NMR Characterization of Bioactive Lignans from *Phyllanthus amarus* Schum & Thorn

M. A. M. Maciel, A. F. Cunha, T. N. C. Dantas
Departamento de Química, Universidade Federal do Rio Grande do Norte, Campus Universitário, 59078-970, Natal, RN, Brazil
mammaciel@hotmail.com

C. R. Kaiser
Instituto de Química, Universidade Federal do Rio de Janeiro, 21941-972, Rio de Janeiro, RJ, Brazil

Keywords: *Phyllanthus amarus Schum & Thom*; Euphorbiaceae; bioactive lignans.

Abstract: *Phyllanthus niruri L. and P. amarus Schum & Thom are targets of current phytopharmacological researches around the world, in which they have been cited as synonymous. However, a recent botanic study indicated significant differences between these species, the main of them were found to be in base, seed and stigma type. In this work P. amarus was unambiguously identified and a phytochemical investigation of its methanolic extract obtained from the whole plant, revealed the presence of six bioactive lignans [isolintetralin (2,3-demethoxy-seco-isolintetralin diacetate), demethylenedioxy-niranthin, 5-demethoxy-niranthin, niranthin, phyllanthin and hypophyllanthin] and one triterpene (2Z, 6Z, 10Z, 14E, 18E, 22E-farnesil farnesol).*

Introduction

Phyllanthus (Euphorbiaceae) a native genus of the American continent is a representative medicinal plant with a higher concentration (about 750 species) in tropical and subtropical countries.[1,2] This genus is widely distributed in Brazilian territory and has long been used in folk medicine.[1-3] *Phyllanthus niruri* L. and *Phyllanthus amarus* Schum & Thorn have been frequent targets of ethnopharmacological work all over the world, mainly in U.S.A, Malaysia, Cuba, Peru, Caribbean, China, Nigeria, Africa, India and Brazil. Despite the large number of literature register these species are cited as being synonymous. However, a recent botanic study performed in Brazil, proved to exist significant differences between *Phyllanthus niruri* L. and *Phyllanthus amarus* Schum & Thorn. According to this study, the main differences were evidenced to its base, seed and stigma type. Specifically, *Phyllanthus niruri* L. presents asymmetric base of lamina, capitated stigma and seeds with many *verrucula* in longitudinal lines. Meanwhile, *Phyllanthus amarus* Schum & Thorn has asymmetric base of lamina, but the stigma is not capitated and the seeds are striated.[4,5] Additionally, it is important to highlight that Webster, in 1957, notified that *Phyllanthus niruri* L. species had never been confirmed out of the American continent.[6] Considering these reports the evaluation of the whole published chemistry, pharmacology and therapeutic potential of *Phyllanthus niruri* L. and *Phyllanthus amarus* Schum & Thorn, as well as *Phyllanthus sellowianus* Mull. Arg. is a difficult task, and does not bring satisfactory results, since *P. amarus* and *P. sellowianus* are considered a variation of

P. niruri, or the three of them might also be considered synonymous.

Concerning to their pharmacological potential, many of their traditional therapeutic properties were scientifically validated, being *Phyllanthus niruri* L. an effective agent in uric acid elimination and liver diseases which cause jaundice (icterus), as well as diuretic, antioxidant, antitumoral, anticarcinogenic, antigenotoxic, antimicrobial, antiplasmodial, antiinflammatory, antinociceptive, analgesic, antimalarial, antiviral (including action against HIV), hepatoprotector, hypoglycemic and hypolipidemic[7-17] and *Phyllanthus amarus* Schum & Thorn proved to be diuretic, antiinflammatory, antioxidant, antiviral, antitumoral, antimutagenic, antispasmodic, antiulcerogenic, hepatoprotector, hypoglycemic and hypotensive.[7,8,10,18-31]

The previously mistakable identification of *Phyllanthus amarus* Schum & Thorn, and its medicinal importance motivated the present work, in which this plant was submitted to classical phytochemical evaluation using a specimen collected in the northern region of Brazil.

