QUÍMICA

Submissão: 15/11/2015
Aceite: 06/06/2016
Publicação: 01/12/2016

DOI 10.5935/2446-4775.20160016

Evaluation of bioactive extracts of *Parapiptadenia rigida* and *Piptadenia gonoacantha* using supercritical CO_2

Avaliação de extratos bioativos de *Parapiptadenia rigida* e *Piptadenia gonoacantha* usando CO_2 supercrítico

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Resumo

A avaliação e composição do perfil de extratos das folhas de *Parapiptadenia rigida* e *Piptadenia gonoacantha* usando CO_2 supercrítico em diferentes condições de temperatura (40, 60, 80ºC) e pressão (100, 150, 200 bar) foram estudadas. Os experimentos foram realizados numa unidade contendo, principalmente, um extrator de aço inoxidável e uma válvula micrométrica de amostragem. A melhor pressão para extração foi de 200 bar. Para a espécie *Parapiptadenia rigida*, a melhor temperatura de extração foi 60ºC (0,73%), sendo 80ºC para *Piptadenia gonoacantha* (1,13%). Benzenosulfonamida esteve presente em todos os extratos e confere aos extratos uma característica interessante devido à atividade antifúngica contra patógenos de plantas.

Palavras-chave: *Parapiptadenia rígida*. *Piptadenia gonoacantha*. Fluido supercrítico. Extratos. Benzenosulfonamida. Solubilidade.

Abstract

The evaluation and composition of the leaves extracts profile of *Parapiptadenia rigida* and *Piptadenia gonoacantha* using supercritical CO_2 in different conditions of temperature (40, 60, 80ºC) and pressure (100, 150, 200 bar) were studied. Experiments were performed in a unit mainly containing a stainless steel extractor and a micrometer valve for sampling. The better pressure for the extraction was 200 bar. The best temperature for extraction was 60ºC for *Parapiptadenia rigida* (0.73%), and 80ºC for *Piptadenia gonoacantha* (1.13%). Benzene sulfonamide was presented in all extracts and makes an interesting characteristic of the extracts due to the antifungal activity against plant pathogens.

Keywords: *Parapiptadenia rigida*. *Piptadenia gonoacantha*. Supercritical fluid. Extracts. Benzenesulfonamide. Solubility.

Introduction

The *Piptadenia* genus belongs to Mimosoideae (Leguminosae) family and contains about 133 tropical species commonly found in Brazil and South America. The *Piptadenia* species are known, in Brazil, as “angico” and in Argentina and Paraguay, as “cebil”. The plants of this genus have been used in the tannery industry due to its bark that is rich in tannins. Also, they helped the forests recovery because they can growth in poor and degraded soil (CORREA, 1984; LORENZI, 2002). There are 15 names of scientific species for *Parapiptadenia* genus and among these 6 names of species are accepted (MORIN, 2016).

The *Parapiptadenia rigida* (Benth) Brenan, and the former synonym *Piptadenia rigida*, species is a tree that reaches between 20 and 30 m in height. It is popularly known as angico red and is often found in the Atlantic Forest. The wood is heavy, very hard, and inelastic and has great durability under natural conditions. It can be used in carpentry, joinery, and for firewood and coal. The plant has ornamental features that recommend it for the landscaping in general (LORENZI, 2002).

The *Piptadenia gonoacantha* (Mart.) J.F. Macbr. specimens have characteristics of small spines. It can reach up to 20 m high and it can be found in Atlantic Forest, in Southeast and South regions of Brazil, including the state of Mato Grosso do Sul. Although it is an under appreciated species due to its blade-shaped spines, it is important in reflorestation. The wood is moderately heavy, hard to cut and measured resistance to pest attack. It is used in furniture, interior finishes, making toys, packaging, brains and door panels, wood and coal (LUNZ, 2004; BEDETTI, MODOLO; and DOS SANTOS ISAIAS, 2014).

Although the species *P. rigida e P. gonoacantha* have been used in industry, there are not works in literature regarding the components present in this species able to withstand the attack of *Scolytidae* (species of caterpillar).

One of extraction processes of essential oils that have prominent among other industrial processes is the supercritical fluid extraction. Williams (1981) defined it as a single process where solvents are employed in operational conditions above their critical points to extract soluble components of a mixture.

