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Chemical constituents from three medicinal plants: *Piper renitens*, *Siparuna guianensis* and *Alternanthera brasiliana*

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Abstract: Chemical study of three medicinal plants: from leaves of *Piper renitens* (Miq.) Yunck, Piperaceae, and *Siparuna guianensis* Aubl., Siparunaceae, and from flowers of *Alternanthera brasiliana* (L.) Kuntze, Amaranthaceae, resulted in isolation of nine compounds: three steroids, β -sitosterol, stigmasterol from *P. renitens* and sitosterol-3-O- β -D-glucopyranoside from *A. brasiliana*, the diterpene kaurane ent-kauran-16 α ,17-diol from *P. renitens*, two derivatives kaempferol-methylether, kumatakenine (kaempferol-3,7-dimethylether) and kaempferol-3,7,3'-trimethylether from *S. guianensis* and three flavones, crysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone), triclin (5,7,4'-trihydroxy-3',5'-dimethoxyflavone) and 7-O- β -D-glucopyranoside-5,4'-dihydroxy-3'-methoxyflavone from *A. brasiliana*. Compounds structures were determinate using 1D and 2D ¹H NMR and ¹³C spectral data, mass and IR spectra, comparing with literature data.

Introduction

Plants utilization in treatment, cure and prevention of any kind of disease is a traditional practice in the entire world. The necessity of knowledge from chemical constituents of plants becomes important as many pharmaceutical products that exist today were developed from substances of that origin (Newman et al., 2003).

Piper genus is included as one of the most important in Piperaceae family and is well distributed in tropical and sub-tropical regions of the planet. Chemical and pharmaceutical studies of this genus are related to the isolation of various secondary metabolites classes (amides, flavonoids, lignans, terpenes, propenylphenols, alkaloids and cyclopetenediones derivatives) and to its use in treatment of some diseases (Parmar et al., 1997; Facundo et al., 2003). In Mexico and Brazil, leaves from *P. amalago* are used to treat stomachache and some infections. Leaves and stems from *P. marginatum* and *P. tuberculatum* are used against snakebite and as sedatives (Chaves et al., 2006; Araújo-Junior et al., 1999). *P. renitens* (Miq.) Yunck is known by the Amazonian rainforest natives as "bararupyangihua" and tea from leaves is used mainly to cure fever, infant diarrhea and

skin inflammations (Ribeiro et al., 1999). Chemical volatile constituents from their leaves were related this specie (Soleane et al., 2007).

Siparuna (formerly Monimiaceae) contains about 72 species of shrubs, straggling shrubs, and trees (Renner et al. 1997). *S. guianensis* Aubl., Siparunaceae, is a small tree found in various parts of Brazilian forests, popularly known as "capitui", "negramena", "erva-santa", "limão-bravo" and others (Zoghbi et al., 1998). Several species of this genus are used in traditional medicine to handling stomach disorders and skin diseases (Leitão et al., 1999). Literature reports the use of *S. guianensis* leaves in popular medicine to cure various diseases, including, sinusitis, fever, rheumatism, migraine, body aches and as anti-inflammatory (Valentini et al., 2010; Rodrigues, 2006). In state of Rondônia, Brazil (an oriental Amazonian state), there are some reports of using leaves tea in treatment of malaria and against rheumatic pains. Chemical studies had revealed presence of volatile compounds, alkaloids and flavonoids (Braz-Filho et al., 1976; Chiu et al., 1982; Machado et al., 1994).

Alternanthera brasiliana (L.) Kuntze is a herbaceous plant from the Amaranthaceae family, popularly known as "penicilina", "terramicina" and "perpetua-do-mato", tea from leaves is widely used to

treat several pathologies, including coughing, diarrhea and for its anti-inflammatory and analgesic properties (Delaporte et al., 2005; Araújo & Onofre, 2011). In state of Rondônia, Brazil, tea from leaf is used for its anti-inflammatory properties. Chemical study of the leaves of *A. brasiliana* had revealed the presence of six di- and triglycosyl kaempferol derivatives and quercetin (Brochado et al., 2003).

