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Classification of *Aristolochia* species based on GC–MS and chemometric analyses of essential oils

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Abstract

Essential oils were obtained from roots of 10 *Aristolochia* species by hydrodistillation and analysed by GC–MS. A total of 75 compounds were identified in the analysed oils. Multivariate analyses of the chemical constituents of the roots enabled classification of the species into four morphological groups. These forms of analysis represent an aid in identification of further specimens belonging to these species.

Keywords: *Aristolochia*; Aristolochiaceae; Essential oils; Multivariate analysis; Principal component analysis; Morphology; Monoterpene; Sesquiterpene

1. Introduction

Brazilian Aristolochiaceae is represented by three genera: *Aristolochia*, which consists of approximately 100 species, and *Holostylis* and *Euglypha*, each of which consists of only one species *Holostylis reniformis* and *Euglypha rojasiana* (Hoehne, 1942). The latter have been suggested to be included in the *Aristolochia* genus (González, 1997). With regard to synonyms, more than 300 names have been proposed for these species. *Aristolochia* and *Euglypha* are also represented by both small and large vines, whereas *Holostylis reniformis* is a shrub. In Brazilian traditional medicine, these plants are known as "one thousand men", and have been mainly used as abortifacients, stomachics, antiophidians, antiasthmatics, expectorants, and, recently, in slimming therapies (Lopes et al., 2001). They are generally sold at popular Brazilian markets, under their common names. Thus, there has been little distinction made among the species. In addition to the *Aristolochia* species and Lepidopteras belonging to the specialist Troidini tribe (Papilionidae and Papilioninae) which feed on such plants, aristolochic acids have been detected in Chinese phytomedicines and dietary supplements used in slimming therapy (Ioset et al., 2003). These acids have been associated with Chinese herb nephropathy, which is a kind of severe kidney disease caused by intake of excessive aristolochic acids (Balachandran et al., 2005; Cui et al., 2005; Ishibe et al., 2005; Zang et al., 2005). Therefore, it is essential, for health safety and quality control of related Brazilian herbal medicines, to develop efficient methods for species identification.

In our continuous studies on the Aristolochiaceae family, we have shown that the chemical constituents of Brazilian species differ significantly. Although allantoin, sugars, aristolochic acids, and furofuran and butyrolactone lignans occur in most of these species (Lopes et al., 2001), they also contain specific types of compounds, such as bicubebins in *Aristolochia lagesiana* and *Aristolochia pubescens* (Pascoli et al., 2006), aryltetralone lignans in *H. reniformis* (da Silva and Lopes, 2004, 2006), benzofuran neolignans, and diterpene ester of aristolochic acids in *A. pubescens* (Nascimento et al., 2000; Nascimento and Lopes, 1999, 2000, 2003).

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Furthermore, kaurane diterpenes are found in *Aristolochia elegans* (Luiz et al., 1990), labdane and clerodane diterpenes in *Aristolochia esperanzae*, *Aristolochia galeata*, and *Aristolochia chamissonis* (Lopes et al., 1987, 1988; Lopes and Bolzani, 1988; Bomm et al., 1999), aristolane sesquiterpenes, dibenzoquinolizinium, and tetrahydroisoquinoline alkaloids in *Aristolochia arcuata* (Watanabe and Lopes, 1995; Francisco et al., 2003), benzylberbine alkaloids in *Aristolochia gigantea* (Lopes, 1992; Lopes and Humpfer, 1997), alkamides in *Aristolochia gehrtii* (Navickiene and Lopes, 2001), and bi- and tetraflavonoids in *Aristolochia ridicula* (Carneiro et al., 2000; Machado and Lopes, 2005). All of these specific compounds could thus be markers for differentiation among these species.

Despite the significant number of Brazilian *Aristolochia* species, the volatile compounds they contain are known for only a few species. Reports on essential oils from South American Aristolochiaceae species have been limited to *Aristolochia argentina* (Priestap et al., 2003), *A. elegans* (Vila et al., 1997), *Aristolochia giberti* (Priestap et al., 2002; Canela et al., 2004), *Aristolochia birostris*, *Aristolochia cymbifera*, *A. gigantea*, *Aristolochia longa*, *Aristolochia macroura*, *Aristolochia papillaris*, *Aristolochia rodriguesia*, *Aristolochia triangularis* (Leitão et al., 1991; Priestap et al., 1990), and *Aristolochia odoratissima* (Usubillaga et al., 2001). The goals of the present study were to investigate the nature of essential oils from roots of 10 *Aristolochia* species, and correlate their oil compositions to morphological groups for the identification of these species. The variation in the chemical constitutions of the essential oils was examined by taking into account the various species and duration of extraction. In addition, the data were compared with those described in the literature (Vila et al., 1997) for the oil obtained from the roots of *A. elegans*.