One of the advantages of supercritical fluid extraction is the possibility of easy recovery of solvent after the extraction process, just by adjusting the temperature and pressure, and the solvent could be continually recycled (STAHL, QUIRIN; and GERARD, 1987).

This process, in which the products extracted have a high purity due to the absence of organic solvent, is generally fast and does not cause environmental damage when using carbon dioxide as supercritical solvent. A solvent widely used in supercritical fluid extraction is carbon dioxide. It has low critical temperature (Tc=31.04 °C) and pressure (Pc=73.8 bar), allowing the extraction of thermolabile products without changing the properties of the extracts. Also, it is inert, offers no risk of secondary reactions, harmless, non-explosive, non-polluting, and non-toxic. The process of separation of solvent and extracted product is simple, because CO_2 is a gas at ambient temperature and pressure and has low cost (HERRERO, CIFUENTES; and IBAÑEZ, 2006).

The extraction is much easier because the properties of the fluids in the supercritical state, such as compressibility similar to a gas, density similar of a liquid, ability to dissolve solutes like a liquid, viscosity like of gas and values of diffusivity intermediate

between gas and liquid varying with density. The properties of a fluid in a critical condition are very sensitive to small changes in pressure, temperature and, consequently, in density (PEREIRA; and MEIRELES, 2010).

The objective of this research was to evaluate the efficiency of the extraction of leaves of *Parapiptadenia rigida* and *Piptadenia gonoacantha* using CO_2 in a supercritical state in different operational conditions of temperature and pressure and the composition profile related to bioactive components.

Materials and methods

Materials

The leaves samples of *Parapiptadenia rigida* and *Piptadenia gonoacantha* were collected in the Forest Institute (FI) of the Federal Rural University of Rio de Janeiro by Professor Acácio Geraldo de Carvalho and Professor José Aguiar Sobrinho. A voucher specimen (RBR 6939) and (RBR 21438), respectively, has been deposited at RBR Herbarium, Biology Institute (Universidade Federal Rural do Rio de Janeiro). After collection, the leaves were dried at ambient temperature and stored in a well-ventilated location.

Carbon dioxide with a minimum purity of 99.9% (White Martins, Rio de Janeiro) was used as solvent in the supercritical extraction.

Experimental methodology

Hydrodistillation

The hydrodistillation extraction of the leaves was done with 348 g of *P. rigida* and 191 g of *P. gonoacantha* using 1000 mL of distilled water in a period of three hours in the boiling point of water.

At the end of extraction, the mixture of extracted oil and water was submitted to an extraction with dichloromethane (3 x 50 mL) in a separation funnel. The organic fraction was treated with anhydrous sodium sulphate in excess. The extract fraction mass obtained was 17 mg for *P. rigida* and10 mg for *P. gonoacantha*. After that, the solutions were filtrated and concentrated.

Supercritical fluid extraction

The extraction of *P. rigida* and *P. gonoacantha* with supercritical carbon dioxide was performed at Applied Thermodynamics and Biofuel Laboratory (DEQ/UFRRJ). The experimental apparatus consists of a stainless steel 316S extractor with 42 mL of capacity. The extractor contains two canvas of 260 meshes to prevent the entrainment of material. A high-pressure pump (Palm model G100), specific for pumping CO_2, was responsible for feeding the solvent into the extractor. A thermostatic bath (model Haake K15) was coupled to the extractor to control the temperature and a manometer was installed on line for controlling the pressure.

About 4.5 g of vegetable material was fed into the extractor and then the thermostatic bath was turned on, reaching the desired temperature. The experiments were performed under the operational conditions of 100, 150 and 200 bar for pressure and 40, 60 and 80°C for temperature. The maximum time of extraction was 80 min, when it was observed the saturation of the extraction curve. Sampling occurred at a maximum flow of 16.45 mL/min, controlled by a rotameter, and was performed at each 5 min using the technique of decompression through the valve. With the pressure reduction, the sample is recovered in a polypropylene tube. The yield was calculated as a ratio between the extracted oil mass and the initial plant mass fed into the extractor.