Experimental

The infrared spectra were registered on Fourier transform BioRad Excalibur Series FTS 3000MX spectrophotometer and KBr was used for solid substances with approximately 1% of sample. The absorption frequencies were measured in units of wave number (cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in Varian Gemini spectrometer, at 300 and 75 MHz, respectively. The signal of the solvent, which was employed to solubilize the samples, as used as a reference pattern. Sigma and Merck deuterated solvents were used in NMR spectroscopy. Merck silica gel (230-80 Mesh) and, Queel and Vetec, Brazil, solvents (MeOH, hexane and ethyl acetate) were utilized for the chromatographic column separations. The TLC was realized with Vetec, Brazil, silica gel 60H, chloroform, hexane and ethyl acetate. TCL revelation procedure was performed with sulphuric acid and methanol (1:1).

Plant material

Phyllanthus amarus was collected in Natal, the capital city of Rio Grande do Norte, Brazil. The plant identification was unambiguously performed by Dr. Maria Iracema Bezerra Loiola and a voucher specimen (no. 1645) has been deposited in Herbarium of the Departamento de Botânica, Ecologia and Zoologia da UFRN.

The dried vegetal material (124.5 g of the whole plant) was submitted to extraction (by percolation method) with MeOH. The obtained filtrate was reduced under vacuum leading 13.6% of the *Phyllanthus amarus* alcoholic extract (16.9g). The chromatographic procedure was performed with silica gel (230-80 Mesh) column, giving a total of 43 fraction. The triterpene 2Z, 6Z, 10Z, 14E, 18E, 22E-farnesil farnesol was identified in the F_{11-16} fraction group, eluted with a mixture of hexane:$CHCl_3$ (90:10). The obtained lignans were identified in the fraction F_{36} to F_{43}, in that F_{36-40} fraction group was eluted with a mixture of hexane:EtOAc in polarity gradient (50:50 – 0:100); whilst F_{41-43} fraction group was eluted using EtOAc.

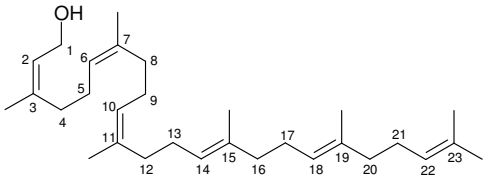
Results and discussion

The phytochemical study performed with the methanolic extract obtained from the whole plant of *Phyllanthus amarus* Schum & Thorn lead to the identification of the triterpene 2Z, 6Z, 10Z, 14E, 18E, 22E-farnesil farnesol and six bioactive lignans: isolintetralin (2,3-demethoxy-*seco*-isolintetralin diacetate), niranthin, 5-demethoxy-niranthin, demethylenedioxy-niranthin, phyllanthin and hypophyllanthin (Figure 1), which presented antioxidant, antiinflammatory and hepatoprotector effects.[32-35] The triterpene was identified in the F_{11-16} fraction group [the infrared (IR) data had shown absorptions of the OH (3410 cm^{-1}), CH_3, CH_2 and CH-alkyl groups (2918 and 2851 cm^{-1})], and its NMR data showed to be coherent to the literature data.[36]

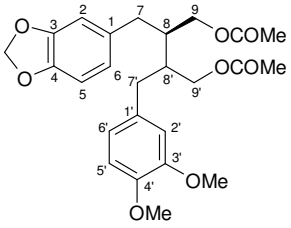
The lignans were identified in the F_{36-40} and F_{41-43} fraction groups. The IR spectrum of the isolintetralin, demethylenedioxy-niranthin, 5-demethoxy-niranthin, niranthin, phyllanthin and hypophyllanthin lignans mixture showed distinctive bands of CH_2 and CH-alkyl groups, through a large and intense band in 2926 cm^{-1} that suggested the presence of more than one substance, C=C aromatic ring groups (1591, 1508 and 1454 cm^{-1}) and C-O groups (1250, 1112 and 1030 cm^{-1}). In this spectrum, the hydroxyl groups of demethylenedioxy-niranthin absorbed in 3502 and 3306 cm^{-1}, and the ester carbonyl moiety equivalent for the 5-demethoxy-niranthin lignan, in 1726 cm^{-1}.