2. Results and discussion

Hoehne (1942) proposed an artificial key for the South American Aristolochiaceae species based on vegetative organs and the external morphology of the inflorescence and flowers, and classified *Holostylis* as a monophyletic genus, and *Aristolochia* species in *Gymnolos*, *Orthoaristolochia* subgenus and five subseries. The Pseudostipulosae (I) subdivision includes Peltiflorae (A), Bilabiatae (B), and Caudatae (C) subseries, whereas Extipulatae (II) includes the Volubilis (A) and Fruticulosae (B) subseries. According to this key, *A. gigantea* and *A. elegans* are included in I-A, *A. galeata*, *Aristolochia malmeana*, and *A. esperanzae* are in I-B, and *A. chamissonis*, *A. lagesiana*, *Aristolochia melastoma*, *A. pubescens*, and *A. arcuata* are in II-A subseries. The relationships among the most diverse lineage in *Aristolochia*, considering the congruence between the *trn*L and *trn*F gene tree and morphological data, remain to be substantiated (Neinhuis et al., 2005). Silva-Brandão et al. (2006) proposed a molecular phylogeny based on the plastid gene *mat*K and the region between the genes *trn*L and *trn*F. In addition, they proposed a chemical relationship for *Aristolochia* based on the pattern of sesquiterpenes identified in CH_2Cl_2 extracts from the leaves of 12 *Aristolochia* species that were not in agreement with the phylogenetic relationships.

Although, according to González and Stevenson (2000), the series and subseries within *Aristolochia* defined by Hoehne (1942) are not tenable, this system was considered in this work, since the morphological characteristics are informative within *Aristolochia* for the practical purpose of species identification. The presence or absence of pseudostipules is an interesting characteristic because it is possible to separate two groups of species using only the vegetative plant (I and II subdivisions proposed by Hoehne).

Essential oils with an intense odour were obtained from the roots of ten *Aristolochia* species by hydrodistillation and analysed by gas chromatography–mass spectrometry (GC–MS) (Tables 1 and 2).

Intraspecific variability was also examined by processing of data obtained by using diverse extraction duration per species (Tables 1 and 2). The results were compared with the morphology and taxonomy of the genera to obtain evidence of possible chemotaxonomic significance.

Table 1
List of the plants investigated with provenance, abbreviations used and voucher specimens

Species	Code[a]	Locality	Voucher specimen
A. arcuata	arc 2R	Araraquara, SP	ESA88280
A. chamissonis	cha 2R	São Joaquim da Barra, SP	ESA88274
A. elegans	ele 2R	Descalvado, SP	ESA88279
A. esperanzae	esp 2R	Ituiutaba, MG	ESA88881
A. galeata	gal 2R	Descalvado, SP	ESA88278
A. galeata	gal 4R	Descalvado, SP	ESA88278
A. gigantea	gig 2R	Araraquara, SP	ESA88281
A. lagesiana	lag 2R	Ituiutaba, MG	ESA88885
A. malmeana	mal 2R	Ituiutaba, MG	ESA88883
A. melastoma	mel 2RS	Araraquara, SP	ESA88883
A. pubescens	pub 2R	Ituiutaba, MG	ESA88882
A. pubescens	pub 4R	Ituiutaba, MG	ESA88882

[a] Code: species name, duration of extraction (2 or 4 h), and part of plant (roots and stems).