Chromatographic analysis

All samples obtained from hydrodistillation and supercritical fluid extraction, were analyzed by gas

chromatograph coupled to a mass spectrometer (GC-MS) using a Shimadzu equipment (model QP 2010), with helium as a carrier gas. A Factor Four/VF-5 ms capillary column with 30 m of length, 0.25 mm of inner diameter and 0.25 µm of thickness were used. The flow rate of helium was 1 mL/min and the temperature programmer used was an increasing step of 10ºC/min from 100 to 290 ºC. The injector temperature was 250ºC, the detector temperature was 310ºC and split was the injection mode at ratio 1:20 with an injection volume of 2 µL at 10% in dichloromethane. The mass spectra were produced through electronic impact (70 eV). The database in the NIST08 library was used to compare and identify the components.

Results and discussion

Analysis of the experimental operational conditions

For determination of yield the processes were performed in triplicate and the average results are shown in **TABLE 1**, for the two species.

TABLE 1. Yields of the extract of *Parapiptadenia rigida* and *Piptadenia gonoacantha* with supercritical CO_2.

Pressure (Bar)	Temperature (°C)		
	40	60	80
Parapiptadenia rigida			
100	0.44 ± 0.11*	0.40 ± 0.07	0.54 ± 0.12
150	0.60 ± 0.17	0.57 ± 0.03	0.79 ± 0.18
200	0.64 ± 0.21	0.73 ± 0.12	0.75 ± 0.05
Piptadenia gonoacantha			
100	0.31 ± 0.21	0.42 ± 0.13	0.65 ± 0.04
150	0.68 ± 0.22	0.56 ± 0.10	1.05 ± 0.13
200	0.85 ± 0.24	0.67 ± 0.08	1.31 ± 0.15

* standard deviation

According to the results, the best extraction conditions were obtained at 60 °C and under pressure of 200 bar with the yield of 0.73%, for *P. rigida* leaves. For *Piptadenia gonoacantha*, the best result was obtained at 80 ºC and 200 bar (1.13%).

For the two species, at 60ºC, the yield decreases when compared the experimental results at constant pressure. This behavior has been observed for many raw materials studied involving plants and seeds, like macadamia nuts (SILVA et al., 2008).

In all experiments, the best yield results were obtained at higher pressures maintaining the temperature constant. The increase in pressure, at constant temperature, caused an increase in the density of the solvent. Moreover, the increase in the solvency power results in an increase in yield.

The accumulated yield of the extracts in function of the extraction time, for all the pressures studied, can be seen in **FIGURES 1** and **2**, respectively for *P. rigida* at 60ºC and for *P. gonoacantha* at 80ºC.

In order to evaluate the effect of pressure and temperature in the efficiency of the oil extraction, statistic tests were performed using the triplicate measurements obtained for each operational condition. The main parameters of the analysis of variance (ANOVA) *P. gonoacantha* and *P. rigida* are shown in **TABLES 2** and **3**, respectively.

TABLE 2. Analysis of variance of experimental data of *Parapiptadenia rigida* oil extraction ($F_{critical}$ = 3.1789).

T	P	*CV*	F_{calc}	p-valor
40	100	0.024	41.51	2×10^{-11}
	150			
	200			
60	100	0.029	9.13	0.000408
	150			
	200			
80	100	0.029	7.38	0.00152
	150			
	200			

T is the temperature, P is the pressure, *and CV* is the variation coefficient, while F_{calc} and $F_{critical}$ are the calculated and critical *F*-values, respectively.

TABLE 3. Analysis of variance of experimental data of *Piptadenia gonoacantha* oil extraction ($F_{critical}$ = 3.1789).

T	P	*CV*	F_{calc}	p-valor
40	100	2.64×10^{-7}	5.41	0.007212
	150			
	200			
60	100	1.00×10^{-6}	9.35	0.000326
	150			
	200			
80	100	7.82×10^{-6}	8.11	0.000832
	150			
	200			

T is the temperature, P is the pressure, *and CV* is the variation coefficient, while F_{calc} and $F_{critical}$ are the calculated and critical *F*-values, respectively.

Based on the ANOVA results exhibited in **TABLES 2** and **3**, the low values obtained for standard deviations (less than 3%), as well as for all coefficient of variations (less than 5%), indicate the good reproducibility of the experimental procedure, which means that the results presented in this work are reliable.

It can be observed that, when the pressure increased, the efficiency also increased at constant temperature, for the two plants studied. This feature was already expected since as the pressure increases the carbon dioxide density increases as well.