The ^{1}H-NMR data of the lignans mixture (isolintetralin, demethylenedioxy-niranthin, 5-demethoxy-niranthin and niranthin) presented distinctive aromatic hydrogen region [H-Ar: δ 6.72 – 6.53 (m)]; methylenedioxy moieties [O-CH_2-O: δ 5.85 (s)]; methoxyl groups bonded to the aromatic ring (OMe-Ar: δ 3.81 – 3.73, 3.51); methoxyl groups bonded to the alkyl moieties (OMe-alkyl: δ 3.31 – 3.19); methynic groups [CH: δ 2.00 – 1.94 (m)]; methylenic groups [CH_2: δ 3.00 – 2.54, 2.26 (m)]; and hydroxylic groups [particularly the demethylenedioxy-niranthin OH: δ 5.66 (s) and 5.58 (s)]. Isolintetralin, and 5-demethoxy-niranthin (detected in the F_{36-40} fraction group) only differ in C-9 and C-9' (CH_2-alkyl) substituted groups, which contain ester and methoxyl moieties, respectively. The observed ^{1}H-NMR differences data of these lignans are coherent with the literature data, as well as their ^{13}C-NMR data.[35,37] Niranthin and demethylenedioxy-niranthin only differ in C-3 and C-4 substituted groups, which contain cyclic dioxymethylene and hydroxyl moieties, respectively. Significant differences were observed among the C-2, C-4, C-6 and C-7 carbon absorbances, as a result of mesomeric effects (electron delocalization) caused by the oxygen of the cyclic or hydroxyl group, which might have encountered in a hydrogen binding. The observed differences were in accordance with previously reported data.[35] The ^{1}H-NMR spectrum of the F_{41-43} fraction group confirmed the presence of aromatic hydrogen; deoxymethylene moieties; methoxyl groups bonded to the aromatic ring; methoxyl groups bonded to alkyl moieties; methynic and methylenic groups; and hydroxylic groups, suggesting the presence of isolintetralin, demethylenedioxy-niranthin, 5-demethoxy-niranthin and niranthin mixed with two other lignans phyllanthin and hypophyllanthin. These last lignans were specially detected through the

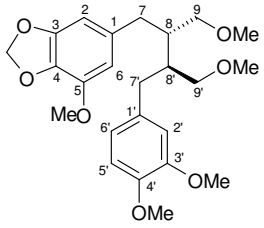
^{13}C-NMR data analysis; the attributed data was in accordance with previously reports data.[33] The assignments of the ^{1}H and ^{13}C NMR spectra of the triterpene farnesil farnesol were performed by one- and two-dimentional NMR analysis. The following describes other absorbance evidences that support the presence of this triterpene in *Phyllanthus amarus* Schum & Thorn, as well as the presence of those cited above lignans, which ^{13}C NMR spectra data are presented below.



