Quim. Nova, Vol. 35, No. 11, 2165-2168, 2012

Artigo

TRITERPENES FROM *Minquartia guianensis* (Olacaceae) AND *IN VITRO* ANTIMALARIAL ACTIVITY[#]

Lorena Mayara de Carvalho Cursino e Cecilia Veronica Nunez*
Laboratório de Bioprospecção e Biotecnologia, Instituto Nacional de Pesquisas da Amazônia, Av. André Araujo, 2936, 69060-001 Manaus – AM, Brasil
Renata Cristina de Paula e Maria Fernanda Alves do Nascimento
Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Av. Pres. Antonio Carlos, 6627, 31270-901 Belo Horizonte – MG, Brasil
Pierre Alexandre dos Santos
Faculdade de Ciências Farmacêuticas, Universidade Federal do Amazonas, Rua Alexandre Amorim, 330, 69010-300 Manaus – AM, Brasil

Recebido em 24/5/12; aceito em 21/9/12; publicado na web em 9/11/12

Minquartia guianensis, popularly known as acariquara, was phytochemically investigated. The following triterpenes were isolated from the dichloromethane extract of leaves: lupen-3-one (**1**), taraxer-3-one (**2**) and oleanolic acid (**3**). The dichloromethane extract of branches yielded the triterpene 3β-methoxy-lup-20(29)-ene (**4**). The chemical structures were characterized by NMR data. Plant extracts, substance **3**, squalene (**5**) and taraxerol (**6**), (**5** and **6** previously isolated), were evaluated by *in vitro* assay against chloroquine resistant *Plasmodium falciparum*. The dichloromethane extract of leaves and the three triterpenes assayed have shown partial activity. Thus, these results demonstrated that new potential antimalarial natural products can be found even in partially active extracts.

Keywords: *Minquartia guianensis*; triterpenes; *Plasmodium falciparum.*

INTRODUCTION

Minquartia guianensis Aubl. popularly known as acariquara, acari, arariúba, among others,[1] occurs in the Amazon region, Nicaragua, Panama and Costa Rica.[2] It belongs to the Olacaceae which has 24 genus and 150 species.[3] From this family, several chemical classes have been isolated such as alkaloids,[4-9] proanthocyanidins,[10,11] chromones,[12] steroids,[13] flavonoids,[12,14] isoprenoids,[12] polyisoprenoids,[15] diterpenes,[16,17] sesquiterpenes,[9] triterpenes,[10,13,18-20] and glycerol-derivatives.[12]

Previous phytochemical studies on *M. guianensis* reported the isolation of triterpenes,[18,20] xanthone and minquartynoic acid.[20,21] The minquartynoic acid isolated from bark chloroform extract showed activity against *Plasmodium falciparum* and *Leishmania major*.[22]

This paper describes the isolation and identification of four triterpenes which have been isolated from dichloromethane extracts of leaves and branches as well as reporting the antimalarial activity evaluation of the plant extracts and three triterpenes, two of which were previously isolated from this species.[18]

RESULTS AND DISCUSSION

Chemistry

Fractionation of leaf and branch extracts yielded four known triterpenes. Triterpenes **1-3** were purified from the dichloromethane extract of leaves while triterpene **4** was isolated from the dichloromethane extract of branches (Figure 1). The identifications were carried out by ^{1}H and ^{13}C (APT and DEPT) NMR spectral data analysis, including 2-D NMR experiments (COSY, HSQC and HMBC).

Compounds **1** and **2** have shown yellow spots in TLC with anisaldehyde sulphuric acid reagent. The ^{1}H-NMR spectrum exhibited seven more intense signals between 0.7 and 1.7 ppm suggesting the presence of triterpene. Two intense signals were observed at δ_H 4.55 (1H, *dd, J* = 2.5 and 1.0 Hz) and 4.67 (1H, *d, J* = 2.5 Hz) indicating the presence of the lupane skeleton. Another signal with low intensity was observed at δ_H 5.54 (1H, *dd*, *J* = 8.0 and 3.0 Hz) indicating signals of the taraxerane skeleton.

