PharmacologyOnLine August 30, 2016

Archives • 2016 • vol.2 • 38-41

IDENTIFICATION AND *IN VITRO* EVALUATION OF ANTIFUNGAL ACTIVITY OF ALKALOIDS FROM *SIPARUNA SESSILIFLORA* KUNTH A. DC. LEAVES

Guevara, A. J.[1]; Pardo, A. I.[2], Díaz, J. E.[1]; Arias-Palacios, J.[2]; Celis C.[1]; Loaiza, A. E.[1*]

[1]Department of Chemistry, Pontificia Universidad Javeriana, Bogotá, Colombia
[2]Department of Microbiology, Faculty of Sciences, Pontificia Universidad Javeriana, Bogotá, Colombia

[*]aloaiza@javeriana.edu.co

Abstract

Total alkaloid extract of the leaves of *Siparuna sessiliflora* was analyzed by gas chromatography coupled to a mass detector (GC-MS) and nuclear magnetic resonance (NMR). Ushinsunine and 4'-O-methyl-N-methylcoclaurine, were identified as major compounds, along with liriodenine, corydine, N-methyllaurotetanine, boldine, N-methylcoridaldine, nornuciferine, stepholidine and nantenine. The alkaloids ushinsunine, 4'-O-methyl-N-methylcoclaurine and liriodenine were isolated and their identities confirmed by MS and NMR. Antifungal activity of alkaloid total extract was evaluated against *Fusarium oxysporum, Alternaria* sp. and *Aspergillus niger*, displaying low antifungal activity against these strains.

Keywords: Alkaloids, Antifungal activity, *Siparuna sessiliflora.*

Introduction

The Siparuna genus (family Siparunacea), is widely distributed in the southern hemisphere, mainly in tropical regions of South America. Some species of this genus, are used empirically in traditional medicine by several indigenous communities, reason why many of these species are considered promising [1,2]. This genus is characterized for producing secondary metabolites such as sesquiterpenes, benzyltetrahydroisoquinoline, aporphine alkaloids and flavonoids [1].

The *Siparuna sessiliflora* species (common name limoncillo de monte) is used by some indigenous communities in Colombia to treat a variety of health problems such as fever, headache, rheumatism, herpes and insect bites [2], however, there are only a few reports related to the phytochemical studies and the evaluation of its biological activity [3,4].

In order to contribute to the knowledge of this species, we undertook a study focused on the identification and evaluation of the antifungal activity of the alkaloids present in their leaves. Here in we report the results found in this regard.

Methods

General experimental procedures

^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance spectrometer (300 and 75 MHz, respectively) in $CDCl_3$. All chemical shifts were quoted in respect to residual proton signals of $CDCl_3$ and methanol-d_4. GC-MS analyses were performed on an Agilent 6850 series II gas chromatograph coupled to an Agilent 5975B VL mass spectrometer (electron ionization, 70 eV) equipped with split/splitless inlet (split relation 20:1, 280°C), Agilent 6850 series automatic injector, and Agilent HP-5MS column (30 m × 0.25 mm × 0.25 µm); initial oven temperature 100°C, then a temperature ramp of 15°C/min to 180°C (hold 1 min); and then a temperature ramp of 5°C/min to 315°C (hold 8 min); total run time 41.33 min. Column chromatography (CC) was performed with silica gel (130–270 mesh). Preparative thin-layer chromatography (pTLC) and thin-layer chromatography (TLC) were performed on silica gel plates GF_{254} (20 × 20 cm with a layer thickness of 1.0 mm and 0.2 mm) and developed under UV (254 nm) fluorescence.

Plant Material

Leaves of the species *Siparuna sessiliflora* were collected in the environmental path Mogambo, on the sidewalk Brasil, municipality of Viotá (Cundinamarca, Colombia) and a voucher of the species was included in the collection of the herbarium of the Pontificia Universidad Javeriana with identification number 013690.

Preliminary phytochemical screening

A sample of 50 g of dried and ground leaves was used to carry out preliminary screening tests to stablish the presence or absence of components like saponins, phenolic compounds, alkaloids, tannins, flavonoids and triterpenes [5].

Alkaloids extraction

A sample of 844 g of dried and ground plant material was extracted with petroleum ether at room temperature to remove non polar compounds and after that, was placed into a Soxhlet using dichloromethane and refluxed for 7 days. The solvent was evaporated under low pressure and the residue was extracted with 5% HCl. The aqueous phase was basified with NH_4OH to pH ≥ 8 and again extracted with dichloromethane. The organic phase was dried with anhydrous Na_2SO_4, filtered and concentrated to produce 1.047 g (0.124%) of total alkaloid extract.