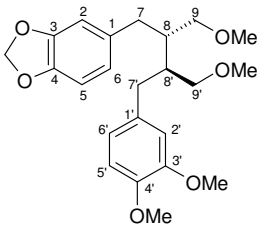
Farnesil farnesol



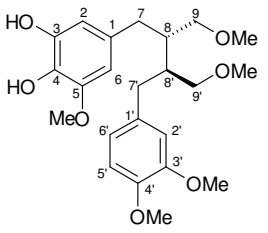
Isolintetralin



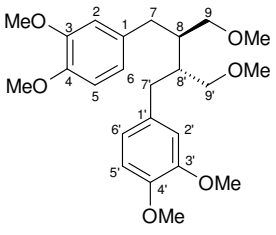
Niranthin



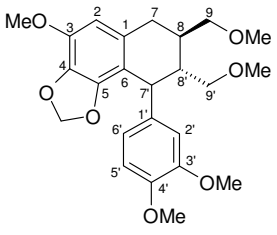
5-Demethoxy-niranthin



Dimethylenodioxy-niranthin



Phyllanthin



Hypophyllanthin

Figure 1. *Phyllanthus amarus* chemical constituents

2Z, 6Z, 10Z, 14E, 18E, 22E-farnesil farnesol: ^{1}H-NMR ($CDCl_3$) *δ*: 4.06 (H-1), 5.40 (H-2), 2.02 (H-4), 2.05 (H-5), 5.07 (H-6), 2.02 (H-8), 2.05 (H-9), 5.07 (H-10), 2.02 (H-12), 2.05 (H-13), 5.07 (H-14), 2.05 (H-16), 2.02 (H-17), 5.07 (H-18), 2.05 (H-20), 2.02 (H-21), 5.07 (H-22), 1.66 (H-24), 1.57 (H-25), 1.57 (H-26), 1.57 (H-27), 1.66 (H-28), 1.66 (H-29), 1.73 (H-30). ^{13}C-NMR ($CDCl_3$) *δ*: 59.18 (C-1), 125.19 (C-2), 139.98 (C-3), 32.11 (C-4), 26.57 (C-5), 125.10 (C-6), 135.54 (C-7), 26.57 (C-9), 125.10 (C-10), 135.54 (C-11), 32.11 (C-12), 26.57 (C-13), 124.57 (C-14), 135.41 (C-15), 39.54 (C-16), 29.84 (C-17), 124.42 (C-18), 135.06 (C-19), 39.54 (C-20), 26.93 (C-21), 124.30 (C-22), 135.06 (C-23), 25.70 (C-24), 16.18 (C-25), 14.30 (C-26), 14.47 (C-27), 22.88 (C-28), 22.88 (C-29), 22.88 (C-30).

2,3-Demethoxy-*seco*-isolintetralin diacetate (isolintetralin): ^{13}C-NMR ($CDCl_3$) *δ*: 133.69, 132.14 (C-1', C-1), 112.23, 110.87 (C-2', C-2), 147,18, 147.01 (C-3', C-3), 148.90, 148.78 (C-4', C-4), 112.23, 111.03 (C-5', C-5), 121.91, 121.18 (C-6', C-6), 35.03, 35.54 (C-7', C-7), 40.79 (C-8', C-8), 55.84, 55.93 (O**Me**-Ar), 101.31 (O-**CH_2**-O).

5-Demethoxy-niranthin: ^{13}C-NMR ($CDCl_3$) *δ*: 133.69 (C-1'), 135.77 (C-1), 112.23 (C-2'), 110.87 (C-2), 148.90 (C-3'), 147.18 (C-3), 147.01 (C-4'), 111.03 (C-5'), 108.12 (C-5), 121.18 (C-6'), 121.91 (C-6), 35.03 (C-7'), 35.54 (C-7), 42.00 (C-8'), 40.79 (C-8), 59.05, 58.89 (O**Me**-alquil), 56.56, 56.48 (O**Me**-Ar), 101.23 (O-**CH_2**-O).

Niranthin: ^{13}C-NMR ($CDCl_3$) *δ*: 135.77 (C-1), 101.31 (C-2), 133.69 (C-4), 148.78 (C-5), 108.16 (C-6), 35.03 (C-7), 40.79 (C-8), 133.69 (C-1'), 112.23 (C-2'), 147.18 (C-3'), 148.90 (C-4'), 111.03 (C-5'), 121.18 (C-6'), 35.03 (C-7'), 40.79 (C-8'), 72.72 (**CH_2**-OMe), 101.23 (O**CH_2**O), 58.89 (O**Me**-alquil), 55.99, 55.93, 55.84 (O**Me**-Ar).

Demethylenedioxy-niranthin: ^{13}C-NMR ($CDCl_3$) *δ*: 135.77 (C-1), 108.12 (C-2), 148.78 (C-3), 148.90 (C-4), 148.78 (C-5), 106.55 (C-6), 37.75 (C-7), 40.79 (C-8), 133.69 (C-1'), 112.23 (C-2'), 147.18 (C-3'), 147.01 (C-4'), 111.03 (C-5'), 121.91 (C-6'), 35.03(C-7'), 40.79 (C-8'), 71.36, 72.72 (C-9 and C-9'-**CH_2**-OCH_3), 58.89, 55.99 (O**Me**-alquil), 55.93, 55.84 (O**Me**-Ar).

Phyllanthin: ^{13}C-NMR ($CDCl_3$) *δ*: 133.75 (C-1, C-1'), 112.27 (C-2, C-2'), 148.85 (C-3, C-3'), 147.08 (C-4, C-4'), 111.07 (C-5, C-5'), 121.25 (C-6, C-6'), 35.11 (C-7, C-7'), 40.88 (C-8, C-8'), 72.78 (C-9), 72.66 (C-9'), 55.92, 56.00 (OMe-Ar), 58.98 (O**CH_3**-9, 9').

