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Assessment of toxicity and differential antimicrobial activity of methanol extract of rhizome of *Simaba ferruginea* A. St.-Hil. and its isolate canthin-6-one

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ABSTRACT

Ethnopharmacological relevance: *Simaba ferruginea* A. St.-Hil., Simaroubaceae, popularly known as “calunga” is a typical subtropical shrub used in Central Brazil mainly for infection, anti-inflammatory, analgesic and gastric duodenal-ulcers. It presents in its composition the alkaloid canthin-6-one, an alkaloid indole β-carboxylic.

Aim: This study aims to investigate the toxicity, antimicrobial activities of methanol extract of *Simaba ferruginea* (MESf) and canthin-6-one by using different experimental models.

Methods: The present study evaluated the phytochemical analysis by high performance liquid chromatography (HPLC), toxicological potential of MESf and canthin-6-one, using the cytotoxicity, genotoxicity assays with CHO-K1 cells and *in vivo* acute test in mice. Antimicrobial activity was evaluated by the broth microdilution assays, while the antimicrobial mechanism of action was also assessed using different *in vitro* bacterial and fungal models.

Results: The HPLC analysis of MESf revealed the presence of canthin-6-one, kaempferol and morin. Differential *in vitro* toxicities were observed between MESf and canthin-6-one. In the cytotoxicity assay, MESf presented toxicity against CHO-K1, while canthin-6-one did not. In the case of *in vitro* genotoxicity, both showed to be potentially genotoxic. In the *in vivo* toxicity study, both MESf (up to 1000 mg/kg) and cantin-6-one (up to 100 mg/kg) caused no toxicologically relevant alterations and are thus considered not to be toxic. MESf was shown to be relatively safe with NOAEL (100 mg/kg) when administrate in mice. Both MESf and canthin-6-one also showed differential antimicrobial activities. On one hand, MESf demonstrated good spectrum of antibacterial action against *Staphylococcus aureus* (MIC 12.5 μg/mL) and *Escherichia coli* (MIC 25 μg/mL) and moderate activity against *Enterococcus faecalis* and *Shigella flexneri* (MIC 200 μg/mL) but no antifungal effect. On the hand, canthin-6-one showed no antibacterial activity, except against *Staphylococcus aureus* (100 μg/mL), but potent *in vitro* fungicidal activity against clinically important *Aspergillus niger* and *Candida* species at MFC intervals ranging from 3.12 to 25 μg/mL. Both MESf and canthin-6-one were bacteriostatic in action. MESf antimicrobial mechanism of actions are associated with changes in the permeability of bacterial membranes, evidenced by the increased entry of hydrophobic antibiotic in *Shigella flexneri*, intense K^+ efflux (*Shigella flexneri*, *Staphylococcus aureus*) and nucleotides leakage (*Staphylococcus aureus*). In the antifungal mode of action, canthin-6-one inhibited *Saccharomyces cerevisiae* growth and including alteration in the cell membrane of *Neurospora crassa*.

Conclusion: The results of this work demonstrated the differential antimicrobial activities of MESf and its alkaloid isolate, canthin-6-one with antibacterial and antifungal activities, respectively. The present study support the popular use of *Simaba ferruginea* in combatting afflictions related to bacterial infections, and demonstrate

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that canthin-6-one as a promising antifungal agent. Both MESf and canthin-6-one are considered non-toxic based on the *in vitro* toxicological study.

1. Introduction

The bacterial and fungal infections represent a major cause of morbidity and mortality worldwide, particularly those acquired in the hospitals. The drugs of microbial or plant origin, account for over 30% of current sales of drugs. Due to increased microbial resistance, the introduction of new antibiotics has become an absolute necessity. In this regards, plants continue to be an important source in the search for new antibiotics, because they are capable of producing substances used as defence mechanism against predation by microorganisms, insects and herbivores (Aoyama and Labinas, 2012).

Brazil has different phyto-geographical domains, which include the Atlantic Forest, Pantanal, *Caatinga, Cerrado* and Amazon Rainforest, and has arouse scientific interest, since this rich source of therapeutic products is negligibly exploited (Faxina et al., 2015). Mato Grosso, is the state with the largest portion of *Cerrado* area in Brazil with an area of 422,125 km^2. The *Cerrado* of Mato Grosso is rich in medicinal species; many of which are used in folk medicine by the local communities to treat several diseases, a pointer for the potential development of herbal medicine and discovery of compounds for use in several applications (Pan et al., 2013).

Simaba ferruginea A. St.-Hil. (*S. ferruginea*), belongs to Simaroubaceae family, which comprises 32 genera and about 200 species. The genus *Simaba* presents 49 species distributed throughout the American continent, with the exception of Argentina, Chile and Uruguay with the highest occurrence in Brazilian territory. *S. ferruginea* popularly known as "calunga" or "feo-da-terra", is a native and endemic plant of Brazilian Cerrado, with scientific synonyms of *Quassia ferrugiena* D. Dietr., *Picrodendron calunga* Mart. Ex. Engl. and *S. hiensis* Moric, 3–5 m high, slightly branched. Its distribution includes the states of Pernambuco, Bahia, Minas Gerais, Goiás and Mato Grosso (Flora do Brasil – *Simaba ferruginea*).