Considering the experimental apparatus used, and the operational conditions investigated in this work, the calculated *F*-values and p-values indicate that the values of temperature and pressure were effective, meaning that the combination of them produced a high content oil of bioactive components.

FIGURE 1. Extraction curve of *Parapiptadenia rigida* at 60°C and at pressures of 100, 150 and 200 bar.

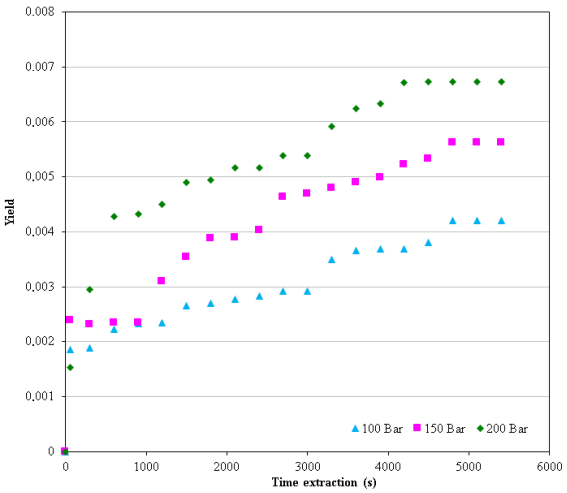
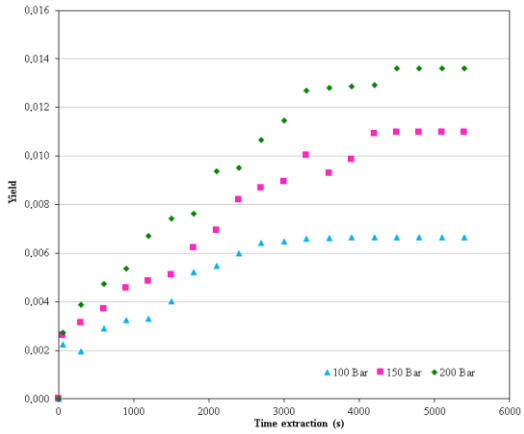


FIGURE 2. Extraction curve of *Piptadenia gonoacantha* at 80°C and at pressures of 100, 150 and 200 bar.



For results at 60 and 80ºC, the yield increased with the increase in temperature, for *P. rigida* extraction. This occurred because the vapor pressure of the solutes increased, favoring the extraction process.

At 80ºC, it was observed a *crossover* behavior between the curves of 100 and 200 bar, showing the effects of density and vapor pressure of the solute in the extraction efficiency.

TABLE 4. show the experimental results of solubility of the extract of *P. rigida* and *P. gonoacantha*. The results show that the solubility ranged from 0.0109 to 0.0357 (g extract/L CO_2) for the first one and from 0.0095 to 0.0422 (g extract/L CO_2) for the *P. gonoacantha*. It was observed that the solubility increases with increasing pressure, at constant temperature, except at 80°C at pressures of 150 and 200 bar, for the *P. rigida*. The solubility behavior was in an opposite way when compared with others results (STAHL, QUIRIN; and GERARD, 1987). The increase in temperature, at constant pressure, leads to an increase in solubility, except at 200 bar. This may be related to the competitive effects of the vapor pressure of the solute and solvent density. The combined effect of both will determine the behavior of the variation of solubility with the operational conditions.

TABLE 4. Experimental solubilities (g extract/L CO_2) of extracts of *Parapiptadenia rigida* and *Piptadenia gonoacantha* with supercritical CO_2.

Pressure (bar)	Temperature (ºC)		
	40	60	80
Parapiptadenia rigida			
100	0.0109	0.0150	0.0161
150	0.0179	0.0214	0.0259
200	0.0357	0.0259	0.0211
Piptadenia gonoacantha			
100	0.0095	0.0140	0.0204
150	0.0209	0.0181	0.0335
200	0.0261	0.0205	0.0422

Identification of bioactive substances

By GC-MS, **TABLES 5** and **6** show the identified components and the relative integrated areas of each component, in percentage, in the extracts obtained from the different operational conditions, for *P. rigida* and *P. gonoacantha*, respectively, using supercritical fluid.