Hypophyllanthin: ^{13}C-NMR ($CDCl_3$; 50MHz; *δ* ppm)]: 131.96 (C-1); 138.15 (C-1'); 106.55 (C-2); 111.84 (C-2'); 143.52 (C-3); 148.85 (C-3'); 133.75 (C-4); 147.08 (C-4'); 147.08 (C-5); 110.75 (C-5'); 115.25 (C-6); 120.60 (C-6'); 33.30 (C-7); 42.08 (C-7'); 35.62 (C-8); 45.59 (C-8'); 75.47 (C-9, **CH_2**-OCH_3); 71.87 (C-9'); 56.63; 55.92 (O**Me**-Ar); 58.98; 59.12 (O**CH_3**-9; 9'); 101.33 (O-**CH_2**-O).

Conclusion

The present phytochemical study performed with *Phyllanthus amarus* Schum & Thorn (a specimen collected in the northern region of Brazil) indicated a strong chemical compatibility with other *Phyllanthus* specimen. Six special metabolites from the lignan class, and one acyclic triterpene were identified and characterized by NMR spectra data. The lignans phyllanthin and hypophyllanthin were previously isolated from *Phyllanthus amarus* Schum & Thorn[32,33] (a specimen from different origin); isolintetralin (2,3-demethoxy-seco-isolintetralin diacetate)[34], niranthin, 5-demethoxy-niranthin, and demethylenedioxy-niranthin were isolated from *Phyllanthus niruri* L. and other specimens from *Phyllanthus* genera[35,37,] and the triterpene (2Z, 6Z, 10Z, 14E, 18E, 22E-farnesil farnesol) from *Phyllanthus niruri* L.[36] Since *Phyllanthus niruri* L. and *Phyllanthus amarus* Schum & Thorn are not any more considered synonymous, this present work bring satisfactory phytochemical results, contributing to the scientific advancement of this broadly used medicinal species *P. amarus*.

Acknowledgment

The authors thank Professor Dr. Maria Iracema Bezerra Loiola (UFRN) for the botanic identification and CNPq for scholarship and financial support.

References

1. C. Castro-Chaves, A. M. F. Cunha, G. M. C. Cruz, D. V. Oliveira, *Rev. Bras. Farmacog.* **12** (2002) 2.
2. M. P. Guerra, R. O. Nodari, *Farmacognosia da Planta ao Medicamento*. UFRGS, UFSC, Porto Alegre, Florianópolis (2003).
3. F. J. A. Matos, M. P. Sousa, M. E. O. Matos, M. I. L. Machado, A. A., Craveiro *Constituintes químicos ativos e propriedades biológicas de plantas medicinais brasileiras*. UFC ed., Fortaleza, Brazil (2004).
4. M. J. Silva, *O gênero Phyllanthus L. (Phyllanthaceae Martynov) no Nordeste do Brasil, com ênfase nas espécies de Pernambuco*. Dissertação de Mestrado, Recife, PE, UFPE (2006).
5. M. J. Silva, M. F. Sales, O gênero *Phyllanthus* L. (Phyllantheae – Euphorbiaceae Juss.) no bioma caatinga do estado de PE-Brasil. *Rodriguésia* **55** (2004) 101.
6. G. L. Webster, *J. Arnold Arboretum* **38** (1957) 51.
7. A. R. S. Santos, R. O. P. De Campos, O. G. Miguel, V. C. Filho, A. C. Siani, R. A. Yunes, J. B. Calixto, *J. Ethnopharmacol.* **72** *(*2000) 229.
8. J. B. Calixto, A. R. Santos, V. Cechinel Filho, R. A. Yunes, *Med. Res. Rev.* **18** (1998) 225.
9. R. Harish, T. Shivanandappa, *Food Chemistry* **95** (2006) 180.
10. B. Gilbert, J. L. P. Ferreira, L. F. Alves, *Monografias de plantas medicinais brasileiras e aclimatadas*. Curitiba: Abifito (2005).
11. H. M. Subeki, K. Takahashi, M. Yamasaki, O. Yamato, Y. Maede, K. Katakura, S. Kobayashi, C. Trimurningshi, T. Yoshihara, *J. Nat. Prod.* **68** (2005) 537.
12. R. K. Cimanga, L. Tona, N. Luyindula, K. Mesia, M. Lusakibanza, C. T. Musuamba, S. Apers, T. De Bruyne, S. Van Miert, N. Hermans, J. Totté, L. Pieters, A. J. Vlietinck, *J. Ethnopharmcol.* **95** (2004) 399.
13. A. K. Khanna, F. Rizvi, R. Chander, *J. Ethnopharmacol.* **82** (2002) 19.