Table 2
Composition of essential oils from *Aristolochia* species

I (seg)[a]		Compound (%)	Species (code)[b]											
			arc 2R	cha 2R	ele 2R	esp 2R	gal 2R	gal 4R	gig 2R	lag 2R	mal 2R	mel 2RS	pub 2R	pub 4R
857	**1**	Unknown 1	–	–	–	–	–	–	–	0.8	–	–	–	–
923	**2**	α-Thujene	–	–	–	–	6.1	–	–	–	–	–	–	–
928	**3**	Tricyclene	–	–	–	–	–	–	–	–	–	0.6	–	–
934	**4**	α-Pinene	5.4	–	1.2	–	–	18.6	2.6	0.7	–	19.1	6.5	7.3
948	**5**	Camphene	8.0	4.1	2.3	–	48.8	32.4	1.6	1.1	46.3	15.3	24.8	36.1
953	**6**	Sabinene	–	–	–	–	–	–	–	–	–	0.3	–	–
974	**7**	β-Pinene	2.3	1.0	0.6	–	–	7.3	–	–	–	12.6	6.4	9.1
978	**8**	6-Methyl-5-hepten-2-ol	–	–	–	–	–	–	–	4.1	–	–	–	–
979	**9**	Octanal	–	1.2	–	–	–	–	–	–	–	–	–	–
985	**10**	β-Myrcene	–	–	–	–	–	–	–	–	–	1.3	–	–
1011	**11**	α-Terpinen	–	–	–	–	8.6	–	–	–	–	–	–	–
1013	**12**	α-Phellandrene	–	–	–	–	–	–	60.9	–	–	–	–	–
1014	**13**	*o*-Cymene	38.6	–	–	–	12.4	–	–	–	–	6.2	–	–
1017	**14**	Allyl tiglate	–	–	–	–	–	–	–	1.9	–	–	–	–
1023	**15**	Limonene	8.7	–	0.5	–	10.5	9.8	–	–	10.3	34.5	2.4	2.8
1042	**16**	*trans*-Ocimene	–	–	–	–	–	–	–	–	–	2.0	–	–
1054	**17**	γ-Terpinene	–	–	–	–	13.6	–	–	–	–	–	–	–
1082	**18**	Terpinolene	–	–	–	–	–	–	–	–	4.3	–	–	–
1088	**19**	β-Linalool	–	2.6	–	–	–	–	16.6	–	–	–	–	–
1118	**20**	*cis*-*p*-2-Menthen-1-ol	–	–	–	–	–	5.0	–	–	–	–	–	–
1118	**21**	Camphor	–	10.3	–	–	–	–	–	2.0	–	–	–	–
1152	**22**	Borneol	–	1.8	1.4	–	–	–	–	2.5	–	–	–	–
1198	**23**	β-Cyclocitral	–	10.3	–	–	–	–	–	–	–	–	–	–
1219	**24**	Isobornyl formate	6.0	–	–	–	–	–	–	–	–	–	–	–
1219	**25**	Methyl β-cyclogeranate	–	–	–	–	–	–	–	4.1	–	–	–	–
1222	**26**	*cis*-Carveol acetate	–	–	–	–	–	3.2	–	–	–	–	–	–
1248	**27**	Methyl citronellate	–	2.7	–	–	–	–	–	–	–	–	–	–
1252	**28**	Linalyl acetate	–	–	–	–	–	–	–	–	–	–	14.2	11.0
1274	**29**	Isobornyl acetate	13.2	3.9	3.8	–	–	23.8	–	–	–	5.3	45.7	33.7
1315	**30**	*p*-Mentha-1(7),8(10)-dien-9-ol	–	2.4	1.2	–	–	–	–	–	–	–	–	–
1372	**31**	Longicyclene	–	–	0.4	–	–	–	–	–	11.8	–	–	–
1375	**32**	Ylangene	–	–	1.7	–	–	–	–	–	–	–	–	–
1389	**33**	β-Patchoulene	–	–	–	8.3	–	–	–	–	–	–	–	–
1389	**34**	α-Gurjunene	–	–	–	–	–	–	–	2.5	–	–	–	–
1400	**35**	Tetradecane	–	–	–	–	–	–	7.6	–	–	–	–	–
1408	**36**	Isocaryophyllene	–	–	–	–	–	–	–	1.4	–	–	–	–
1413	**37**	Isosativene	–	–	–	–	–	–	–	–	10.2	–	–	–
1415	**38**	β-Caryophyllene	–	4.8	–	–	–	–	–	12.1	–	0.6	–	–
1433	**39**	γ-Elemene	–	–	–	–	–	–	3.0	–	–	–	–	–
1441	**40**	β-Gurjunene	15.8	–	–	–	–	–	–	–	17.1	–	–	–
1443	**41**	Aristolene	–	–	0.8	–	–	–	–	–	–	–	–	–
1446	**42**	α-Aromadendrene	–	–	–	31.5	–	–	–	–	–	–	–	–
1449	**43**	β-Aromadendrene	–	–	1.0	–	–	–	–	–	–	–	–	–
1455	**44**	α-Caryophyllene	–	–	–	–	–	–	–	1.1	–	–	–	–
1457	**45**	Aromadendrene, dehydro	–	–	–	–	–	–	–	–	–	1.5	–	–