From the TABLES it can be seen that the extracts are characterized by complex mixtures. The majority components identified in the *P. rigida* extract, using carbon dioxide, according to the peak area, are: 4,6-dimethyl-dodecane, N-butyl-benzene-sulfonamide (NBBS), Bis(2-ethyl-hexyl) hexadecanoate, 2-hydroxy-1-(hydroxy-methyl)ethyl hexadecanoate, 2,3-dihydroxy-propyl octadecanoate and sitostenone.

In the extract of *P. gonoacantha*, there were identified as majorities the NBBS, 2-hydroxy-1-(hydroxy-methyl) ethyl hexadecanoate, *N*-(2-ethoxy-phenyl)-*N*'-(1-phenyl-ethyl)-oxalamide, 2,3-dihydroxy-propyl octadecanoate, squalene, nonacosane, 1,30-triacontanediol and 1-heptacosanol.

The high content of NBBS in the *P. rigida* extract makes an interesting characteristic of the oil due to the antifungal activity against plant pathogens. The NBBS isolated from *Pseudomonas sp*. AB2, an antifungal bacteria family, showed ED_{50} (effective dosage) of 33, 41, 73 and 102 ppm against *Rhizoctonia solani, Phytophthora capsici, Pythium ultimum* and *Botrytis cinerea*, respectively. This effect generated by NBBS can effort that the species *P. rigida* produces this metabolite to defend against these types of plant pathogens (KIM et al., 2000).

The NBBS was also extracted from *Angelica sinensis* (Oliv.) Diels (DENG et al., 2006). The same component isolated from the kernel of *Prunus africana* (Hook.f.) Kalkman (synonym *Pygeum africanum* Hook.f.) presented an elevated antiandrogenic activity, inhibiting the cells growing responsible for the prostate cancer, due to the fact that they do not accept the treatment with hydroxyflutamide (PAPAIOANNOU et al., 2010; ROELL; and BANIAHMAD, 2011).

In this study, it was observed that the NBBS content decreased with the pressure increasing when compared from 40 to 60ºC. The total yield using supercritical fluid increases with the pressure increasing. For the temperature of 80°C, the NBBS content and the yield increased with the increase in pressure. In the *P. gonoacantha* extract, the main component is the NBBS derivative with a molecular weight of 290 g/mol. It was observed in the extracts obtained at 150 bar and 60°C, with the highest concentration (53.70%). At 40°C, the highest content of NBBS derivative was found in the oil extracted at 100 bar; it was observed a decreasing at 150 bar and a slight increasing at 200 bar. At 80°C, only at 150 bar it was observed the presence of NBBS derivative in the extracts (see **TABLES 5** and **6**). For *P. rigida*, higher contents of NBBS derivative were obtained at 80ºC and 200 bar.

The NBBS was also found in other natural raw materials, as in the orange pomace oil extracted using supercritical fluids, with concentrations of 88.8 and 91.4% at 40°C and 200 bar and at 40°C and 300 bar, respectively (BENELLI et al., 2010). The NBBS derivative was also observed in the peach almond oil, extracted using supercritical fluid, with the concentrations of 97.0 and 98.8% at 50°C and 300 bar and at 40°C and 300 bar, respectively (MEZZOMO, MANTÍNEZ; and FERREIRA , 2009).

The compositions of *P. rigida* and *P. gonoacantha* extracts, respectively, by hydrodistillation, were apparently different, as showed in **TABLE 7**. The majority components present in the extracts using supercritical fluid were not observed in the extracts obtained by hydrodistillation, with the exception of hexadecanoic acid. The composition of the oil obtained by hydrodistillation is characterized by the presence of low molecular weight compounds commonly present in essential oils, like *m*-cumenol, *cis*-pulegol and ionones, beyond volatile compounds such as aldehydes, ketones, alcohols, acids and sesquiterpene.

The majority of the components of *P. rigida* oil were the hexadecanoic acid, phytol and the octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propanoate and the majorities of *P. gonoacantha* oil were the hexadecanoic acid, phytol, and the linolenic acid.

Acknowledgments

To CNPq, CAPES and FAPERJ, for financial support.

