Quantification of Antimalarial Quassinoids Neosergeolide and Isobrucein B in Stem and Root Infusions of *Picrolemma sprucei* Hook F. by HPLC-UV Analysis

Rita C. S. Nunomura[1], Ellen C. C. Silva[1],
Sergio M. Nunomura[2], Ana C. F. Amaral[3],
Alaíde S. Barreto[3], Antonio C. Siani[3] and Adrian M. Pohlit[2]
[1]Department of Chemistry, Amazon Federal University (UFAM), Amazon,
[2]Coordination of Research in Natural Products,
Amazon National Institute (INPA), Amazon,
[3]Laboratory of Natural Products, Farmanguinhos,
Oswaldo Cruz Institute Foundation (FIOCRUZ), Rio de Janeiro,
Brazil

1. Introduction

Natural products have been very important to ensure the survival of the man, since the ancient times, especially as remedies to treat different diseases. Today, despite the development of new therapies and new ways of drug development (combinatorial chemistry, ie); natural products continue to play a highly significant role in the drug discovery and development process. (Newman, Cragg, 2007).

Even though fewer drugs have been approved as therapeutical agents lately, nature still inspires the drug development for neglected diseases (malaria, tuberculosis and leishmania) and alternative therapies such as phytotherapy. In both cases, medicinal plants, plants that have been used by the folk medicine for years, are mostly studied. The World Health Organization (WHO) recognized the importance of phytotherapy and the conservation of medicinal plants that stated "the importance of conservation is recognized by WHO and its Member States and is considered to be an essential feature of national programmes on traditional medicines" (Akerele, 1991).

The successful use of some medicinal plants by local population for years, in many cases for centuries, in the treatment of diseases or symptoms associated to some diseases is the basis of the development of drugs or other therapeutical products from them. For instance, artemisinin, a very potent antimalarial, including for drug-resistant malaria strains, was isolated from *Artemisia annua* L., a plant from the traditional Chinese medicine used as remedy for chills and fever for more than 2000 years (Agtmael *et al.* , 1999).

On the other hand, there is an increasing interest for medicines from nature. This interest in products of plant origin is due to several reasons as possible side-effects from synthetic

drugs and the awareness that "natural products" are harmless. The world market for phytomedicinal products was estimated in U$ 10 billion in 1997, with an annual growth of 6.5%. In Germany, 50% of phytomedicinal products are sold on medical prescription and the cost being refunded by health insurance. This includes pharmaceutical formulations as plant extracts or purified fractions called phytomedicines or herbal remedies. In many countries, phytomedicines or herbal remedies are controlled as synthetic drugs and they have to fulfill the same criteria of efficacy, safety and quality control (Rates, 2001).

However the quality control of phytomedicines poses a significant challenge due to the complexity of a vegetable extract and column chromatography has proved to be a very helpful and powerful technique. The quality control of *Gingko biloba* L. formulations is good example of this challenge. *Gingko* leaves contains as active compounds flavonoids and terpene lactones (gingkgolides and bilobalide) along with long-chain hydrocarbons, alicyclic acids, cyclic compounds, sterols, carotenoids, among others. Most of the quality control of *Gingko* preparations are based on column chromatography and that was reviewed elsewhere already (Sticher, 1992; van Beek, 2002).

Column chromatography, especially high performance liquid chromatography (HPLC), has been extensively used in the quality control of plant extracts and phytomedicines formulations, because of its characteristics. The chosen technique must be able to identify the interested compounds (active principles) that are normally not volatile and, in some cases, occur at very small concentrations. Ideally this technique should also be capable to quantify the interested compounds, so one can establishes dosages for the phytomedicine formulation. The required efficiency and selectivity for qualitative and quantitative analysis of the effective components can be achieved by HPLC. Li *et al*. (2011) have recently reviewed the use of different chromatography techniques, such as HPLC, in the quality control of Chinese medicine.

Although, HPLC is a very powerful technique applied in the quality control of medicinal plants, it is necessary to properly identify the active principles of the medicinal plant. This is achieved combining the use of HPLC or other separation technique with a biological test. The search for antimalarials from medicinal plants is one of the most successful examples of this combination as mentioned earlier. In the Amazon region, there are a large number of plants popularly used against malaria or associated symptoms (fever for instance). Milliken (1997) has identified over hundreds of antimalarial plants used by local population in the Amazon region. Many of these plants remained up to now without a study that could confirm their antimalarial activity.

From the fewer plants studied so far, *Picrolemma sprucei* Hook. f., has been studied by our research group. Herein we described the use of HPLC in the quality control of the antimalarial quassinoids, neosergeolide and isobrucein B, the active principles of this species.

Picrolemma sprucei Hook. f. (*P. pseudocoffea* Ducke is a commonly cited pseudonym) is a widely distributed and important Amazonian medicinal plant. It is known in the Amazon region by common names which call attention to its resemblance to the coffee plant: *sacha-café* in Peru (Duke & Vasquéz 1994), *caferana* in Brazil (Silva et al. 1977) and *café lane* or *tuukamwi* in French Guiana (Grenand et al. 1987). Infusions of roots, stems, and leaves of *P. sprucei* are traditionally used in different dosages and preparations for the treatment of

malaria fevers (Bertani et al. 2005, Vigneron et al. 2005, Milliken 1997), gastrointestinal problems and intestinal worms (Moretti et al. 1982, Duke & Vasquéz 1994). Also, the sale of this plant is sometimes restricted by local vendors due to its use in provoking spontaneous abortions.