1462	**46**	α-Patchoullene	–	–	–	–	–	–	–	2.0	–	–	–	–
1472	**47**	Isoeugenol, methyl ether	–	–	–	–	–	–	–	–	–	0.6	–	–
1481	**48**	Germacrene D	–	3.1	–	–	–	–	–	–	–	–	–	–
1484	**49**	Isoledene	–	–	–	–	–	–	–	3.1	–	–	–	–
1490	**50**	δ-Selinene	–	–	–	10.2	–	–	–	–	–	–	–	–
1495	**51**	Viridiflorene	–	–	–	42.2	–	–	–	–	–	–	–	–
1495	**52**	Germacrene A	–	5.8	–	–	–	–	–	–	–	–	–	–
1507	**53**	Valencene	–	–	3.7	7.9	–	–	–	–	–	–	–	–
1518	**54**	Bicyclogermacrene	–	–	–	–	–	–	2.1	1.6	–	–	–	–
1530	**55**	Unknown 2	–	–	4.3	–	–	–	–	–	–	–	–	–
1536	**56**	γ-Maaliene	–	–	–	–	–	–	–	3.4	–	–	–	–
1541	**57**	Elemol	–	–	–	–	–	–	–	1.8	–	–	–	–
1551	**58**	Unknown 3	–	–	0.6	–	–	–	–	–	–	–	–	–
1545	**59**	Eudesma-3,7(11)-diene	–	–	–	–	–	–	–	2.9	–	–	–	–
1549	**60**	Germacrene B	–	–	–	–	–	–	5.7	–	–	–	–	–
1557	**61**	*trans*-Nerolidol	–	3.6	49.6	–	–	–	–	3.6	–	–	–	–
1566	**62**	Ledol	–	7.0	–	–	–	–	–	3.0	–	–	–	–
1576	**63**	Aromadendrene oxide-2	–	–	10.6	–	–	–	–	–	–	–	–	–
1576	**64**	Caryophyllene oxide (−)-B	–	–	–	–	–	–	–	3.5	–	–	–	–
1580	**65**	Globulol	–	3.7	–	–	–	–	–	–	–	–	–	–
1582	**66**	Viridiflorol	2.1	–	–	–	–	–	–	–	–	–	–	–
1591	**67**	Guaiol	–	4.0	–	–	–	–	–	–	–	–	–	–
1598	**68**	*Epi*-globulol	–	–	–	–	–	–	–	13.0	–	–	–	–
1609	**69**	Cubenol	–	–	2.3	–	–	–	–	–	–	–	–	–
1613	**70**	δ-Cadinol	–	1.8	–	–	–	–	–	–	–	–	–	–
1614	**71**	1,6-Humuladien-3-ol	–	–	–	–	–	–	–	12.1	–	–	–	–
1620	**72**	Aristol-9-en-3-ol	–	–	1.8	–	–	–	–	–	–	–	–	–
1623	**73**	Unknown 4	–	4.6	–	–	–	–	–	–	–	–	–	–
1632	**74**	α-Eudesmol	–	5.1	–	–	–	–	–	–	–	–	–	–
1638	**75**	τ-Cadinol	–	1.6	–	–	–	–	–	–	–	–	–	–
1641	**76**	β-Bisabolol	–	–	1.5	–	–	–	–	–	–	–	–	–
1650	**77**	Aromadendrene oxide-1	–	–	6.4	–	–	–	–	–	–	–	–	–
1685	**78**	Eudesmen-7(11)-en-1-ol	–	–	–	–	–	–	–	15.9	–	–	–	–
2075	**79**	8,14-Cedranediol	–	14.5	–	–	–	–	–	–	–	–	–	–
2399	**80**	Unknown 5	–	–	1.2	–	–	–	–	–	–	–	–	–
		Total	100.1	99.9	96.9	100.1	100.0	100.1	100.1	100.2	100.0	99.9	100.0	100.0
		Aliphatic												
		Alkanes, Alkenes	–	–	–	–	–	–	7.6	–	–	–	–	–
		Alcohols	–	–	–	–	–	–	–	4.1	–	–	–	–
		Aldehydes, Ketones	–	1.2	–	–	–	–	–	–	–	–	–	–
		Fatty acids, aliphatic esters	–	–	–	–	–	–	–	1.9	–	–	–	–
		Terpenoids												
		Monoterpene hydrocarbons	63.0	5.1	4.5	–	100.0	68.1	65.1	1.7	60.9	92.0	40.1	55.3