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TABLE 5. Chemical composition of *Parapiptadenia rigida* extracts using supercritical fluid

Compounds	RT (min)	Relative area (%)								
		Extraction conditions[a]								
		1A	1B	1C	2A	2B	2C	3A	3B	3C
5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-,(*R*)-2(4*H*)-benzofuranone	9.68	-	-	-	-	-	-	-	-	1.05
3-Methyl-5-propylnonane	10.23	-	-	-	-	1.36	0.89	-	-	-
2,6,10-Trimethyl-dodecane,	10.78	-	-	-	1.58	1.54	1.38	-	-	-
Nonanedioic acid	10.90	-	-	-	-	-	-	-	-	1.57
4,6-Dimethyl-dodecane	11.45	-	-	-	10.43	6.49	6.08	-	-	-
10-Methyl-nonadecane	11.92	-	-	-	1.06	0.92	0.93	-	-	-
Isotridecanol	12.08	-	-	-	-	-	-	-	0.91	-
4-(2,4-Dimethylcyclohex-3-enyl)but-3-en-2-one	12.55	-	-	-	-	-	-	-	0.87	-
n-Octadecane	12.59	-	-	-	5.00	3.69	3.48	-	-	-
N-Butyl-benzenesulfonamide	12.66	19.92	17.24	-	10.79	10.86	7.93	2.84	13.86	22.53
N-Butyl-benzenesulfonamide derivative (m/z 290)	12.96	-	-	16.89	-	-	-	-	-	-
Hexahydrofarnesyl acetone	13.07	-	1.24	-	3.28	2.55	1.76	-	1.50	2.35
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	13.89	-	-	-	-	-	-	-	0.86	-
Phytol	13.93	-	-	0.81	-	-	-	-	-	1.14
n-Hexadecanoic acid	14.35	-	-	5.74	-	-	-	4.33	4.16	7.48
2-Hexyl-1-decanol	14.73	-	-	-	-	-	-	-	-	0.95
cis-13-Octadecenal	16.17	-	-	0.96	-	-	-	-	-	1.16
n-Octadecanoic acid	16.35	-	-	5.15	-	-	-	3.45	2.73	5.44
Ethyl 9-hexadecenoate	16.61	-	-	1.12	-	-	-	-	-	1.20

Linoleic acid	16.96	-	-	-	-	-	-	-	-	0.84
4,8,12,16-Tetramethylheptadecan-4-olide	18.13	-	-	1.63	-	0.87	0.65	-	-	-
Bis(2-ethylhexyl) hexanedionate	18.38	3.68	4.89	-	12.03	5.40	3.60	-	-	-
Eicosyl 2-ethylbutyrate	19.25	-	-	-	-	-	-	-	1.23	-
2-Hydroxy-1-(hydroxymethyl)ethyl hexadecanoate	19.54	-	-	4.94	-	-	-	27.93	15.24	4.14
2,3-Bis[(trimethylsilyl)oxy]propyl eicosanoate	20.78	-	-	-	-	-	-	-	-	1.65
2-Deoxy-bis(thiononyl)-dithioacetal *d*-ribose	20.99	-	-	-	-	-	-	1.33	1.50	-
2,3-Dihydroxypropyl octadecanoate	21.33	-	-	3.36	-	-	-	24.89	13.74	2.61
Nonacosane	23.01	-	-	-	1.24	2.80	2.19	-	-	-
Triacontane	25.85	0.63	2.13	0.75	0.67	3.30	2.39	-	-	-
Stigmasterol acetate	26.04	-	-	2.08	-	-	-	1.61	1.12	2.34
1,30 Triacontanediol	28.88	2.16	3.00	5.49	-	4.06	4.19	7.81	3.38	4.94
1-Heptacosanol	30.53	-	-	3.76	-	-	-	5.65	-	1.44
Sitosterol	31.24	2.01	3.94	1.07	-	2.16	2.33	-	-	-
4,22-Stigmastadiene-3-one	33.15	8.78	6.84	4.09	3.45	5.15	4.81	-	1.19	3.87
Lupeol	34.04	3.29	2.72	1.21	-	-	-	-	-	1.44
Sitostenone	35.25	36.22	29.63	14.95	15.18	20.27	19.60	-	6.98	13.98
Friedeline	38.75	4.51	6.94	5.35	-	5.59	4.05	-	-	3.94
4-*tert*-Butylcalix[4]arene	39.38	-	-	1.70	-	-	-	6.22	4.27	1.65
Stigmastane-3,6-dione	41.33	-	-	2.18	-	-	-	-	-	-

Extraction conditions[a]: 1A (40°C/100 bar); 1B (40°C/150 bar); 1C (40°C/200 bar); 2A (60°C/100 bar); 2B (60°C/150 bar); 2C (60°C/200 bar); 3A (80°C/100 bar); 3B (80°C/150 bar); 3C (80°C/200 bar).

