolation of 13-O-coumaroylplumieride (43 g), plumieride (7.5 g), 13-O-caffeoylplumieride (60 mg), 1 α -plumieride (20 mg) and protoplumericin A (9 g). A further purification step involving droplet counter-current chromatography and a mixture of chloroform/methanol/water (4:6:5, ascending mode) led to the isolation of 13-deoxyplumieride (200mg), plumenoside (50 mg) and 8-isoplumieride (700 mg).

4.6. Capillary electrophoresis

For analytical purposes, iridoids can be analyzed by capillary electrophoresis. A method to separate nine iridoids described in [131] uses a Hewlett-Packard (HP^{3D} CE) capillary electrophoresis system coupled to a photodiode array detector (210 nm and 230 nm) and equipped with a fused-silica capillary tube (90 cm $\times$ 75 μ m I.D.). The distance to the detector was 81.5 cm. Other conditions: sample injection at 50 mbar for 3 s and further deionized water injection at 50 mbar for 3 s; constant voltage, 16 kV (positive to negative); cartridge temperature, 30 $^{\circ}$ C; electrolyte (buffer), 50 mM sodium borate and 30 mg/mL 2,6-di-O-methyl- β -cyclodextrin (DM- β -CD); run time, 32 min. Before the analyses, the capillary column was sequentially purged with 0.5 M NaOH, 0.1 M NaOH, deionized water and buffer solution. The iridoids studied eluted in the following order: geniposide, loganin, shanzhiside, aucubin, catalpol, harpago-

side, gardenoside, geniposidic acid and loganic acid. All were commercially purchased and only loganin, loganic acid and gardenoside were described for the Apocynaceae family. Several conditions of analyses were studied, including different pH, surfactants, concentrations of sodium borate and the addition of cyclodextrins (CD) to the buffer, and it was concluded that the less polar DM- β -CD added to 50 mM borate solution was the most suitable running buffer. In this condition, only aucubin and catalpol could not be separated, even with the addition of organic solvents and/or valine, urea and barium ion. The greatest advantage of capillary electrophoresis compared to HPLC analyses (the most commonly used technique) is its speed.

According to [132], capillary electrophoresis can be used to analyze a mixture of eleven iridoid glycosides: unedoside, harpagide, methyl catalpol, morroniside, asperuloside, griselinoside, catalpol, ketologanin, verbenalin, loganin and 10-cinnamoyl catalpol. Only loganin was found in the Apocynaceae family. For the analyses, iridoids were diluted in purified water. A Hewlett-Packard ^{3D}CE system coupled to a diode array detector and equipped with an air-cooling device was used. The fused-silica capillary tube measured 80 cm in length, 50 μ m in I.D. and 375 μ m in O.D. Distance to the detector was 71.5 cm only for UV detection (197 nm, 235 nm, 239 nm and 283 nm). When coupled to a mass spectrometer system (Bruker ESQUIRE) with an electrospray ionization source, the drying gas was nitrogen at 200 °C and flow-rate 100 L/h. In this case, the distance between injector and UV detector was 20 cm. Other conditions: sample injection at 50 mbar for 5 s (only UV detection) or 25 s (with MS system); voltage, +20 kV; cartridge temperature, 25 °C; electrolyte solution, 20 mM ammonium acetate with 100 mM sodium dodecyl sulfate (SDS), pH 9.5; sheath liquid, 1 mM lithium acetate mixture to water/methanol (1:1 v/v) at a flow rate of 200 μ L/h. When the MS system was used: scan range, 100-550 *m/z*; cut-off, 80 *m/z*; glass capillary exit, 95 V; skimmer, 32 V; electrospray voltage for the capillary, -4.0 kV; for the cylinder, -1.8 kV; for the end plate, -3.5 kV. In the comparison among the counterions sodium dodecyl sulfate (SDS), ammonium dodecyl sulfate and lithium dodecyl sulfate, diluted in water and running buffer, the best resolution for separating iridoid glucosides, lower noise in the MS system, and better repeatability and sensitivity were found with SDS in the running buffer. The volatility of ammonium acetate in buffer enables MS analyses, and concentrations higher than 20 mM did not represent better resolution. Quite the contrary, higher SDS concentrations furnished better results. In the study of the influence of pH, the best one was 9.5, although its influence in the range of 8.7-10.0 was lower than the SDS effect. Good linearity was observed for all the iridoids glucosides analyzed, but in different ranges.

The literature describes chromatographic techniques related to the characterization, isolation and purification of iridoids. Most reports show the open column technique as the principal technique used to isolate this class. Also, there have been few studies on counterflow and capillary electrophoresis chromatographies. In general, there has been little scientific investment in the area of obtaining iridoids of the Apocynaceae family, despite the great pharmacological importance of this class of constituents.

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