Oxygenated monoterpenes	19.2	34.0	6.5	–	–	32.0	16.6	8.6	–	5.3	59.9	44.7

(continued on next page)

Table 2 (*continued*)

I (seg)[a]	Compound (%)	Species (code)[b]											
		arc 2R	cha 2R	ele 2R	esp 2R	gal 2R	gal 4R	gig 2R	lag 2R	mal 2R	mel 2RS	pub 2R	pub 4R
	Sesquiterpene hydrocarbons	15.8	13.6	7.6	100.1	–	–	10.8	30.0	39.1	2.1		
	Oxygenated sesquiterpenes	2.1	41.4	72.2	–	–	–	–	52.8	–	–	–	–
	Aromatics	–	–	–	–	–	–	–	–	–	0.6	–	–
	Others/unknown	–	4.6	6.1	–	–	–	–	0.8	–	–	–	–

[a] *I*: linear retention index.

[b] For abbreviations of species, see Table 1.

More than 50 essential oils were obtained from the roots of the plants under diverse durations of extraction (0.5, 2, and 4 h). The extraction yields for 2 and 4 h changed significantly with regard to either yield or composition, as exemplified by oils from *A. pubescens* and *A. galeata* (Fig. 1, Table 2). Extractions for only 30 min showed even greater variations: the results were not reproducible, and most of the oils were characterized by a 2,2-dimethyldecane derivative. Thus, statistical analysis was carried out using 2 and 4 h extractions, and involved 80 compounds (75 known). Under these conditions, the yield of the derived essential oils ranged from 0.09% up to 2.38% (% v/w). The best results obtained by the principal component analyses (PCA) were achieved for oils using 2 h of extraction. The GC–MS analysis showed that under these experimental conditions, the largest compound diversity was obtained for the oils extracted from the roots of *A. chamissonis* (22 compounds), *A. elegans* (21 compounds), and *A. lagesiana* (24 compounds).

Except for *A. esperanzae*, camphene (**5**) was identified in all investigated oils, being the major compound in the oils of *A. galeata* and *A. malmeana*. The most abundant compound in the oils differed for other plants (*o*-cymene (**13**) for *A. arcuata*, 8,14-cedranediol (**79**) for *Aristolochia chamissonis*, *trans*-nerolidol (**61**) for *A. elegans*, viridiflorene (**51**) for *A. esperanzae*, α-phellandrene (**12**) for *A. gigantea*, eudesm-7(11)-en-1-ol (**78**) for *A. lagesiana*, limonene (**15**) for *A. melastoma*, and isobornyl acetate (**29**) for *A. pubescens*), which could aid in the differentiation among these species.

The highest concentration of monoterpene hydrocarbons (60.9–100.0%) was observed in the oils of *A. arcuata*, *A. galeata*, *A. gigantea*, *A. malmeana*, and *A. melastoma*, whereas the oil of *A. pubescens* showed a predominance of oxygenated monoterpenes (59.9%) (Table 2). That of *A. esperanzae* showed a predominance of sesquiterpene hydrocarbons (100%), whereas those of *A. chamissonis*, *A. elegans*, and *A. lagesiana* showed a predominance of oxygenated sesquiterpenes (41.4%, 72.2%, and 52.8%, respectively).