Purification and identification of alkaloids

Total alkaloid extract was analysed by GC-MS and compounds were primarily identified by comparison of standard spectra library NIST05a.L and NMR profile. This extract (1 g) was partitioned by CC with 10 g of silica gel inactivated with 1 mL of water, using the following solvents: hexane (150 mL), dichloromethane (150 mL), ethyl acetate (200 mL) and methanol (200 mL). Ethyl acetate fraction (586.7 mg) was chromatographed by CC using silica gel (30 g) inactivated with 3 mL of water, eluting with a gradient of dichloromethane–ethyl acetate-methanol to afford three subfractions: Fr. 1 (173.4 mg), Fr. 2 (207.8 mg) and Fr. 3 (105.9 mg). Fr. 1 was separated by pTLC to obtain two compounds (compound **1**: 54.5 mg and compound **2**: 23.4 mg). Fr. 2 was separated by CC to obtain compound **3** (123.4 mg). These isolated compounds were analysed by NMR and GC-MS.

Antifungal susceptibility testing

These assays were carried by M38-A broth microdilution method [6], changing RPMI broth by Muller Hinton supplemented with glucose 2% [7], against *Aspergillus niger*, *Fusarium oxysporum* and *Alternaria* sp., at concentrations from 0.75 mg/mL to 4 mg/mL.

Results and Discussion

Preliminary phytochemical screening

To characterize the qualitative chemical profile, preliminary phytochemical tests were accomplished showing positive results for flavonoids, phenolic compounds, triterpenes and alkaloids; and negative for tannins and quinones.

Purification and identification of alkaloids

Total alkaloid extract of leaves of *S. sessiliflora* was analysed by GC-MS and this primary approach was complemented with the analysis of NMR profiles, allowing to identify ushinsunine and 4'-O-methyl-N-methylcoclaurine as major compounds. Comparison of MS spectra with library NIST05a.L showed also the presence of liriodenine, corydine, N-methyllaurotetanine, boldine, N-methylcoridaldine, nornuciferine, stepholidine and nantenine.

Column and preparative thin layer chromatography allowed isolating compounds **1**, **2** and **3** whose identities were stablished and confirmed by GC-MS, NMR and the comparison with literature reports, as ushinsunine [8], liriodenine [9] and 4'-O-methyl-N-methylcoclaurine [10] respectively.

Ushinsunine (an aporphinic alkaloid) and 4'-O-methyl-N-methylcoclaurine (an isoquinoline alkaloid) have not been reported before in plants of Siparuna genus, while liriodenine is widely spread in species of this genus according to literature reports [1].

Ushinsunine differentiates, in ^{1}H NMR from its diastereoisomer oliveroline, from the J coupling constant value of hydrogens H-7a and H-8. In oliveroline, these hydrogens have a relative configuration *trans*, showing a coupling constant of 11.5 Hz [11], bigger than the coupling constant of these hydrogens in unshinsunine, which is in the order of 2.7 Hz [8], typical for a relative configuration *cis*.

(-)-Ushinsunine (**1**)

^{l}H NMR (300 MHz, $CDCl_3$, d/ppm, J/Hz): 2.66 (3H, s, N-CH_3), 2.71-2.81 (2H, m, H-5 and H-6), 3.11-3.23 (2H, m, H-5,6), 3.37 (1H, m, H-8), 4.95 (1H, d, J =2.7 Hz, H-7a), 5.98 and 6.13 (1H, d, J = 1.5 Hz, -OCH_2O-), 6.58 (1H, s, H-4), 7.30-7.35 (1H, m, H-10), 7.43-7.48 (1H, m, H-9,11), 8.15 (1H, dd, J = 8.1 and 1.4 Hz, H-12).

^{13}C RMN (75 MHz, $CDCl_3$): 28.63 (C-5), 43.33 (N-CH_3), 53.32(C-6), 66.21 (C-8), 66.98 (C-7a), 100.95 (C-2), 107.76 (C-4), 115.41 (12-b), 121.68, 127.49, 127.96, 128.39, 129.47, 129.84, 130.31, 134.56, 142.97, 147.35.

4'-O-Methyl-N-methylcoclaurine (**3**):

^{1}H RMN (300 MHz, CD_3OD, d/ppm): 2.50 (3H, s, N-CH_3), 2.76-2.78 (1H, m, H-4), 2.78-2.8 (1H, m, H-3), 2.80-2.84 (1H, m, H-1a), 2.90-2.92 (1H, m, H-4), 3.08-3.12 (1H, m, H-1a), 3.2-3.24 (1H, m, H-3), 3.75-3.77 (1H, m, H-1), 3.78 (3H, s, O-CH_3), 3.82 (3H, s, O-CH_3), 6.09 (1H, s, H-8), 6.66 (1H, s, H-5), 6.82 (2H, d, J = 8.6 Hz, H-3' y H-5'), 7.02 (2H, m, d, J = 8.6 Hz, H-2' y H-6').