Studies on the biological activity of infusions and other derivatives of *P. sprucei* have shown that extracts of this plant have important antimalarial and antihelminthic activities. Bertani *et al.* (2005) reported that a *P. sprucei* leaf infusion inhibited 78 % of *Plasmodium yoelli* rodent malaria growth *in vivo* at a dosage of 95 mg/kg. Furthermore, these same authors reported that of a total of 36 preparations from 25 traditionally used antimalarial plants from French Guiana, *P. sprucei* leaf infusion had the greatest *in vitro* activity against the human malaria parasite *Plasmodium falciparum* (median inhibition concentration, IC_{50}=1.43 µg.mL^{-1}). These results indicate *P. sprucei* leaf extracts have potential as antimalarials.

In 2006, Nunomura *et al.* showed that water and ethanol extracts of *P. sprucei* at concentrations of 1.3 g.L^{-1} were lethal (90-95 % mortality) *in vitro* towards larvae of the nematoide species *Haemonchus contortus* (Barber Pole Worm), a gastrointestinal nematode parasite found in domestic and wild ruminants. These studies lend support to popular assertions that infusions and other derivatives of *P. sprucei* have important antimalarial and antihelminthic activities.
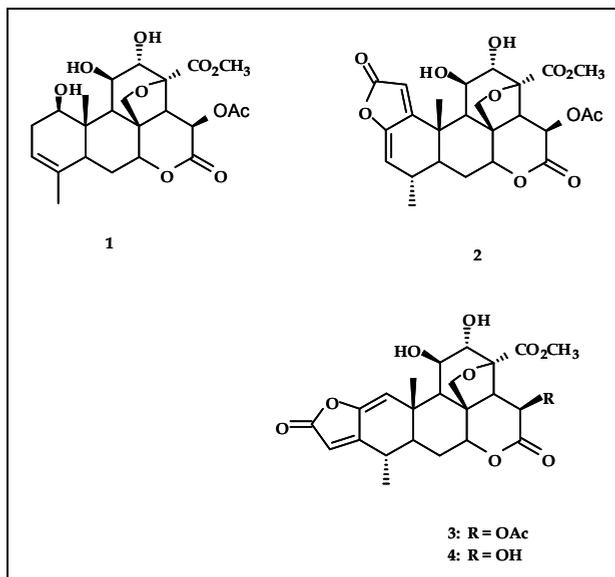

Fig. 1. Quassinoids from *Picrolemma sprucei* Hook.

Two quassinoids have been isolated from *P. sprucei* roots, stems and leaves and identified as isobrucein B (**1**) (Moretti et al. 1982) and neosergeolide (**2**) (Schpector et al.1994, Vieira *et al.* 2000). Quassinoid is the name given to any of a number of bitter substances found exclusively in the Simaroubaceae family (Polonsky 1973). Early reports on *P. sprucei* composition from French Guiana (Moretti *et al.* 1982) described the isolation of sergeolide

(**3**), a structural isomer of **2** and a derivative, 15-deacetylsergeolide (**4**) (Polonsky *et al*. 1984), from the leaves. Since confirmation of the structure of **2** by x-ray crystallography (Schpector *et al*. 1994) and the systematic application of two-dimensional NMR techniques to the identification of components of *P. sprucei* (Vieira *et al*. 2000, Andrade-Neto *et al*. 2007), neither sergeolide nor its derivative have ever again been described and may be erroneous structures.

Chemically, quassinoids are degraded triterpene compounds which are frequently highly oxygenated. Many quassinoids exhibit a wide range of biological activities *in vitro* and/or *in vivo,* including antitumor, antimalarial, antiviral, anti-inflammatory, antifeedant, insecticidal, amoebicidal, antiulcer and herbicidal activities. For instance, bruceantin (**5**), brusatol (**6**), simalikalactone D (**7**), quassin (**8**) and glaucarubinone (**9**) are some of the most well-studied quassinoids and exhibit a wide range of biological activities (Guo *et al*. 2005).
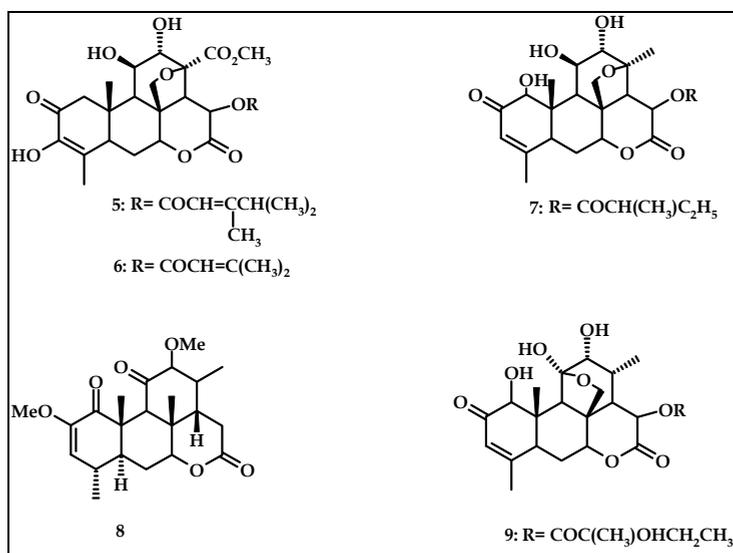

Fig. 2. Quassinoids bruceantin, simalikalactone D, quassin, brusatol and glaucarubinone.