This work describes the isolation and structural characterization of the nine compounds from three medicinal plants popularly used as anti-inflammatory: β -sitosterol **1** and stigmasterol **2** from leaves of *P. renitens*, sitosterol-3-*O*- β -D-glucopyranoside **9** from flowers of *A. brasiliana*, ent-kauran-16 α ,17-diol **3** from leaves of *P. renitens*, kumatakenine (kaempferol-3,7-dimethylether) **4** and kaempferol-3,7,4'-trimethylether **5**, from leaves of *S. guianensis* and crysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone) **6**, triclin (5,7,4'-trihydroxy-3',5'-dimethoxyflavone) **7** and 7-*O*- β -D-glucopyranoside-5,4'-dihydroxy-3'-methoxyflavone **8**, from flowers of *A. brasiliana*. The structures were established by spectroscopic techniques, mainly EIMS and 1D and 2D NMR. This is the first report of the occurrence of these compounds in these plants.

Materials and Methods

Melting points were obtained on a Mettler FP82HT apparatus and are uncorrected. IR spectra were recorded using a Perkin Elmer 1000 FT-IR spectrophotometer. The mass spectra were obtained on a Hewlett-Packard 5971 mass spectrometer by electron impact ionization (70 eV). ^1H and ^{13}C NMR were recorded on a Bruker Avance DRX-500 (500 MHz for ^1H and 125 MHz for ^{13}C); Silica gel 60 (Merck, kiesegel 60 F254, 0.20 mm) were used for analytical TLC. Silica gel 60 (Merck, 230-240 mesh) was used for column chromatography. All compounds were visualized on TLC by spraying with $\text{AlCl}_3/\text{EtOH}$ and vanillin/perchloric acid/ EtOH followed by heating.

Leaves from *Siparuna guianensis* Aubl., Siparunaceae, and *Piper renitens* (Miq.) Yunck, Piperaceae, were collected in Mirante da Serra County, Rondonia State, Brazil on April, 2010, and May, 2009, respectively. The plant material was classified systematically by the botanist Dr. J. Gomes from Instituto Nacional de Pesquisa da Amazônia herbarium where voucher specimens were deposited: 216220 for *S. guianensis* and 213523 for *P. renitens*. Leaves from *P. renitens* (1.1 kg) and *S. guianensis* (1.8 kg) were dried, triturated and submitted to ethanol extraction at room temperature in portions of **2** and **3** L, respectively, for three times. After solvent distillation at reduced pressure, 26.0 g and 41.0 g, respectively, of extracts were obtained. A portion of 22.0 g of the ethanol extract from *P. renitens*

was adsorbed in silica gel (100.3 g) and eluted under reduced pressure in a chromatographic column, using successively hexane, chloroform, ethyl acetate and methanol as solvents.

Chloroform fraction was treated with $\text{MeOH}:\text{H}_2\text{O}$ (90:10) and kept under refrigeration overnight to induce chlorophyll separation. This material was dried and re-chromatographed on a silica gel column and eluting with mixtures of hexane and chloroform of increasing polarity, obtaining a mixture of β -sitosterol **1** and of stigmasterol **2** [32.3 mg (hexane/chloroform 40:60), ^{13}C NMR (CDCl_3) data, see Jácome et al., 2004] and ent-kauran-16 α ,17-diol **3** [22.1 mg (hexane/chloroform 50:50), 187-188 $^\circ\text{C}$, ^{13}C NMR (CDCl_3) data, see Nascimento & Lopes, 2003].

Similarly, a portion of 35.5 g of the ethanol extract from *S. guianensis* were adsorbed by silica gel (90.0 g) and eluted under reduced pressure in a chromatographic column, using successively hexane, chloroform, ethyl acetate and methanol as solvents. Chromatography on a silica gel column of the chloroform fraction (9.3 g) eluting with mixtures of hexane and chloroform of increasing polarity yielded 23 sub-fractions. The subfractions 32 to 54 were combined and re-chromatographed on a silica gel column and eluted with hexane and ethyl acetate mixture with increasing polarity yielded kumatakenin (kaempferol-3,7-dimethylether) **4** [31.8 mg (hexane/ethyl acetate 60:40), 243-245 $^\circ\text{C}$, ^{13}C NMR (CDCl_3) data, see table 1, Silva et al., 2009] and kaempferol-3,7,4'-trimethylether **5** [21.5 mg (hexane/ethyl acetate 55:45), 146-148 $^\circ\text{C}$, ^{13}C NMR (CDCl_3) data, see table 1, Paula et al., 2006].

Flowers from *Alternanthera brasiliana* (L.) Kuntze, Amaranthaceae, were collected in Porto Velho, Rondônia State, Brazil on September 2008. The plant material was classified systematically by the botanist Dra. Ana Cristina R. de Souza from Dr. Ary Tupinamba Pena Pinheiro Herbarium in Faculdade Sao Lucas (Porto Velho, Rondonia State, Brazil) where may be found a voucher specie identified by 445801 descriptor.