PCA were individually applied on datasets of normalized chromatograms from roots oils. These analyses allowed us to draw similarity plots of the corresponding oils to the principal components providing information about the characteristic peaks, which are the most discriminating for the samples observed on the plots (Fig. 1, Supplementary data section: Fig. 2, Table 3). In addition, these results corroborate the previous observation that the duration of extraction is a very important consideration for the identification of these *Aristolochia* species. Moreover, PCA showed that 2 h of hydrodistillation is the best duration when the oils are used for discriminate species. Except for *A. gigantea*, oils characterized by positive score signals for PC3 (principal component 3) correspond to species that showed a predominance of oxygenated compounds (mono- and sesquiterpenes), whereas the negative values correspond to those with a predominance of non-oxygenated

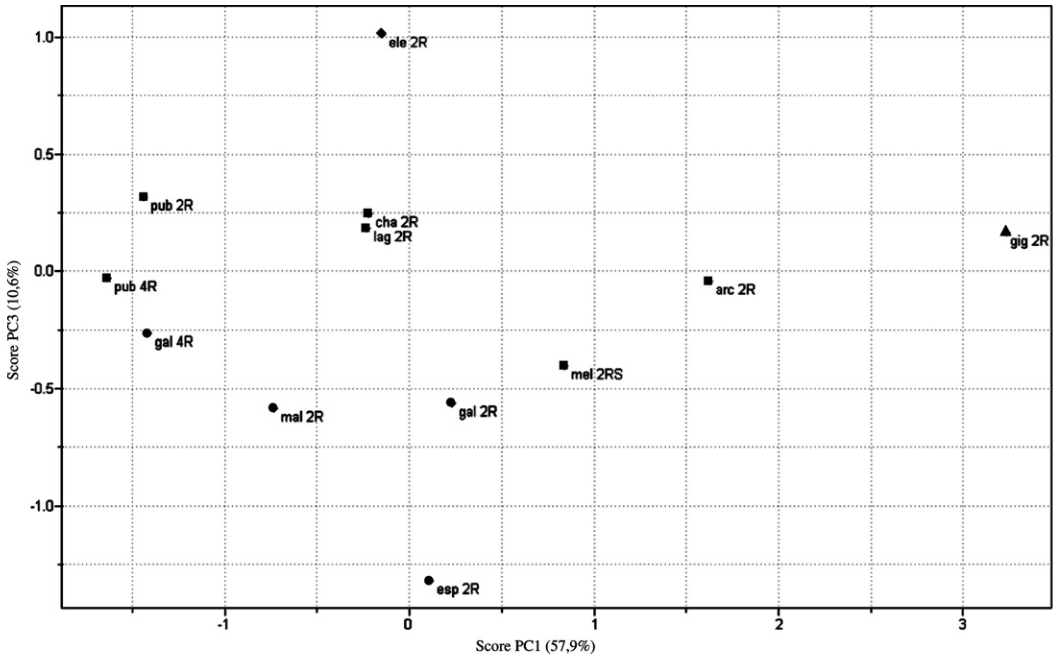

Fig. 1. Principal component analysis (PCA) of chemical constituents of essential oils from the roots of *Aristolochia* species. The principal components (PC1 and PC3) contain ca. 68.5% information (for abbreviations of species, see Table 1).

compounds (Fig. 1, Table 2, and Supplementary data section: Table 3). Four groups were suggested by the plots shown in Fig. 1 and Fig. 2, which were characterized by high loading of PC1 for monoterpenes, such as α-thujene (**2**), and high loading of PC3 for sesquiterpenes, such as germacrene A (**52**), γ-elemene (**39**), and β-gurjunene (**40**). Thus, a group consisting of *A. arcuata*, *A. chamissonis*, *A. lagesiana*, *A. melastoma*, and *A. pubescens* was suggested. *A. gigantea* and *A. elegans* were in two different groups characterized by the highest positive PC1 (3.23) and PC3 (1.01) scores, respectively. *A. esperanzae*, *A. galeata*, and *A. malmeana* may be in a fourth group that was more similar to the first group. If we compare these groups with the morphological classification (subseries) proposed by Hoehne (1942), we can suggest that *A. gigantea* and *A. elegans* should be treated as a separated subseries.

An analysis of the data regarding chemical composition by PCA of the oil described in the literature for *A. elegans* (dried material in Vila et al., 1997) compared to those obtained in this study showed similar values for the roots of *A. elegans*, which suggests that the use of fresh or dried material does not significantly affect the analyses.