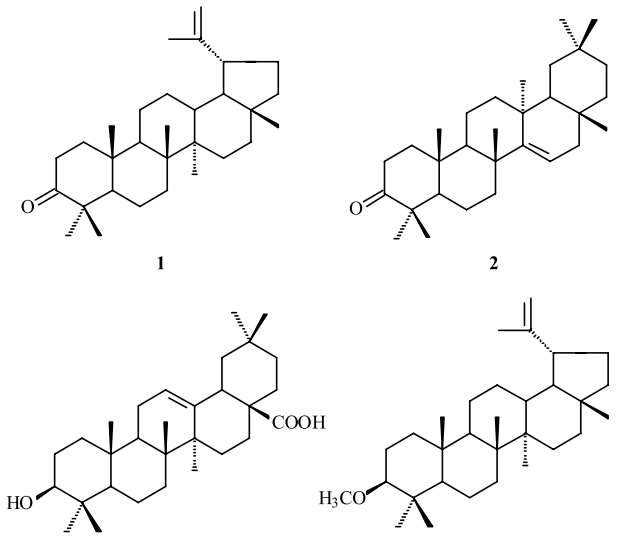


Figure 1. *Chemical structures of the isolated triterpenes from Minquartia guianensis*

The composition of the triterpene mixture was determined by the difference in intensity of signals by ^{13}C-NMR data spectra analysis.[23,24] The ^{13}C-NMR spectrum showed two intense signals at δ_C 151.0 and δ_C 109.6, corresponding to a quaternary carbon and a methylene carbon of the double bond, respectively, confirmed by DEPT spectra. Another signal at δ_C 218.3 was attributed to a carbonyl group. These signals confirmed the presence of the lupane skeleton, with a carbonyl group

*e-mail: cecilia@inpa.gov.br
[#]Artigo em homenagem ao Prof. Otto R. Gottlieb (31/8/1920-19/6/2011)

at position 3. The ^{13}C-NMR spectra also showed two less intense signals at δ_C 157.8 and 117.4, corresponding to a quaternary carbon and a methyne carbon of the taraxerane double bond, respectively, also confirmed by DEPT spectra.

Other data were compared with those available in literature[25,26] and allowed the identification of **1** and **2** as the two triterpenes lupen-3-one and taraxer-3-one, respectively (Table 1). This is the first report of taraxer-3-one occurrence in Olacaceae while lupen-3-one was only previously identified in Olacaceae from leaves of *M. guianensis*.[18]

Table 1. ^{13}C NMR (125 MHz) data of compounds **1-4** in $CDCl_3$

Carbon	δ_C 1	δ_C 2	δ_C 3	δ_C 4
1	40.2	38.6	38.4	40.0
2	34.4	34.3	27.7	25.2
3	218.3	218.3	80.4	88.7
4	47.5	47.8	38.2	38.0
5	55.2	56.0	55.6	55.9
6	19.6	20.2	18.1	20.9
7	33.8	35.3	32.1	34.3
8	41.0	39.1	39.5	38.6
9	50.0	49.0[a]	47.7	50.5
10	37.1	36.0	37.9	37.2
11	21.7	17.7	23.7	22.3
12	25.3	36.9	122.8	29.4
13	38.4	37.9	143.8	38.8
14	43.1	157.8	41.9	42.8
15	27.7	117.4	27.9	27.4
16	35.8	38.0	23.6	35.6
17	43.2	37.5	46.7	43.0
18	48.5	48.9[a]	41.3	48.0
19	48.2	40.9	46.0	48.3
20	151.0	29.0	30.9	150.9
21	30.0	30.0	34.6	31.9
22	39.9	33.3	34.5	40.9
23	26.9	26.3[b]	28.2	28.0
24	21.3	21.6	16.9	16.0
25	16.2	15.0	15.6	16.1
26	16.0	30.9	17.0	16.1
27	14.7	25.8[b]	26.1	14.5
28	18.3	30.2	173.9	18.0
29	109.6	33.6	33.2	109.3
30	20.0	21.6	23.8	19.3
31	-	-	-	57.5

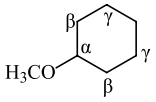
The letters a and b indicate possible exchangeable values

The ^{1}H-NMR spectrum of compound **3** showed several signals in the shielding region between δ_H 0.7 and δ_H 1.4. The deshielding region revealed a signal at δ_H 5.30 (1H, *t, J* = 4.0 Hz), which together with a signal at δ_H 2.82 (1H, *dd, J* = 14.0 and 4.0 Hz) indicated the oleanane skeleton. The ^{13}C-NMR spectral data exhibited signals at δ_C 122.8 and 143.8 corresponding to the carbons in C-12 and C-13, respectively. The signal at δ_C 173.9 was assigned to the carboxyl group (C-28). Other signals were compared with literature data (Table 1).[27,28] Taken together, this data allowed the identification of compound **3** as oleanolic acid which, to the best of our knowledge, is described here for the first time in Olacaceae.