Flowers (0.8 kg) from *A. brasiliana*, were dried, triturated and submitted to ethanol extraction at room temperature in portions of 1.5 L, in three times. After solvent distillation at reduced pressure, 21.3 g of extract were obtained. All the extract were absorbed by silica gel and eluted in a chromatography column with chloroform and ethyl acetate as binary mixtures with increasing polarity yielded 104 sub-fractions. The subfraction **9** (1.2 g chloroform/ethyl acetate 90:10) yielded a solid residue which after recrystallization from acetone yielded crysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone) **6** [21.9 mg, 324-326 $^\circ\text{C}$, ^{13}C NMR ($\text{DMSO}-d_6$) data, see table 1, Awaad et al., 2006]. The subfractions 17 to 34 (2.2 g chloroform/ethyl acetate 85:15) were combined, the resulting fraction was rechromatographed on a silica gel column and eluted with chloroform and ethyl acetate mixture with increasing polarity yielded triclin

(5,7,4'-trihydroxy-3',5'-dimethoxyflavone) **7** [23.1 mg (chloroform/ethyl acetate 82:18), 278-280 °C, ¹³C NMR (DMSO-d₆) data, see table 1, Jiao et al., 2007] and 7-*O*-β-D-glucopyranoside-5,4'-dihydroxy-3'-methoxyflavone **8** [17.4 mg (chloroform/ethyl acetate 40:60), 176-178 °C, ¹³C NMR (DMSO-d₆) data, see table 1, Markham & Moore, 1980]. The subfraction 56 (chloroform/ethyl acetate 90:10) yielded a solid residue which after recrystallization from methanol yielded sitosterol-3-*O*-β-D-glucopyranoside **9** [34.2 mg, 232-235 °C, ¹³C NMR (DMSO-d₆) data, see table 1, Macari et al., 1990].

Table 1. ¹³C-NMR spectral data for compounds **4-8**.

C	4	5	6	7	8
	δ _C	δ _C	δ _C	δ _C	δ _C
2	155.9	156.3	164.2	164.2	164.8
3	138.9	137.9	103.8	104.2	103.9
4	178.8	178.1	182.2	182.2	182.4
5	161.7	160.9	157.9	157.9	157.4
6	97.7	97.8	99.4	99.4	100.2
7	165.4	165.2	164.7	164.2	163.5
8	92.1	92.4	94.4	94.4	95.7
9	156.8	156.4	162.0	161.4	161.7
10	106.1	108.5	104.2	104.2	105.1
1'	122.8	122.6	122.1	120.9	122.8
2'	-	-	111.1	-	111.2
2'/6'	130.1	130.3	-	105.4	-
3'	-	-	148.7	-	148.7
3'/5'	114.1	115.5	-	148.9	-
4'	162.7	161.3	140.1	151.7	140.1
5'	-	-	116.4	-	116.5
6'	-	-	120.9	-	121.1
1''	-	-	-	-	100.8
2''	-	-	-	-	73.7
3''	-	-	-	-	77.8
4''	-	-	-	-	77.1
5''	-	-	-	-	70.3
6''	-	-	-	-	61.3
OMe-3	59.8	60.1	-	-	-
OMe-7	56.1	56.7	-	-	-
OMe-3'	-	-	57.0	56.8	56.7
OMe-4'	-	55.4	-	-	-
OMe-5'	-	-	-	56.6	-

Results and Discussion

The structure of the steroids β-sitosterol **1** and stigmasterol **2**, isolated from the leaves of *P. renitens*, and sitosterol-3-*O*-β-D-glucopyranoside **9**, isolated from

flowers of *A. brasiliana* were assigned based on the analysis of the 1D and 2D ¹H and ¹³C NMR spectra and by comparison with the literature data (Jácome et al., 2004; Macari et al., 1990).

Compound **3** was obtained from the leaves of *P. renitens*, their EIMS spectrum showed the molecular ion peak at *m/z* 306, compatible with the molecular formula C₂₀H₃₄O₂. The IR spectrum displayed absorption bands indicating the presence of OH (3430 cm⁻¹) group. The ¹³C NMR spectrum of compound **3** showed twenty signals, which were attributed to three CH₃ (δ_C 17.7, 21.5, 33.5), ten CH₂ (δ_C 66.4, 53.4, 42.0, 42.1, 40.3, 37.3, 26.4, 20.67, 18.6, 18.3), three CH (δ_C 56.7, 56.2, 45.5) and four C (δ_C 81.9, 44.7, 39.4, 33.2). The ¹H NMR spectrum showed two doublet at δ_H 3.78 (*J*=11.0 Hz) e 3.67 (*J*=11.0 Hz), revealed the presence of a carbinolic methylene (CH₂O-17) and three singlets assigned to methyl groups at δ_H 1.03, 0.85 and 0.81. The peak at δ_C 81.9 and literature data comparison with 16α,17-ent-kauranediol, was decisive to locate the hydroxymethyl and hydroxyl groups at C-16 and consider the same structure for **3** (Nascimento & Lopes, 2003).