Isobrucein B (Fandeur et al. 1985) and neosergeolide (Andrade-Neto *et al*. 2007) display significant *in vitro* antimalarial activity to the human malaria parasite *P. falciparum*. Recently, the *in vitro* antimalarial activities of isobrucein B and neosergeolide were shown to be comparable to antimalarial drugs quinine and artemisinin (Silva *et al*. 2009). According to this same *in vitro* study, isobrucein B and neosergelide are as cytotoxic or as much as an order of magnitude more cytotoxic than the antitumor drug doxorubicine towards several human tumor strains. Additionally, isobrucein B has been shown to have important antileukemic, antifeedant and leishmanicidal (Moretti *et al*. 1982; Nunomura, 2006).

Bertani *et al*. (2005) conveyed concern about the toxicity of infusions and other preparations based on different parts of *P. sprucei* which is recognized in Amazonian traditional medicine in general. Additionally, these authors were critical of the absence of knowledge of the toxicity of infusions prepared from this species and lack of information available on the

quassinoid composition of these infusions in the study on toxicity published by Fandeur *et al*. (1985), which focused only on the acute toxicity and antimalarial activity of isolated quassinoid components o *P. sprucei* and not on toxicity and antimalarial activity of infusions. Additional studies are needed to prove the *in vivo* efficacy and pharmacological activity of these infusions as antimalarials with focus on the dose-effect and dose-response to define the levels of toxicity. The aim of the present study was to develop a method for the quantification of isobrucein B and neosergeolide in *P. sprucei* root and stem infusions based on reversed-phase high performance liquid chromatography (HPLC) and ultraviolet detection (UV).

2. Materials and methods

2.1 Reagents and solvents

Acetonitrile, HPLC grade, was purchased from Mallinckrodt Baker, Inc. (Xalostoc, Mexico). The water used in all experiments was purified on a Milli-Q Plus System (Millipore, Bedford, MA, USA).

2.2 Isolation of isobrucein B (1) and neosergeolide (2)

Two collections were performed on the main campus of the University of Amazonas, in Manaus, Amazonas State, Brazil, in January and July of 1999. Voucher specimens are deposited at the UFAM Herbarium (Silva 5729 & 5730) and INPA Herbarium (223883). Identification was performed by Dr. Wayt Thomas as *Picrolemma sprucei* Hook. f. (Wayt Thomas, personal communication). Roots and stems were cut into small pieces while fresh and allowed to dry in the shade and were then ground. Air-dried powdered stems (890 g) were extracted 3 times by maceration in hexanes at room temperature (1 week per extraction). After concentration, hexane extract (4.79 g) was obtained. Next, the stems were repeatedly infused in boiling water (Polonsky 1982) which resulted in aqueous solution (20 L). The aqueous solution was concentrated (2.0 L) then continuously extracted with chloroform (40 h), that after total evaporation yielded chloroform extract (10.8 g). Chloroform extract was purified on a column of silica gel which was eluted first with chloroform (100 %), then a gradient of chloroform/methanol 99:1–70:30 (600 mL), 70:30–50:50 (600 mL), 50:50–25:75 (600 mL), and 25:75–100 % methanol (600 mL) and resulted in 171 collected fractions that were combined based on thin-layer chromatography (TLC) analysis to yield 11 fractions. Fraction 9 (1.87 g) was purified on a column of silica gel which was eluted first with 100 % hexane, then 80:20 hexane/chloroform (180 mL), 15:80:5 hexane/chloroform/acetone (800 mL), 10:80:10 hexane/chloroform/acetone (400 mL), 10:70:20 hexane/chloroform/acetone (1440 mL), 10:60:30 hexane/chloroform/acetone (500 mL), 10:50:40 hexane/chloroform/acetone (720 mL), acetone (500 mL), and methanol (500 mL) which resulted in 69 fractions that were combined based on TLC analysis. Combined fraction 42-50 (360 mg) was re-crystallized from methanol/water and yielded colorless crystals which were identified as pure **2** (73.9 mg) based on their spectral properties. The supernatant was re-dissolved in methanol and the insoluble material was washed and filtered resulting in **1** (62.0 mg), a white solid, which was identified based on its spectral properties. The isolation of **1** and **2** yielded 0.57% and 0.68 %, respectively. The compounds **1** and **2** were identified on the basis of their IV, MS and NMR (^{1}H, ^{13}C, HOMOCOSY, HMQC, HMBC and NOESY experiments) spectra analysis.