Compound **4** showed a purple color in TLC when revealed with anisaldehyde sulphuric acid reagent. The ^{1}H-NMR spectrum exhibited a doublet at δ_H 4.69 (1H, *J* = 2.0 Hz) and a doublet of doublet at δ_H 4.57 (1H, *J* = 2.0 and 1.0 Hz). At δ_H 3.35 (3H, *s*) a signal of hydrogen of a methoxy group which is linked in C-3 was observed, as confirmed by HMBC. Signals at δ_H 2.63 (1H, *dd*, *J* = 12.0 and 4.0 Hz) revealed protection of the H-3. The ^{13}C-NMR spectrum showed signals at δ_C 150.9 and δ_C 109.3, corresponding to a quaternary carbon (C-20) and a methylene carbon (C-29), respectively, by DEPT, suggesting the presence of a lupane skeleton.[27] The carbinol signal was observed at δ_C 88.7 and the methoxy group appeared at δ_C 57.5. The HSQC experiment showed the couplings of δ_C 109.3 (C-29) to δ_H 4.57 (H-29), δ_C 109.3 (C-29) to δ_H 4.69 (H-29), δ_C 88.7 (C-3) to δ_H 2.63 (H-3) and δ_C 57.5 (C-31) to δ_H 3.35 (H-31). In the HMBC spectrum, the following correlations, among others, were observed: signal at δ_C 88.7 to δ_H 0.95 (^3J), δ_C 88.7 to δ_H 3.35 (^3J), δ_C 48.3 to δ_H 4.69 (^3J), δ_C 19.3 to δ_H 4.69 (^3J) and δ_C 48.3 to δ_H 4.57(^3J). Assignment of H-29 (δ_H 4.57) and H-30 (δ_H 1.68) was determined by ^{1}H-^{1}H COSY spectrum analysis.

As the ^{13}C-NMR data of **4** are not available in the literature consulted, we performed a calculation in order to estimate the chemical shifts of **4** by subtracting the effect of the OH group in C-3 and adding the OCH_3 group in its place, and then calculating the α, β and γ effects on the carbon chemical shift (Table 2). Lupeol is a common triterpene with this skeleton and hence the $\Delta\delta_C$ calculation was based on its ^{13}C-NMR data.[29] These calculations allowed the identification of 3β-methoxy-lup-20(29)-ene which is reported for the first time in Olacaceae.

Table 2. α, β and γ calculated effects by subtracting an OH group and adding an OCH_3, from lupeol



	Lupeol[16]	- OH effect	Calculated (lupane)	+ OMe effect	Calculated (3-β-methoxy-lup-20(29)-ene)
1	38.7	+5	43.7	-4	39.7
2	27.4	-8	19.4	+5	24.4
3	79.9	-41	38.9	+51	89.9
4	38.8	-8	30.8	+5	35.8
5	55.3	+5	60.3	-4	56.3

Antiplasmodial activity

Dichloromethane extracts of leaves and branches from *M. guianensis*, oleanolic acid (**3**), squalene (**5**) and taraxerol **(6)** (Figure 2) (previously isolated)[18] were assayed in two concentrations (50 and 25 μg/mL) against *P. falciparum* (W2) and were found to be partially active except for dichloromethane extract from branches (Table 3).

Compound **3**, isolated here from dichloromethane extract of leaves, caused 51% parasitemia reduction against *P. falciparum* (W2), at 50 μg/mL concentration and 28% reduction at 25 μg/mL. The two compounds previously isolated, squalene (**5**) and taraxerol (**6**), also from the dichloromethane extract of leaves,[18] showed slightly better activity, with 67 and 32% reduction for squalene and 64 and 34% for taraxerol, at 50 and 25 μg/mL, respectively. These results allowed prediction of the IC_{50} range of 25-50 μg/mL for the assayed triterpenes

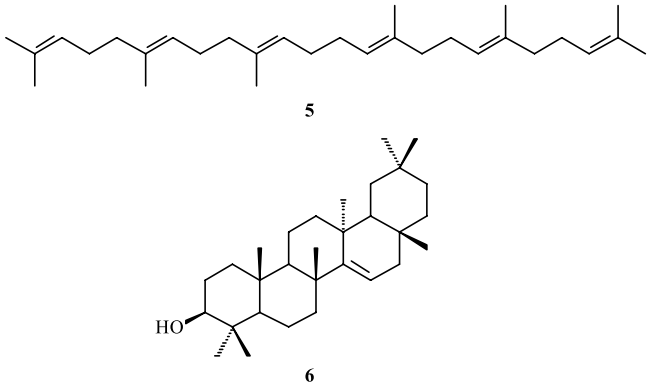


Figure 2. *Chemical structures of the two previously isolated triterpenes from Minquartia guianensis which were assayed against Plasmodium falciparum*