TABLE 6. Chemical composition of *Piptadenia gonoacantha* extracts using supercritical fluid

Compounds	RT (min)	Relative area (%)								
		Extraction conditions[a]								
		1A	1B	1C	2A	2B	2C	3A	3B	3C
n-Tetradecane	11.45	-	-	-	0.85	-	-	-	-	-
4-Methyl-tetradecane	12.11	-	-	-	1.20	-	-	-	-	-
n-Octadecane	12.58	-	-	-	1.48	-	-	-	-	-
n-Methyl-benzenesulfonamide	12.63	-	0.80	1.34	-	-	-	-	-	-
n-Butyl-benzenesulfonamide	12.66	20.38	-	-	-	-	-	-	1.72	-
n-Butyl-benzenesulfonamide derivative (m/z 290)	12.96	-	-	-	35.44	53.7	47.24	-	-	-
Hexahydrofarnesyl acetone	13.08	0.82	-	-	2.97			-	-	-
n-Nonadecane	13.96	-	-	-	2.04			-	-	-
n-Hexadecanoic acid	14.33	-	0.62	-	2.82	6.89	5.42	0.62	0.54	-
cis-9-Hexadecenal	16.20	-	-	-	-	2.19	1.34	-	-	-
n-Octadecanoic acid	16.36	-	-	-	1.54	3.03	2.96	-	-	-
n-Eicosane	16.66	2.04	1.20	5.64	-	-	-	-	-	-
2,7,10-Trimethyldodecane	16.68	0.78	0.52	-	-	-	-	-	-	-
2,6,10,15-Tetramethylheptadecane	17.58	0.65	0.39	-	-	-	-	-	-	-
Heptadecane	17.64	-	2.34	-	-	-	-	-	-	-
n-Heneicosane	17.83	-	-	0.76	-	-	-	-	-	-
4,8,12,16-Tetramethylheptadecan-4-olide	18.13	0.67	0.71	1.23	-	-	-	0.98	0.44	-
Bis(2-ethylhexyl) hexanedionate	18.40	2.83	-	1.45	-	-	-	3.22	3.01	-
n-Docosane	18.45	-	-	-	-	-	-	1.49	0.85	-
n-Tricosane	19.29	-	-	-	-	-	-	1.75	1.27	-

2-Ethylhexyl octadecyl oxalate	19.31	3.26	1.45	4.23	-	-	-	-	-	-
2-Hydroxy-1-(hydroxymethyl)ethyl hexadecanoate	19.56	-	-	-	13.86	4.37	2.68	-	-	-
Oxalic acid diamide *N,N'*-bis(2-ethylphenyl)-	19.61	-	0.79	-	-	-	-	-	-	-
N-(2-Ethoxy-phenyl)-*N'*-(1-phenyl-ethyl)-oxalamide	20.66	6.74	12.48	4.25	0.90	1.86	1.86	-	4.10	7.19
n-Tetracosane	20.95	4.21	5.07	9.04	-	-	-	1.91	2.06	1.35
2,3-Dihydroxypropyl octadecanoate	21.36	-	-	-	12.37	2.90	2.38	-	-	-
n-Pentacosane	21.90	-	-	-	-	-	-	1.42	2.68	-
Squalene	22.13	-	-	-	-	-	-	-	-	22.91
Nonacosane	23.03	11.67	14.25	11.68	-	1.80	1.95	3.49	16.93	13.49
2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	23.55	-	-	-	-	-	-	-	-	1.61
n-Dotriacontane	24.32	2.94	3.90	2.62	-	-	-	0.68	3.25	2.42
n-Triacontane	25.84	7.70	10.14	6.25	-	1.33	1.41	1.32	8.29	6.02
1-Heptatriacotanol	26.39	-	-	-	-	-	-	1.38	-	1.28
Vitamin E	26.75	-	-	-	-	-	0.83	-	-	2.00
n-Tritetracontane	27.76	-	0.89	0.80	-	-	-	-	0.79	-
Octadecanal	28.90	10.31	9.22	8.38	2.60	3.17	4.49	13.98	12.86	5.25
1-Heptacosanol	30.51	-	-	-	-	2.61	5.05	53.98	-	-
2-Nonadecanone	30.82	-	4.99	2.19	-	-	-	-	-	1.49
Sitosterol	31.30	-	-	-	-	2.12	1.90	-	-	-
Lupeone	33.19	-	-	-	-	3.17	2.46	-	-	-
Lupeol	34.04	-	-	-	-	2.17	1.82	-	-	-
Pentadecanal	34.61	2.10	3.40	4.62	-	-	-	2.52	2.09	-
1,2-Epoxy-3-(hexadecyloxy)-propane	37.61	-	-	-	-	-	-	-	-	2.50