14. L. Tona, N. P. Ngimbi, M. Tsalaka, K. Mesia, K. Cimanga, S. Apers, T. De Bruyne, L. Pieters, J. Tollé, A. J. Vlietinck, *J. Ethnopharmacol.* **68** (1999) 193.

15. J. Qian-Cutrone, S. Huang, J. Trimble, H. Li, P. Lin, A. Masud, S. E. Klohr, K. F. Kadow, *J. Nat. Prod.* **59** (1996) 196.

16. T. Ogata, H. Higuchi, S. Mochida, H. Matsumoto, A. Kato, T. Endo, A. Kaji, H. Kaji, *Res. Human Retroviruses* **8** (1992) 1937.

17. P. S. Venkateswaran, I. Millman, B. S. Blumberg, *Proc. Nat. Acad. Sci. USA*. **84** (1987) 274.

18. S. P. Thyagarajan, S. Jayaram, *Indian J. Med. Microbiol.* **10** (1992) 64.

19. N. V. Rajeshkumar, R. Kuttan, *J. Ethnopharmacol.* **73** (2000) 215.

20. C. A. L. Kassuya, A. A. Silvestre, V. L. G. Rehder, J. C. Calixto, *Eur. J. Pharmacol.* **478** (2003) 145.

21. A. K. Kiemer, T. Hartung, C. Huber, A. M. Vollmar, *J. Hepatol.* **38** (2003) 289.

22. K. R. Raphael, R. Kuttan, *J. Ethnopharmacol.* **87** (2003) 193.

23. N. V. Rajeshkumar, K. L. Joy, G. Kuttan, R. S. Ramsewak, M. G. Nair, R. Kuttan, *J. Ethnopharmacol.* **81** (2002) 17.

24. R. K. Raphael, T. A. Ajith, S. Joseph, R. Kuttan, *Teratogenesis, Carcinogeneses, and Mutagenesis* **22** (2002) 285.

25. A. A. Odetola, S. M. Akojenu, *Afr. J. Med. Med. Sci.* **29** (2000) 119.

26. S. Jayram, S. P. Thyagarajan, S. Sumathi, S. Manjula, S. Malathi, N. Mandagopalan *Indian. J. Vir.* **1997**, *13*, 59-64.

27. M. Ott, S. P. Thyagarajan, S. Gupta, *Eur. J. Clinical Investig.* **27** (1997) 908.

28. C. D. Lee, M. Ott, S. P. Thyagarajan, D. A. Shafritz, R. D. Burk, S. Gupta, *Eur. J. Clin. Investig.* **26** (1996) 1069.

29. N. Srividya, S. Periwal, *Indian. J. Exp. Biol.* **33** (1995) 861.

30. B. Gowrishanker, O. H. Vivekanandan, *Mutation. Res.* **322** (1994) 185.

31. B. S. Blumberg, I. Millman, P. S. Venkateswaran, S. P. Thyagarajan, *Cancer Detection and Prevention* **14** (1990) 195.

32. A. Sharma, R. T. Singh, S. S. Handa, *Phytochem. Anal.* **4** (1993) 226.

33. A. Somanbadhu, S. Nityangkuru, C. Mahidol, *J. Nat. Prod.* **56** (1993) 233.

34. Y. L. Huang, C. C. Chen, J. C. Ou, Planta Med. **58** (1992) 473.

35. P. Satyanarayana, S. Venkateswarlu, *Tetrahedron* **47** (1991) 8931.

36. B. Singh, P. K. Agrawal, R. S. Thakur, *Phytochemistry* **28** (1989) 1980.

37. C. C. Chang, Y. C. Lien, C. S. Karin, C. Liu, S. S. Lee, *Phytochemistry* **63** (2003) 825.