Table 3. Percentage of parasitemia reduction caused by *Minquartia guianensis* extracts and compounds **3**, **5** and **6** against chloroquine-resistant *Plasmodium falciparum* (W2 clone)

		% Parasitemia reduction		
Extracts/ compounds	Vegetal part	25 µg/mL	50 µg/mL	Classification
DCM	Leaves	50	100	Partially active
DCM	Branches	0	0	Inactive
Oleanolic acid (**3**)		28	50	Partially active
Squalene (**5**)		32	67	Partially active
Taraxerol (**6**)		34	64	Partially active
Chloroquine		**100**	**100**	**Active**

3, **5** and **6**. However, the IC_{50} could not be determined because of the small amount of the triterpenes. IC_{50} was determined only for the positive control, chloroquine (Figure 3). In the literature available, only compound **3** was assayed against *Plasmodium falciparum*, and exhibited IC_{50} values of 88.8 and 70.6 mg/mL against chloroquine resistant (K1) and sensitive (T9-96) *P. falciparum*, respectively.[30] Our results revealed that **3** is even more active against the chloroquine resistant *P. falciparum* W2 clone. According to Sairafianpour and collaborators, the antiplasmodial activity of oleanolic acid might be due to its incorporation into the erythrocyte membrane, which would affect *Plasmodium* growth.[31]

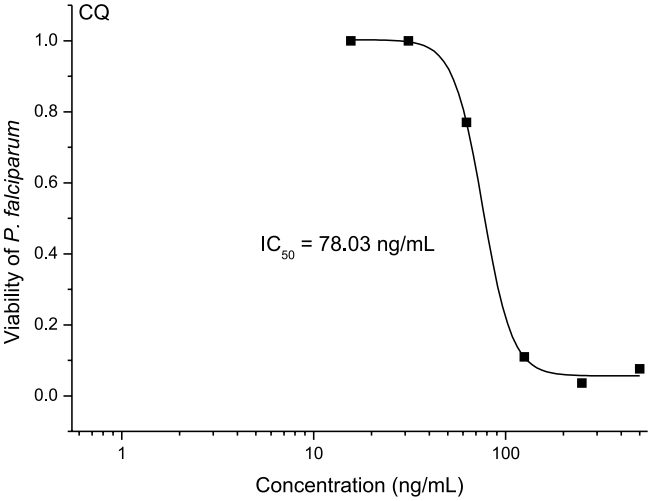


Figure 3. *IC_{50} determination of chloroquine against Plasmodium falciparum*

EXPERIMENTAL

General

The compounds isolated were characterized by their NMR spectra which were recorded on a Varian spectrometer operating at 500 MHz (^1H) and at 125 MHz (^{13}C) using $CDCl_3$ as the sample solvent and internal standard. High performance liquid chromatography (HPLC-DAD) was carried out with analytic and semi-preparative cyanopropyl columns (Luna-Phenomenex®). The fractionations were carried out by the classical techniques including open chromatographic column and comparative thin layer chromatography (TLC) of silica gel 60 (230-400 mesh, ASTM, Merck) and Florisil (100-200 mesh, ASTM, Merck) to guide the fractions analysis.

Plant material

Minquartia guianensis Aubl. leaves and branches were collected at the Reserva Ducke, Manaus, Amazonas, Brazil, in April 2005. A plant voucher specimen was compared with one previously deposited (179.806) in the herbarium of the National Institute of Amazonian Research - INPA, Manaus, AM, Brazil.

Extraction and isolation

M. guianensis dry leaves (289 g) and branches (281 g) were powdered and extracted three times with CH_2Cl_2, using an ultrasound bath (20 min) each time, yielding the crude extracts (3.5 and 8.5 g, respectively).