2.3 Preparation of root and stem infusions

P. sprucei infusions were prepared based on a popular recipe which is used to provoke spontaneous abortions and with which toxic effects are associated according to locals. Stems are the part most commonly used in these remedies. Shade-dried, ground root or stem (9.0 g) was placed in a beaker and boiling deionized water (1.0 mL) was added. The beaker was covered and allowed to stand for 10 min. After this time, the contents of the beaker was filtered hot in a funnel with filter paper which resulted in root and stem infusions. A single infusion was prepared from powdered, dried roots and another from powdered stems obtained from mature plants.

2.4 Calculation of extractives

Infusion as prepared above was totally evaporated using rotary evaporation under vacuum and a heated bath (< 50 °C), then freeze-drying. The resulting dry extract was weighed and divided by the mass of plant material used (9.0 g) in the preparation of each infusion and expressed as a percentage (w/w) of extractives.

2.5 Preparation of samples of infusions for HPLC analysis

Freeze-dried extracts were dissolved in water to yield final concentrations of stem and root extracts of 445 and 911 mg.L^{-1}, respectively.

2.6 Preparation of standard solutions of isobrucein B (1) and neosergeolide (2)

Stock solutions of **1** and **2** were prepared at 0.63 g.L^{-1} and 0.50 g.L^{-1}, respectively, in methanol. Calibration standards were obtained by appropriate dilution of the stock solutions with methanol. For **1**, the concentrations used in calibration were 100, 50, 25, 10 and 5.0 mg.L^{-1}. For **2**, the concentrations used in calibration were 20, 10, 5.0 and 2.5 g.L^{-1}. All standard solutions were stored at -20 °C until analysis and protected from light, remaining stable for at least three months.

2.7 Apparatus and chromatographic conditions

The liquid chromatography system consisted of an LC-10 Shimadzu, with a SPD-10A UV detector, LC-10AVp quaternary pump, SIL- 10A autosampler and a CBM-10A system controller (Kyoto, Japan). A Supelcosil LC-18 analytical column (250 mm × 4.6 mm i.d., 5 µm particle size) from Supelco (Bellefonte, PA, USA) was used for separation of **1** and **2.** The mobile phase consisted of a gradient of acetonitrile:0.05 % aqueous trifluoroacetic acid delivered at 1.0 mL.min^{-1} as follows: initial (t_i=0 min) 10:90, then linear gradient over 20 min to 25:75, and this composition was maintained (isocratic) until the end of each run (t_f=30 min). Flow rate was 1 mL.min^{-1}. Quantification was performed using the detector set at a wavelength of 254 nm. Injection volume was 50 µL.

2.8 Analysis of Infusions by HPLC-UV and calibration curve

Chromatograms of pure **1** and **2** presented retention times of approximately 14 and 25 min, respectively. The peaks corresponding to **1** and **2** were identified in each chromatogram of the infusions with the help of injection of the standard solutions of **1** and **2** or with co-elution (figure 3).
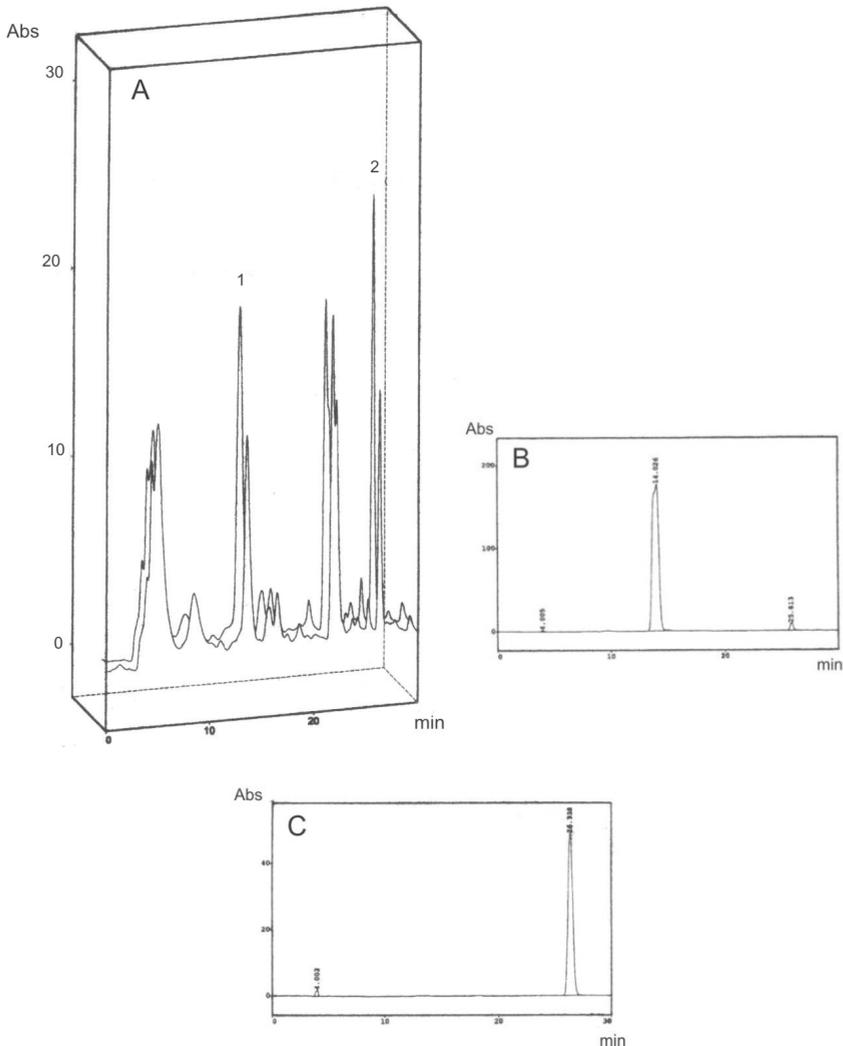
Fig. 3. A: chromatograms of root infusions with (back trace) and without (front trace) addition of neosergeolide and isobrucein B at 254 nm. B: chromatogram of pure isobrucein B (t_R= 14.0 min) at 254 nm. C=chromatogram of neosergeolide (t_R= 25.3 min) at 254 nm.