^{13}C RMN (75 MHz, $CDCl_3$, d/ppm): 24.34 (C-4), 39.14 (C-1a), 40.99 (N-CH_3), 46.05 (C-3), 54.19 (O-CH_3), 54.87 (O-CH_3), 64.55 (C-1), 111.11 (C-5), 113.31 (C-3'), 114.29 (C-8), 128.30 (C-1'), 128.32(C-8a), 130.23 (C-2'), 130.80 (C-4a), 143.7 (C-6), 146.6 (C-7), 158.28 (C-4').

Antifungal activity

Minimal inhibition concentration (MIC) of alkaloid total extract was evaluated against fungus *Alternaria sp*., *Fusarium oxysporum* and *Aspergillus niger*, using broth microdilution susceptibility tests. Total alkaloid extract displayed a MIC of 2 mg/mL against *Fusarium oxysporum* and 4 mg/mL against *Alternaria sp*. It was not found the MIC against *Aspergillus niger* even at the highest concentration evaluated of 4 mg/mL.

In our search, no reports were found about the biological activity of ushinsunine, whereas 4'-O-methyl-N-methylcoclaurine was found to be reported as a potential antihypertensive agent [10].

In conclusion, the chemical identity of the mayor compounds of a total alkaloid extract of the leaves of *Siparuna sessiliflora* species is reported, being the first time that ushinsunine and 4'-O-methyl-N-methylcoclaurineare reported in plants of Siparuna genus. This total extract showed low antifungal activity against *Fusarium oxysporum*, *Alternaria* sp. and *Aspergillus niger*.

Acknowledgments

The authors thank Pontificia Universidad Javeriana (PUJ) for the financial support though the project ID 004360, proposal ID 00004573 and Leonor Rodriguez from Mogambo LTDA Sendero Ambiental for her cooperation.

References

1. Leitão, G.G., Simas, N.K., Soares S.S., de Brito, A.P., Claros, B.M., Brito, T.B., Monache, F.D., Chemistry and pharmacology of Monimiaceae: a special focus on Siparuna and Mollinedia. J Ethnopharmacol 1999; 65: 87-102.
2. Renner, S., Hausner, G., Siparunaceae. Flora neotropica monograph 95. New York: The New York Botanical Garden.2005
3. Mahecha C. Actividad antioxidante y antibacteriana de aceites esenciales extraidos de hojas y frutos de Siparuna sessiliflora [Thesis]. Bogotá, Colombia: Pontificia

Universidad Javeriana; 2010.
4. Padilla, F., Gil, E., Actividad antibacteriana de extractos y fracciones de hojas de Siparuna sessiliflora Kunth A. DC. (limoncillo). Rev Cubana Plant Med 2012; 17: 65–72
5. Dominguez X. Métodos de investigación fitoquímica. Mexico DF: Limusa; 1973.
6. CLSI, Reference method for broth dilution antifungal susceptibility testing filamentous fungi, Approved Standard, 2nd ed., CLSI document M38-A2, 950 West Valley Roadn Suite 2500, Wayne, Pennsylvania 19087, USA, 2008.
7. Fuller, J., Schofield, A., Jiwa, S., et al., Caspofungine test endpoint for Aspergillus isolates shows poor agreement with the reference minimum effective concentration. J Clin Microbiol 2010; 48: 479–482.
8. Chen, C., Chang, F., Wu, Y., The constituents from the stems of annona cherimola. J Chin Chem Soc 1997;44: 313–319.
9. Pang, S., Wang, G., Huang, B., Zhang Y., Qin, L., Isoquinoline alkaloids from Broussonetia papyrifera fruits. Chem Nat Compd 2007; 43: 83–84.
10. Iturriaga-Vásquez, P., Miquel, R., Ivorra, M., D'Ocon M., Cassels, B., Simplified tetrandrine congeners as possible antihypertensive agents with a dual mechanism of action. J Nat Prod 2003; 66: 954–957.
11. Ku, A. F., Cuny, G. D., Synthetic Studies of 7-Oxygenated Aporphine Alkaloids: Preparation of (−)-Oliveroline, (−)-Nornuciferidine, and Derivatives. Org Lett 2015; 17: 1134–1137.