3. Conclusions

The GC–MS and exploratory multivariate analyses of the chemical constituents of the essential oils from roots of 10 *Aristolochia* species, which were obtained after 2 h of hydrodistillation, are good tools for helping to identify and also classify *Aristolochia* species into morphological groups. These analyses could aid in the identification of further specimen belonging to *Aristolochia* genus, including those available in the popular markets.

4. Experimental

4.1. Plant material

The plant materials were collected in February 2004, when the vines were in the blooming stage. All species were collected from their natural habitat (Table 1). Voucher specimens of each plant were determined by Dr. L. Capellari Jr. and deposited in the Herbarium of the ESALQ, Piracicaba, SP, Brazil (Table 1).

A. gigantea develops a strong underground system of subterranean stems and roots (tuberous or rhizomatous roots). They were collected separate from the stems (aerial stems) and were called roots (R) for simplicity. *A. melastoma* is a little runner vine, and from this species essential oils were obtained from the roots and stems.

4.2. Analysis of compounds

Roots of each individual (30 g) were cut into small pieces and stored at −8 °C until oil extraction. The essential oils were obtained by hydrodistillation in 250 ml H_2O for 30 min, 2 h, and 4 h, with simultaneous extraction of the distillate with GC-grade *n*-hexane (1 ml), which enabled separation of the essential oil in an ice-cooled oil receiver, in a modified Clevenger apparatus to reduce hydrodistillation over-heating artifacts. The oils were collected with the addition of GC-grade *n*-hexane (1 ml) and dried over anhydr. Na_2SO_4. The solutions were then dried over a sieve, analysed by GC–MS and/or stored at −8°C. The composition of the volatile constituents was established by GC–MS analyses. GC–MS analyses were performed on a Shimadzu GCMS-QP5050A system in EI mode (70 eV) equipped with a split/splitless injector (220 °C), at a split ratio of 1/10, using a VF-1MS fused-silica capillary column (30 m × 0.25 mm i.d.; film thickness: 0.25 μm). The oven temperature was programmed from 60 °C (5 min) to 280 °C at a rate of 4 °C/min and held at this temperature for 10 min. Helium was used as a carrier gas at a flow rate of 0.8 ml/min. The injection volume of each sample was 2 μl. Retention indices for all compounds were determined according to the equation proposed by Van den Dool and Kratz (1963), using *n*-alkanes as standards. Adjusted retention times for each peak were determined by subtracting the retention time of methane from the retention time of each peak. Components were identified based on comparison of their mass spectra with those at the Mass Spectrometry Data Centre (Houss and Road, 1991), Wiley and NBS Libraries (Massada, 1976) and those described by Adams (1995), as well as by comparison of their retention indices with data in the literature (Skaltsa et al., 2001). In several cases, the essential oils were co-injected with compounds previously isolated from these species or purchased standard compounds.

4.2.1. Standard compounds

Aldrich kits containing: 24 standard hydrocarbons/C_5–C_{30}, straight-chain alkanes (Aldrich 29,850-6); 19 fatty acids/C_6–C_{24}, straight-chains (Aldrich 29,851-4); fatty acid methyl esters/C_6–C_{24} straight-chain (Aldrich 29,851-4).

Compounds isolated and identified by spectroscopic methods (mainly by MS, ^1H and ^{13}C NMR) from these plants: spathulenol, β-caryophyllene, caryophyllene oxide, *trans*-nerolidol, vanillin, kobusone, calarene, and 2-oxocalarene.

4.2.2. Statistical analysis

The principal component analysis (PCA) was used as a statistical method to suggest the structure of the set and to analyse the variables in relation to the characteristics being studied. Overall, 80 characteristics (chemical compounds, of which 75 were identified and five were unknown) were analysed in 10 species and 53 oils. In order to reduce the scattering effects and to compare samples, the chromatograms were normalized by reducing areas under each chromatogram to a value 1 (Bertrand and Scotter, 1992). The plots defined by PC1 and PC3 for the 80 characteristics were performed on chromatographic data using the Pirouette® version 3.11(Infometrix Inc., 2003). Fig. 1, as well as Fig. 2 and Table 3 (Supplementary data section) show the results obtained using a original data matrix X (80 × 12) described in Table 2, 80 variables, 12 samples, nine optimal factors, 1st derivative, and the variances of PC1 (21.260) and PC3 (3.880) account for 57.89% and 10.57%, respectively, of total variance.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2007.07.007.

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