Several injections of standard solution were performed and then average areas were calculated for each individual concentration injected for isobrucein B (1) and neosergeolide (2). The calibration curves in the determination of **1** and **2** in *P. sprucei* stem and root infusions (Figure 4A and 4B, respectively) used in the determination of these components in *P. sprucei* stem and root infusions were obtained by linear regression performed on the average areas versus standard sample concentrations Y and X, respectively (figure 3) at 254 nm.

After calibration with standard samples of isobrucein B and neosergeolide, *P. sprucei* root and stem infusions were analyzed. Samples of infusions were analyzed in triplicate and the average values of the areas corresponding to the quassinoids neosergeolide and isobrucein B were calculated. From these average areas, the concentration of each quassinoid was calculated in the root and stem infusions using the linear equation generated during calibration of each quassinoid.
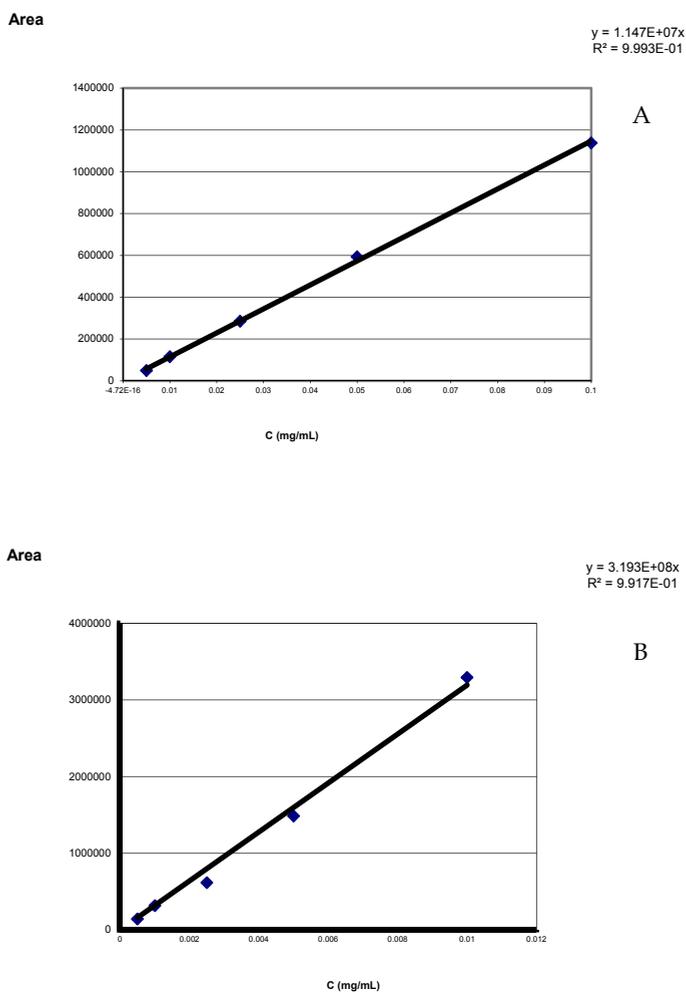
Area

$y = 1.147E+07x$
$R^2 = 9.993E-01$

A

Area

$y = 3.193E+08x$
$R^2 = 9.917E-01$

B

Fig. 4. A: Calibration curve of isobrucein B (**1**); B: Calibration curve of neosergeolide (**2**).

3. Results and discussion

The quassinoids isolated from *P. sprucei* were identified by NMR techniques and compared to literature (Moretti, *et al.* 1982, Vieira, *et al.* 2000). The chemical shifts of NMR ^{1}H and ^{13}C of **1** and **2** are presented in tables 1 and 2 respectively.