Compounds **4** and **5** were isolated from the leaves of *S. guianensis* and were identified as *O*-methylated derivatives of kaempferol. These compounds show positive test for flavonoids using AlCl₃/EtOH in TLC plate. The IR spectrum displayed O-H stretching bands at 3350 cm⁻¹ for **4** and 3375 cm⁻¹ for **5**, and conjugated carbonyl group absorptions at 1675 cm⁻¹ and 1667 cm⁻¹, respectively. The ¹H NMR spectra of compounds **4** and **5** showed two meta-coupled doublets for A-ring, at δ_H 6.55 (*d, J*=2.2 Hz, H-6), 6.13 (*d, J*=2.2 Hz, H-8) for **4** and 6.35 (*d, J*=2.0 Hz, H-6), 6.44 (*d, J*=2.0 Hz, H-8) for **5** and two orto-coupled doublets, for B-ring, at δ_H 8.02 (*d, J*=8.8 Hz, H-2'/6') and 6.99 (*d, J*=8.8 Hz, H-3'/5') for **4** and 8.08 (*d, J*=7.9 Hz, H-2'/6'), 7.02 (*d, J*=7.8 Hz, H-3'/5') for **5**, commonly observed in the kaempferol nucleus (Pizzolatti et al., 2003). Also, one signal for a chelated hydroxyl (OH-5) was observed at δ_H 12.76 for **4** and 12.53 for **5**. The ¹³C NMR spectra of compounds **4** and **5** showed signals for methoxyl groups at δ_C 59.8 and 56.1 for **4** and at δ_C 60.1, 56.7 and 55.4 for **5**. The downfield shift of the methoxyl groups at δ_C 59.8 for **4** and 60.1 for **5**, suggests a crowded position such as C-3 (Silva et al., 2009); this assignment is supported by the absence of the characteristic absorption at δ_H 6.80 of a vinylic hydrogen in flavones (Fossen & Andersen, 2006). A correlation peak in HMBC between C-7 (δ_C 165.4) and a methoxy group (δ_H 3.77) completed the elucidation of the structure of compound **4**. Therefore, compound **4** was established as kumatakenin (kempferol-3,7-dimethylether) (Silva et al., 2009).

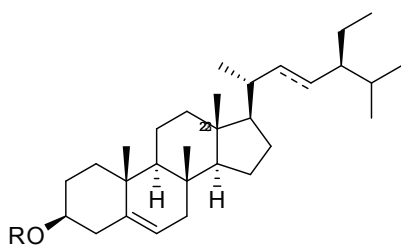
The location of the two methoxyl groups at C-7 and C-4' for compound **5**, was supported by analysis of the HMBC spectrum, where a correlation between

C-7 at δ_c 165.2 and a methoxyl group at δ_H 3.87 and a correlation between C-4' at δ_c 161.3 and a methoxyl group at δ_H 3.86 were observed. On this basis the compound **5** was unambiguously identified as kaempferol-3,7,3'-trimethylether (Paula et al., 2006).