Extraction conditions[a]: 1A (40°C/100 bar); 1B (40°C/150 bar); 1C (40°C/200 bar); 2A (60°C/100 bar); 2B (60°C/150 bar); 2C (60°C/200 bar); 3A (80°C/100 bar); 3B (80°C/150 bar); 3C (80°C/200 bar).

TABLE 7. Chemical composition of *Parapiptadenia rigida* and *Piptadenia gonoacantha* extracts by hydrodistillation

Compounds	RT (min)*	%*	RT (min)**	% **
Benzeneacetaldehyde	3.454	3.02	-	-
2-pyridineacetic acid hexahydro-1-methyl	-	-	4.294	1.18
m-cumenol	-	-	5.356	0.82
cis-pulegol	-	-	5.543	0.66
Nonanoic acid	-	-	5.876	1.55
Geranic acid	-	-	7.136	5.51
Decanoic acid	-	-	7.233	1.13
m-Benzyloxybenzaldehyde	7.791	3.02	-	-
trans-alpha-Ionone	-	-	8.119	0.99
cis-geranylacetone	-	-	8.352	0.69
2-Phenyl-1,3-dioxan-5-yl-(9E,12E,15E)-9,12,15-octadecatrienoate	8.498	1.44	-	-
7-Methoxy-2,2,4,8-tetramethyltriciclo-undecane	-	-	8.534	5.05
trans-β-Ionone	-	-	8.867	1.67
trans-Ionone epoxide	-	-	9.155	6.73
2,6-Bis(1,1-dimethylethyl)-4-methyl-phenol,	9.29	1.69	9.29	1.20
Dicyclohexyl methanone,	-	-	9.37	0.70
5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	-	-	9.45	0.53
5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-(R)-2(4H)-benzofuranone,	9.679	1.69	-	-
Cinnamic acid m-methyl	-	-	9.714	4.24
Dodecanoic acid	9.746	2.27	10.144	1.52

5,6,7,7a-Tetrahydro-4,4,7a-trimethyl 2(4H)-benzofuranone,	-	-	9.806	3.77
Tetradecanoic acid	12.131	1.45	12.509	1.54
1-Tetradecene	-	-	12.757	1.63
Hexahydrofarnesyl acetone	13.066	2.59	13.861	0.97
1-Nonadecene	-	-	13.069	1.65
Methyl pentadecanoate	13.951	1.79	-	-
Isophytol	-	-	14.179	0.99
Hexadecanoic acid	14.361	9.89	14.441	13.05
Ethyl hexadecanoate	-	-	14.66	2.08
n-Nonadecanol-1	-	-	15.621	1.07
Linoleic acid	15.696	1.65	15.7	0.67
Phytol	15.858	13.44	15.872	7.90
Octadecanoic acid	16.338	1.89	-	-
1,3-Dioxolane, 2-(phenylmethyl)-	16.409	1.56	-	-
Pregna-4,16-diene-3,20-dione	18.761	2.00	-	-
Eicosane	19.289	1.47	-	-
Heneicosane	20.947	1.62	-	-
Tetracosane	23.001	2.47	-	-
4-*tert*-Butylcalix[4]arene	23.36	3.88	-	-
Octadecyl 3-(3,5-ditert-butyl-4-hydroxyphenyl) propanoate	24.739	12.84	-	-

*Parapiptadenia rigida
** Piptadenia gonoacantha