Carbon	δ (C)	δ (H)	δ Literature (CDCl₃/Py-5%)[1]	
			C	H
1	81.1	4.17 (s)	81.3	4.26
2	197.0	-	197.6	-
3	124.3	6.11 (q; 2.8; 1.0)	124.5	6.11
4	163.0	-	162.6	-
5	51.6	2.92 (d; 12.1)	43.4	2.91
6	28.5	1.86 (ddd; 14.7; 12.1; 2.6); 2.40 (ddd; 14.7; 2.8; 2.8)	28.2	1.86; 2.41
7	83.1	4.75 (d)	81.7	4.75
8	45.5	-	45.8	-
9	42.8	2.34 (d; 4.0)	42.4	2.38
10	47.5	-	47.7	-
11	72.4	4.75 (sl)	74.3	4.75
12	75.8	4.28 (s)	75.1	4.12
13	80.5	-	81.7	-
14	43.5	3.04 (d; 12.4)	52.3	3.03
15	66.6	6.31 (sl)	67.8	6.30
16	167.0	-	167.6	-
29	22.5	1.96 (d; 1.0)	22.4	1.95
19	11.6	1.18 (s)	11.3	1.18
30	73.3	3.75 (dd; 7.7; 2.0); 4.81 (d; 7.7)	73.0	3.75; 4.81
21	172.6	-	169.5	-
1′	169.0	-	170.7	-
2′	20.4	2.09 (s)	20.5	2.08
OMe (5′)	20.4	3.84 (s)	-	3.83
H-(OH-1)	-	4.54 (s)	-	-
H-(OH-12)	-	3.25 (s)	-	-

[1]Moretti et al. (1982).

Table 1. Chemical shifts in NMR ^{1}H (500 MHz, CDCl₃) and NMR [13]C (125 MHz, CDCl₃) of isobrucein B (**1**).

Carbon	δ (C)	δ (H)	δ Literature (CDCl₃/CD₃OD-5%)[1]	
			C	H
1	162.8	-	161.89	-
2	149.7	-	148.91	-
3	115.9	5.71 (dd, 2.0, 2.0)	116.76	5.76 (t, 2.0)
4	31.60	2.40 (m)	31.07	2.41 (ddq, 1.6, 2.0, 6.8)
5	45.70	1.82 (d, 2.0)	45.26	1.86 (ddd, 1.6, 12.4, 14.0)
6a	29.0	2.22 (dd, 2.0, 2.0)	28.80	2.27 (dt, 14.0, 2.0)
6b		1.76 (dd, 2.0, 2.0)		1.73 (dt, 14.0, 2.0)
7	83.6	4.85 (d, 2.0)	83.55	4.76 (d, 2.0)
8	47.5	-	46.74	-
9	39.6	2.52 (d, 2.0)	39.06	2.34 (brd, 4.0)
10	42.7	-	42.06	-
11	75.0	4.59 (d, 6.0)	73.60	4.56 (d, 4.0)
12	77.1	4.32 (d, 6.0)	76.00	4.23 (brs)
13	82.3	-	81.74	-
14	51.1	3.29 (ddd, 15.0, 2.0, 2.0, 12.0)	49.63	3.24 (brd, 11.0)
15	68.5	6.04 (d, 12.0)	67.00	6.09
16	167.5	-	168.24	-
18	170.5	-	171.25	-
19	18.6	1.64 (s)	18.12	1.60 (s)
29	19.9	1.18 (d, 6.0)	19.55	1.20 (d, 6.8)
30a	74.2	4.78 (d, 8.0)	73.6	4.78 (d, 8.0)
30b		3.81 (dd, 8.0, 2.0)		3.77 (d, 8.0)
1′	169.0	-	170.40	-
2′	20.6	1.98 (s)	20.49	2.09 (s)
3′	171.7	-	171.56	-
4′	113.4	6.26 (s)	113.17	6.26 (d, 2.0)
OMe (5′)	52.9	3.78 (s)	53.02	3,84 (s)

[1] Vieira, et al. (2000)

Table 2. Chemical shifts in NMR ¹H (200 MHz, CDCl₃) and NMR ¹³C (50 MHz, CDCl₃) of neosergeolide (**2**).

The authenticity of standards is a key-step in quantitative analysis, especially in plant extract analysis. In most cases, authentic standards are not available commercially and this strengths the importance of liquid chromatography. Liquid chromatography enables the isolation of authentic standards at different scales (from microgram until gram scale) and at very high purity that can be used later to perform quantitative analysis. In our study, combining open-column and planar chromatography, we were able to isolate several milligrams of each pure standard, as can be observed at figure 3, that were then used in the quantitative analysis of the quassinoids **1** and **2** in root and stem infusions of *P. sprucei* by HPLC.

The structural authenticity of each standard can be confirmed by the use of modern spectroscopy techniques as MS and NMR. Although these techniques are considered complementary, normally NMR is much more informative. For instance, in our study, HMBC experiments furnished conclusive information that not sergeolide, but neosergeolide was isolated.

As described in the experimental section, samples of stem and root infusions were prepared using approximately 9 g of crushed, dried stems are infused with 1 L of boiling water. HPLC analysis of *P. sprucei* stem and root infusions resulted in the concentrations presented in table 3.

Quassinoid	Root infusion		Stem infusion	
	mg.L^{-1}	μM	mg.L^{-1}	μM
isobrucein B (**1**)	32.0	67.0	14.0	29.0
neosergeolide (**2**)	0.79	1.6	0.38	0.75

Table 3. Concentrations of isobrucein B (**1**) and neosergeolide (**2**) in *P. sprucei* stem and root infusions determined by HPLC-UV at 254 nm.