The dichloromethane extract of leaves was fractionated on a chromatographic column using silica gel 60 as the stationary phase, and eluted with solvents of increasing polarity (hexane, AcOEt and MeOH). From this column, 2 fractions were re-fractionated (fractions 10-13 and 23-29). Fraction 10-13 (425 mg) was rechromatographed on a chromatographic column using Florisil as a stationary phase, and eluted with gradients of hexane, AcOEt and MeOH; subfraction 4 yielded the mixture of compounds **1** and **2** (2.3 mg) by recrystallization. Fraction 23-29 (480 mg) was submitted to a chromatographic column using silica gel 60 and eluted with a gradient of CH_2Cl_2 and acetone. Subfractions 9-11 (25 mg) were purified by HPLC eluted with acetonitrile (isocratic) on a cyanopropyl column (Phenomenex®), 250 x 10 mm, 5 µm with 25 µL of injection volume, yielding compound **3** (2 mg) (Rt = 40.2 min).

The dichloromethane extract of branches was fractionated on a chromatographic column using silica gel 60 as the stationary phase, and eluted by a gradient of hexane, AcOEt and MeOH. Fraction 3-5 (74.8 mg) was rechromatographed on silica gel 60 using a gradient of hexane, CH_2Cl_2 and AcOEt. Sub-fractions 8-14 (27 mg) were recrystallized yielding compound **4** (3.2 mg).

Plasmodium falciparum continuous culture and *in vitro* assay

The *P. falciparum* W2 clone was kept in a continuous culture at 37 ºC on human erythrocytes using the candle jar method in RPMI medium supplemented with 10% human plasma (complete medium), as previously described.[32] Synchronization of the parasites was achieved by sorbitol treatment[33] and parasitemias were determined microscopically in Giemsa-stained smears.

In vitro assays were carried out with erythrocytic cultures of chloroquine resistant *Plasmodium falciparum* (W2 clone), by the HRP2 method.[34] Briefly, the assay was started by parasite-drug incubation: 20 µL of each extract or substance diluted (stock sample concentration: 50 µg/mL, in DMSO) was placed in 96-well microplates in

duplicate, which contained 180 µL of infected erythrocyte suspension (1.5% hematocrit, 0.05% parasitemia). Controls without drugs were used, with infected erythrocytes (positive control) or infected erythrocyte, frozen 24 h after starting the assay (background). The microplates were then incubated in a 5% CO_2 atmosphere at 37 ºC for 48 h. After this period, the microplates were frozen (-20 ºC for at least 24 h), in order to promote erythrocyte lysis. The cell lysis content (100 µL/well) was transferred to 96-well plates, previously incubated overnight with anti-HRP2 capture antibody (MPFM-55A ICLLAB®) and blocked with 200 µL/well of blocking solution (PBS/BSA 2%). After 1 h of incubation, the plates were washed and incubated with 100 µL/well of the peroxidase-conjugated secondary antibody for a further 1 h (MPFG55P-ICLLAB®). The plates were again washed and incubated for 10 min in the presence of TMB substrate solution. The reaction was stopped with HCl 1 N and the absorbance measured on a spectrophotometer (450 nm). The percentage reduction of parasite growth was calculated from the absorbance measure.

ACKNOWLEDGEMENTS

The authors thank CT-Agro/CNPq/MCTI (520281/2007-1 and 562892/2010-9) and PPBio/CNPq/MCTI (558321/2009-7) for the fractionation financial support, to the Phytochemistry Laboratory of Faculty of Pharmacy/UFMG and the *Rede de Produtos Naturais para Quimioterapia Antimalárica* (Natural Products Net for Antimalarial Chemotherapy), PRONEX/CNPq (555655/2009-1) and FAPEMIG (CDS APQ 01129-10), coordinated by Profa. Dra. A. B. de Oliveira, for the *in vitro* assays financial support.