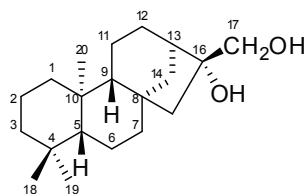
Compounds **6-8** were isolated from the flowers of *A. brasiliana* and were identified as three flavones. These compounds show positive test for flavonoids using $AlCl_3/EtOH$ in TLC plate. The IR spectra of compounds **6-8** displayed absorption bands indicating hydroxyl and conjugated carbonyl groups at 3387 and 1673 cm^{-1} , respectively (Silva et al., 2006). The HMBC spectra of compounds **6** and **8** exhibited correlations between the hydrogen signal at δ_H 6.91 (s, H-3) for **6**, 6.90 (s, H-3) for **7** and 6.90 (s, H-3) for **8**, and the three quaternary carbon resonances at δ_c 122.1 (C-1'), 111.1 (C-2) and 182.2 (C-4) for **6**, 120.9 (C-1'), 105.4 (C-2) and 182.2 (C-4) for **7** and 122.8 (C-1'), 111.2 (C-2) and 182.4 (C-4) for **8**, respectively, which allowed us to unequivocally assign this hydrogen at position **3** of the flavone nucleus (Fossen & Andersen, 2006). 1H NMR spectra of compounds **6-8** showed signals at δ_H 12.98 for **6**, 12.91 for **7** and 12.93 for **8** assigned to the chelated hydroxyl group at C-5, two meta-coupled doublets at δ_H 6.21 (d, $J=2.0$ Hz, H-6) and 6.50 (d, $J=2.0$ Hz, H-8) for **6**, 6.20 (d, $J=1.9$ Hz, H-6), 6.54 (d, $J=1.9$ Hz, H-8) for **7** and 6.46 (d, $J=1.9$ Hz, H-6) and 6.89 (d, $J=1.9$ Hz, H-8) for **8**, suggesting an A-ring substituted at positions C-5 and C-7. 1H NMR spectra of compounds **6** and **8** showed one broad singlet and two doublets at δ_H 7.56 (bs, H-2'), 6.95 (d, $J=8.9$ Hz, H-5')

and 7.55 (d, $J=8.9$ Hz, H-6') for **6** and 7.56 (bs, H-2'), 6.96 (d, $J=8.8$ Hz, H-5') and 7.58 (d, $J=8.8$ Hz, H-6') for **8**, suggesting a B-ring substituted at positions C-3' and C-4'. The 1H NMR spectra of compounds **7** showed one signal at δ_H 7.56 (bs), compatible with a B-ring substituted at positions C-3', C-4' and C-5'. The 1H and ^{13}C NMR spectra of compounds **6-8** showed signals at δ_H 3.91 (3H, s), for **6**, 3.90 (6H, s) for **7** and 3.92 (3H, s) for **8** and δ_c 57.0 for **6**, 56.6 and 56.8 for **7** and 56.7 for **8** assigned to methoxyl groups. For compound **8**, additional signals were observed at δ_H 5.06 (1H, d, $J=7.3$ Hz, H-1''), 3.46 (1H, m, H-2''), 3.35 (1H, m, H-3''), 3.30 (1H, m, H-4''), 3.21 (1H, m, H-5''), 3.75 (1H, dd, $J=11.6$ and $J=2.2$), 3.51 (1H, dd, $J=11.6$ and $J=2.2$ Hz) and δ_c 100.8, 77.8, 77.1, 73.7, 70.3, and 61.3, which supported the presence of an *O*- β -D-glucose moiety. The HMBC spectra of compounds **6-8** exhibited unambiguous correlations between the hydrogens of OCH_3 -3' (δ_H 3.91) and C-3' (δ_c 148.6) for **6**, OCH_3 -3'/5' (δ_H 3.90) and C-3'/5' (δ_c 148.8) for **7** and OCH_3 -3' (δ_H 3.91) and C-3' (δ_c 148.7) for **8**. The HMBC spectrum of **8** exhibited also a correlation between the anomeric hydrogen at δ_H 5.06 (1H, d, $J=7.3$ Hz, H-1'') and C-7 (δ_c 163.5).

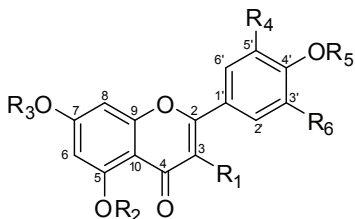
Comparison of these data and the literature leads to the following conclusion: (a) compound **6** is crysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone) (Awaad et al., 2006), (b) compound **7** is tricrin (5,7,4'-trihydroxy-3',5'-dimethoxyflavone) (Jiao et al., 2007) and (c) compound **8** is 7-*O*- β -D-glucopyranoside-5,4'-dihydroxy-3'-methoxyflavone (Markham & Moore, 1980).



- 1** 22,23 dihydro; R=H
2 $\Delta^{22,23}$, R=H
9 22,23 dihydro; R= β -D-glucopyranoside



3



- 4** $R_1=OCH_3$; $R_2=R_4=R_5=R_6=H$; $R_3=CH_3$
5 $R_1=OCH_3$; $R_2=R_4=R_6=H$; $R_3=R_5=CH_3$
6 $R_1=R_2=R_3=R_4=R_5=H$; $R_6=OCH_3$
7 $R_1=R_2=R_3=R_5=H$; $R_4=R_6=OCH_3$
8 $R_1=R_2=R_4=R_5=H$; $R_6=OCH_3$; $R_3=O-\beta$ -D-glucopyranoside

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