Consistent with the data presented in table 1 the concentrations of both **1** and **2** are at least twice as large in root infusions as in stem infusions. Interestingly, the percentage of extractives of roots during infusion (5.1 %) is twice that of stems (2.5 %) which would seem to be related to the greater concentration of these constituents in the root infusion.

Comparison of root and stem infusions shows that **1** is about 40 times as concentrated as **2** in both stem and root teas on a molar basis. These data suggest that the more relevant active principle in stem and root infusions analyzed is **1**.

4. Conclusion

The HPLC analysis of infusions (aqueous extracts) of stems and roots of *P. sprucei* revealed higher quantities of isobrucein B than neosergeolide, 40 fold, for both infusions. Considering this information and *in vitro* activity of both compounds, it is very likely that isobrucein B plays more important role for the antimalarial activity than neosergeolide.

More research is needed to describe seasonal, regional and specimen specific variation in *P. sprucei* quassinoid composition which should have a direct influence on the composition of stem and root infusions prepared from samples of different origins. Knowledge of the extent of these variations, especially as they influence quassinoid composition in infusions, is of fundamental importance given the valuable medicinal and dangerous toxic properties of these widely used Amazonian remedies.

The high performance liquid chromatography has proved to be a powerful tool in the plant extract analysis. The possibility to perform qualitative and quantitative analysis, by HPLC, enables the development of new phytoterapeutical products from the Amazon biodiversity.

5. Acknowledgment

6. References

Agtmael, M.A.; Eggelte, T.A.; Boxtel, C.J. (1999). Artemisin drugs in the treatment of malaria: from medicinal herb to registered medication. *Trends in Plant Science*,vol. 20, pp. 199-205, ISSN 1366-2570.

Andrade-Neto, V.F.; Pohlit, A.M.; Pinto, A.C.S.; Silva, E.C.C.; Nogueira K.L.; Melo, M.R.S; Henrique, MC; Amorim, R.C.N.; Silva, L.F.R.; Costa, M.R.F.; Nunomura, R.C.S.; Nunomura, S.M.; Alecrim, W.D.; Alecrim, M.G.; Chaves, F.C.M.; Vieira, P.P.R. (2007). *In vitro* inhibition of *Plasmodium falciparum* by substances isolated from Amazonian antimalarial plants. *Memorias do Instituto Oswaldo Cruz* vol. *102*, No. 3, pp. 359-365, ISSN 0074-0276

Bertani, S.; Bourdy, G.; Landau, I.; Robinson, J.C.; Esterre, P.; Deharo, E. (2005). Evaluation of French Guiana traditional antimalarial remedies. *Journal of Ethnopharmacology* Vol. 98, pp. 45-54, ISSN 0378-8741.

Duke, J. A.; Vasquez, R. (1994). *Amazonian ethnobotanical dictionary*, CRC Press, ISBN 0849336643, Florida (USA), 215 pp.

Fandeur, T.; Moretti, C.; Polonsky, J. (1985). *In vitro* and *in vivo* assessment of the antimalarial activity of sergeolide. *Planta Medica,* vol. 51, pp.20-23, ISSN 0032-0943.

Grenand P, Moretti C, Jacquemin H 1987. *Pharmacopées traditionnelles en Guyane, Guyane Française*. Collection Mémoires 108, ORSTOM, Cayenne, French Guiana, 569 pp.

Guo, Z.; Vangapandu, S.; Sindelar, R.W.; Walker, L.A.; Sindelar, R.D. (2005). Biologically active quassinoids and their chemistry: Potentianl leads for drug design. *Current Medicinal Chemistry,* Vol. 12, pp. 173-190, ISSN 1568-0142.

Li, Y.; Wu,T.; Zhu, J.; Wan, L.; Yu, Q.; Li, X.; Cheng, Z.; Guo, C. (2010). Combinative method using HPLC fingerprint and quantitative analyses for qualitaty consistency evaluation of an herbal medicinal preparation produced by different manufaturers.

Journal of Pharmaceutical and Biomedical Analysis, vol. 52, pp. 597-602, ISSN 0731-7085.

Li, Y.; Zhao, J.; Yang, B. (2011). Strategies for quality of Chinese medicines. *Journal of Pharmaceutical and Biomedical Analysis*, vol. 55, pp. 802-809, ISSN 0731-7085.

Milliken, W. (1997). *Plants for malaria. Plants for fever: medicinal species in Latin America - a bibliographic survey*, Kew Publishing, United Kingdom, 122 pp.

Moretti, C.; Polonsky, J.; Vuilhorgne, M.; Prange, T. (1982). Isolation and structure of sergeolide, a potent cytotoxic quassinoid from Picrolemma pseudocoffea. *Tetrahedron Letters*, Vol. 23, pp. 647-650, ISSN 0040-4039.

Nunomura, R.C.S.; Silva, E.C.C.; Oliveira D.F.; Garcia, A.M.; Boeloni, J.N.; Nunomura, S.M.; Pohlit, A.M. (2006). In vitro studies of the anthelmintic activity of *Picrolemma sprucei* Hook. f. (Simaroubaceae). *Acta Amazonica*, Vol. 36, pp. 327-330, ISSN 0044-5967.