REFERENCES

1. Camargo, J. L. C.; Ferraz, I. D. K.; *Informativo Técnico Rede de Sementes da Amazônia* **2005**, 10.
2. http://www.tropicos.org/NameDistributions.aspx?nameid=22900119, accessed in May 2012.
3. Souza, V. C.; Lorenzi, H.; *Botânica sistemática: Guia ilustrado para identificação das famílias de fanerógamas nativas e exóticas no Brasil, baseado em APG II*, 2ª ed., Instituto Pantarum: Nova Odessa, 2008.
4. Polonsky, J.; Varenne, J.; Prangé, T.; Pascard, C.; *J. Chem. Soc., Chem. Commun.* **1981**, *15,* 731.
5. El-Seedi, H. R.; Gohil, S.; Perera, P.; Torssell, K. B. G.; Bohlin, L.; *Phytochemistry* **1999**, *52*, 1739.
6. El-Seedi, H. R.; Larsson, S.; Backlund, A.; *Biochem. Syst. Ecol.* **2005**, *33*, 831.
7. Valera, G. C.; De Budowisk, J.; Monache, F. D.; Marini-Bettòlo, G. B.; *Rendiconti* **1977**, *62*, 363.
8. Govindachari, T. R.; Viswanathan, N.; *Phytochemistry* **1972**, *11*, 3529.
9. Wiart, C.; Martin, M-T.; Awang, K.; Hue, N.; Serani, L.; Laprévote, O.; Pais, M.; Rhamani, M.; *Phytochemistry* **2001**, *58*, 653.
10. Dirsch, V.; Wiemann, W.; Wagner, H.; *Pharm. Pharmacol. Lett.* **1992**, *2*, 184.
11. Dirsch, V.; Neszmélyi, A.; Wagner, H.; *Phytochemistry* **1993**, *34*, 291.
12. Abe, F.; Yamauchi, T.; *Phytochemistry* **1993**, *33*, 1499.
13. Montrucchio, D. P.; Miguel, O. G.; Miguel, M. D.; Monache, F. D.; Carvalho, J. L. S.; *Visão Acadêmica* **2005**, *6*, 48.
14. Jerz, G.; Waibel, R.; Achenbach, H.; *Phytochemistry* **2005**, *66*, 1698.
15. Spitzer, V.; Marx, F.; Maia, J. G. S.; Pfeilsticker, K.; *Fat. Sci. Technol*. **1995**, *97*, 37.
16. Tang, W.; Hioki, H.; Harada, K.; Kubo, M.; Fukuyama, Y.; *J. Nat. Prod.* **2008**, *71*, 1760.
17. Tang, W.; Kubo, M.; Harada, K.; Hioki, H.; Fukuyama, Y.; *Bioorg. Med. Chem. Lett*. **2009**, *19*, 882.
18. Cursino, L. M. C.; Mesquita, A. S. S.; Mesquita, D. W. O.; Fernandes, C. C.; Pereira Jr, O. L. P.; Amaral, I. L.; Nunez, C. V.; *Acta Amazonica* **2009**, *39,* 181.
19. Forgacs, P.; Provost, J.; *Phytochemistry* **1981**, *20*, 1689.
20. El-Seedi, H. R.; Hazell, A. C.; Torssel, K. B. G.; *Phytochemistry* **1994**, *35*, 1297.
21. Marles, R. J.; Farnsworth, N. R.; Neill, D. A.; *J. Nat. Prod.* **1989**, *52*, 261.
22. Rasmussen, H. B.; Christensen, S. B.; Kvist, L. P.; Kharazmi, A.; Huansi, A. G.; *J. Nat. Prod.* **2000**, *63*, 1295.
23. Olea, R. S. G.; Roque, N. F.; *Quim. Nova* **1990**, *13*, 278.
24. Brochini, C. B.; Nunez, C. V.; Moreira, I. C.; Roque, N. F.; Chaves, M. H.; Martins, D.; *Quim. Nova* **1999**, *22*, 37.
25. Nunez, C. V.; *Tese de Doutorado,* Universidade de São Paulo, Brasil, 2000.
26. Saraiva, R. C. G.; Pinto, A. C.; Nunomura, S. M.; Pohlit, A. M.; *Quim. Nova* **2006**, *29*, 264.
27. Mahato, S. B.; Kundu, A. P.; *Phytochemistry* **1994**, *37*, 1517.
28. Gohari, A. R.; Saeidnia, S.; Hadjiakhoondi, A.; Abdoullahi, M.; Nezafati, M.; *J. Med. Plants* **2009**, *8*, 65.
29. Silverstein, R. M.; Webster, F. X.; Aguiar, P. F.; *Identificação espectrométrica de compostos orgânicos,* 6ª ed., LCT: Rio de Janeiro, 2000.
30. Steele, J. C. P.; Warhurst, D. C.; Kirby, G. C.; Simmonds, M. S. J.; *Phytother. Res.* **1999,** *13,* 115.
31. Sairafianpour, M.; Bahreininejad, B.; Witt, M.; Ziegler, H. L.; Jaroszewski, J. W.; Staerk, D.; *Planta Med.* **2003**, *69,* 846.
32. Trager, W.; Jensen, J. B.; *Science* **1976**, *193*, 673.
33. Lambros, C.; Vanderberg, J. P.; *J. Parasitol.* **1979**, *65*, 418.
34. Noedl, H.; Wersdorf, W. H.; Miller, R. S.; Wongsrichanalai, C.; *Antimicrob. Agents Chemother*. **2002**, *46*, 1658.