In Mato Grosso, the stem bark and rhizome are used by the population for the treatment of dyspepsia, fever, oedema, wound healing, gastro-duodenal ulcers, dysentery, rheumatic pain, treating wound, obesity, diabetes and other ailments in the forms of cold maceration in water or by decoction and teas. A cup of tea (infusion) made from its dried rhizome is taken twice a day (Bieski et al., 2015; Almeida et al., 2011; Ribeiro et al., 2017).

There are reports on the preliminary studies of its antifungal and analgesic activities (Noldin et al., 2005) and its pharmacological studies demonstrated potent anti-ulcerogenic activity in experimental models (Almeida et al., 2011).

A total of 114 chemical constituents have been isolated from plants of the genus *Simaba* Aubl., which belong to the quassinoids, alkaloids, coumarins, triterpenes, steroids classes and other metabolites (Barbosa et al., 2011). Among these compounds, canthin-6-one, is one of the major constituents of *S. ferruginea* plant and it was first isolated in 1952 from the plant *Pentaceras australis* (Showalter, 2013).

Canthin-6-one is a β-carbonyl indole alkaloid, an amorphous dark yellow, liposoluble and photosensitive solid, generally found mainly in plants of Simaroubaceae and Rutaceae families. Canthin-6-one alkaloid was isolated for the first time by our group from methanol extract of *S. ferruginea* rhizome (MESf) and has been reported to present various biological and pharmacological activities like antibacterial (O'Donnell and Gibbons, 2007; Chen and Chen, 2013), antifungal (Noldin et al., 2005; Lagoutte et al., 2008; Dejos et al., 2014), anti-ulcerogenic (Almeida et al., 2011; Showalter, 2013), anti-inflammatory, antiproliferative, leishmanicidal, antiviral, analgesic, anti-parasitic, antitumor, antidiabetic (Noldin et al., 2005; Amura et al., 2003), and aphrodisiac activities (Kim et al., 2016).

Based on the popular uses of *S. ferruginea* rhizome reported in the literature, we carried out assessment of MESf and canthin-6-one antimicrobial activities against selected human pathogens (bacteria and fungi) of medical importance. We also investigated in this work the toxicity of MESf and canthin-6-one using different *in vitro* models (cytotoxicity, genotoxicity assays) in CHO-K1 cells and *in vitro* acute oral toxicity assessment in mice. In the latter, histopathological analyses was also performed.

2. Materials and methods

2.1. High-performance liquid chromatography (HPLC) analysis

The experiment was performed with an HPLC Shimadzu chromatograph model LC-10 Avp Series equipped with an LC-10 CE pump, DGU-14A degasser, UV–vis (SPD-10A) detector, column oven (CTO-10A) equipped with manual injection Rheodyne (loop, 20 µL), and CLASS LC-10 integrator. The sample was analyzed using a reverse-phase Phenomenex Luna 5 µm C18 (2) (250 × 4.6 mm^2) column with direct-connect C18 Phenomenex Security Guard Cartridges (4 × 3.0 mm^2) filled with similar material as the main column. Chromatographic separation of alkaloids was undertaken *via* isocratic elution with a mobile phase consisting of Milli-Q water, acetonitrile and formic acid (250:250:0.25 v/v). The flow rate eluent was 1.0 mL/min at 40 °C with UV detection at 370 nm for duration of 30 min. Chromatographic separation of flavonoids was undertaken via gradient elution with a mobile phase consisting of A = 0.2% de formic acid in Milli-Q water; B = 0.2% of the formic acid in methanol. The gradient programming was: 0-0.01 min, 5% B; 0.01-5 min, 25% B; 5-10 min, 45% B; 10-16 min, 45% B; 16-20 min, 80% B; 20-25min, 80% B; 25-30 min, 80% B; 30-35 min, 100% B; 35-35.1 min, 100% B. Flowrate: 1.0 mL/min at 35 °C; UV detection at 280 nm.

The alkaloid and flavonoids were identified by comparing the retention times of samples and authentic standards of canthin-6-one, morinn and kaempferol. The content of the compounds was expressed as µgmg of extract (MESf), which was calculated after correlating the area of the analyze with the calibration curves of the standard constructed at concentrations of 0.5–10 µg/mL, with R^2 adjusted at 0.9991. The extract solutions and standard were prepared with mobile phase and filtered through a Millipore® (0.22 mM pore size) membrane.

2.2. Isolation of the alkaloid canthin-6-one

Botanical identification, collection of the rhizomes, preparation of the MESf and isolation of canthin-6-one from *S. ferruginea* have been previously described (Almeida et al., 2011). Due to the low yield of canthin-6-one (0.04% w/w) in the MESf, additional quantities of this alkaloid were purchased from Alpha Chimica (Chatenay-Malabry, France), with register no. 479-43-6. The purity of the purchased compound was confirmed by Gas chromatography–mass spectrometry (GC-MS), Hydrogen-1 nuclear magnetic resonance (^{1}H NMR) and Carbon-13 nuclear magnetic resonance (^{13}C NMR) (Noldin et al., 2005). The plant name was checked at the www.theplantlist.org website on the 23rd of August 2015, and its collection was realized after due authorization by Biodiversity Information Authorization (SISBIO) of the Instituto Brazileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA)/ Ministerio do Meio Ambiente (MMA) under approval no. 23124-1.