Rates, S.M.K. (2001). Plants as source of drugs. *Toxicon*, vol. 39, pp. 603-613. ISSN 0041-0101.

Schpector, J. Z.; Castellano, E.E.; Rodrigues, Filho E.; Vieira, I.J.C. (1994). A new quassinoid isolated from *Piclolemma pseudocoffea*. *Acta Crystallographica C*, Vol. 50, pp. 794-797, ISSN 0108-2701.

Silva, E.C.C. (2006). *Isolamento, transformação química e atividade biológica in vitro dos quassinóides neosergeolida e isobruceína B*. Dissertação de Mestrado, Universidade Federal do Amazonas, Manaus, Amazonas, 191 pp.

Silva E.C.C.; Cavalcanti, B.C.; Amorim, R.C.N.; Lucena, J.F.; Quadros, D.S.; Tadei, W.P.; Montenegro, R.C.; Costa-Lotufo, L.V.; Pessoa, C.; Moraes, M.O.; Nunomura, R.C.S.; Nunomura, S.M.; Melo, M.R.S.; Andrade-Neto, V.F.; Silva, L.F.R.; Vieira, P.P.R.; Pohlit, A.M. (2009). Biological activity of neosergeolide and isobrucein B (and two semi-synthetic derivatives) isolated from the Amazonian medicinal plant *Picrolemma sprucei* (Simaroubaceae). *Memorias do Instituto Oswaldo Cruz*, Vol. 104, pp. 48-55, ISSN 0074-0276.

Silva, M.F.; Lisbôa, P.L.B.; Lisbôa, R.C.L.; (1977). *Nomes vulgares de plantas amazônicas*. INPA, Belém, PA, Brasil, 222 pp.

Sticher, O. (1993). Quality of Ginkgo Preparations. *Planta Medica*, 59, pp 2-11, ISSN 0032-0943.

Street, R.A.; Stirk, W.A.; Van Staden, J. (2008). South African traditional medicinal plant trade – Challenges on regulating quality, safety and efficacy. *Journal of Ethnopharmacology*, vol.119, pp. 705-710, ISSN 0378-8741

Van Beek, T.A. (2002). Chemical analysis of *Ginkgo biloba* leaves and extracts. *Journal of Chromatography A*, vol. 967, pp. 21-55, ISSN 0021-9673.

Vieira, I.J.C.; Rodrigues-Filho, E.; Fernandes, J.B.; da Silva, M.F.G.F.; Vieira, P.C. (2000). Complete ^{1}H and ^{13}C chemical shift assignments of a new C_{22}-quassinoid isolated from *Picrolemma sprucei* Hook. by NMR spectroscopy. *Magnetic Resonance in Chemistry*, Vol. 38, pp. 805-808, ISSN 0749-1581.

Vigneron, M.; Deparis, X.; Deharo, E.; Bourdy, G. (2005). Antimalarial remedies in French Guiana: a knowledge attitudes and practices study. *Journal of Ethnopharmacology*, Vol. 98, pp. 351-360, ISSN 0378-8741.

WHO (1998). Quality control methods for medicinal plant materials. ISBN 92 4 154510 0, Geneva (Switzerland), 127 pp.
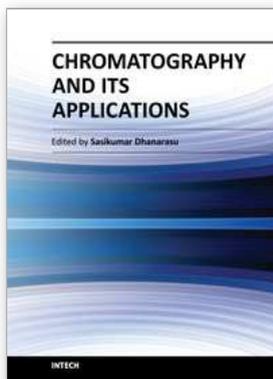
Chromatography and Its Applications

Edited by Dr. Sasikumar Dhanarasu

Chromatography is a powerful separation tool that is used in all branches of science, and is often the only means of separating components from complex mixtures. The Russian botanist Mikhail Tswett coined the term chromatography in 1906. The first analytical use of chromatography was described by James and Martin in 1952, for the use of gas chromatography for the analysis of fatty acid mixtures. A wide range of chromatographic procedures makes use of differences in size, binding affinities, charge, and other properties. Many types of chromatography have been developed. These include Column chromatography, High performance liquid chromatography (HPLC), Gas chromatography, Size exclusion chromatography, Ion exchange chromatography etc. In this book contains more details about the applications of chromatography by various research findings. Each and every topics of this book have included lists of references at the end to provide students and researchers with starting points for independent chromatography explorations. I welcome comments, criticisms, and suggestions from students, faculty and researchers.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Rita C. S. Nunomura, Ellen C. C. Silva, Sergio M. Nunomura, Ana C. F. Amaral, Alaíde S. Barreto, Antonio C. Siani and Adrian M. Pohlit (2012). Quantification of Antimalarial Quassinoids Neosergeolide and Isobrucein B in Stem and Root Infusions of Picrolemma sprucei Hook F. by HPLC-UV Analysis, Chromatography and Its Applications, Dr. Sasikumar Dhanarasu (Ed.), ISBN: 978-953-51-0357-8, InTech, Available from: http://www.intechopen.com/books/chromatography-and-its-applications/quantification-of-antimalarial-quassinoids-neosergeolide-and-isobrucein-b-in-stem-and-root-infusions

INTECH
open science | open minds