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Secondary metabolites from the mistletoes *Struthanthus marginatus* and *Struthanthus concinnus* (Loranthaceae)

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1. Subject and source

Mistletoe is the common name for hemi-parasite plants belonging to several families of the order Santalales, where Loranthaceae is the largest pantropical plant family, with approximately 70 genera and 800 species around the world. Members of this family parasitize a broad range of Gymnosperms and Angiosperms (Deeni and Sadiq, 2002; Costa et al., 2010) and can cause important damages to their hosts leading to great economic losses (Deeni and Sadiq, 2002; Costa et al., 2010). In Brazil there are approximately 10 genera of Loranthaceae with around 100 species (Souza and Lorenzi, 2008). Species of the genus *Struthanthus* (Loranthaceae) are used worldwide and in the Brazilian folk medicine to treat many diseases (Coe and Anderson, 1996; Otero et al., 2000a,b; Stalcup, 2000; Scarpa, 2004; Vieira et al., 2005; Lorenzana-Jiménez et al., 2006; Trojan-Rodrigues et al., 2012). In Brazil, some species of *Struthanthus* are related for the treatment of respiratory tract diseases (Vieira et al., 2005), and some authors refer to their use as a maceration specifically to treat pneumonia and tuberculosis (Stalcup, 2000; Reif, 2007; Silva, 2008). Aerial parts of *Struthanthus marginatus* (Desr.) G. Don (Loranthaceae; voucher specimens RB 468941 and RFA 34490) were collected parasitizing a specimen of *Vernonia* sp. (Asteraceae, RB 469120) in Petrópolis, Rio de Janeiro State, Brazil, and leaves of *Struthanthus concinnus* Mart. (Loranthaceae; voucher specimens RB 469119 and RFA 34484) were collected over a specimen of *Morus alba* (Moraceae, RB 468944) in Nova Friburgo, Rio de Janeiro State, Brazil. Plants were identified by Dr. Carlos Henrique Reif.

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2. Previous work

The chemistry of the genus *Struthanthus* is practically unknown, with only one single report on the isolation and identification of a secondary metabolite – rutin, isolated from *Struthanthus subtilis* (Cordero et al., 2003). Other works refer to phytochemical prospection of different classes of secondary metabolites and report positive reactions on various plant parts of *S. marginatus* for alkaloids, saponins, steroids and triterpenoids, tannins, flavonoids, catechins, sugars, polysaccharides, proteins and aminoacids (Pissinate, 2006; Freire et al., 2011). In another study with this species, the carotenoid content was measured, and lutein-epoxide, lutein, β-carotene, violaxanthin-cycle pigments and neoxanthin were described (Matsubara et al., 2003). High levels of trace elements, including silicon, manganese, iron, copper and zinc were described for *S. marginatus* (Pereira and Felcman, 1998; Freire et al., 2011). Flavonoids, tannins and saponins were detected in the hydroalcoholic extract of fresh leaves *Struthanthus vulgaris* (Vieira et al., 2005), alkaloids in *Struthanthus cassythoides* (Coe et al., 2010), and high amounts of condensed tannins in the leaves, stems, roots and haustorial system of *S. vulgaris* (Salatino et al., 1993). In contrast with the scarce and vague literature information on the chemistry of the genus, there are some reports on the biological activities for some representatives of the genus. The oral administration of extracts from *S. marginatus* leaves provided protection against gastric lesions induced by all the ulcerogenic agents employed without any toxicity detected, which could justify its popular use for gastric disturbances (Freire et al., 2011). Other biological activities include antimicrobial against Gram positive and Gram negative bacterial samples for the hydroalcoholic extract from fresh leaves of *S. vulgaris* (Vieira et al., 2005); the neutralization of the *Bothrops atrox* venom for *Struthanthus orbicularis* extracts (Otero et al., 2000b); dose dependent hypotension and cardiotoxic effects in anesthetized mice for the methanolic extract of *Struthanthus venetus* leaves (Lorenzana-Jiménez et al., 2006); and brine shrimp toxicity for *S. cassythoides* extracts (LC50 1574 μg/mL) (Coe et al., 2010). For *S. concinnus*, despite its wide distribution in Brazil and the reports of its popular use, no phytochemical or biological activity works have been found in the literature.

3. Present study

The plants were dried at room temperature and fragmented in a knife mill. Aerial parts of *S. marginatus* (579.26 g) and leaves of *S. concinnus* (262.20 g) were extracted exhaustively with pure ethanol, which was evaporated under reduced pressure. The residue was suspended with water and then partitioned successively with water and hexane, dichloromethane, ethyl acetate (EtOAc) and n-butanol. The hexane extract of *S. marginatus* (5.9 g) was submitted to purification in silica gel column chromatography (CC), eluted with mixtures of increasing polarities of hexane, EtOAc and methanol (MeOH) to afford 33 fractions (SMH-1–SMH-33). Fractions SMH-9, SMH-13 and SMH-24 were submitted to recrystallization yielding purified compounds characterized as sitosterol (Della Greca et al., 1990) in mixture with a minor quantity of stigmasterol; (**1**) 3-*O*-n-acil-lup-20(29)-en-3β,7β,15α-triol (Fukunaga et al., 1988); and (**2**) 3-*O*-[6′-*O*-n-acil-β-glucosil]-sitosterol (Gomes and Alegrio, 1998) (Fig. 1). The compound structures were established after comparison of their ^{1}H and ^{13}C NMR data with those from the literature. Fraction SMH-7 was submitted to gas chromatography coupled with mass spectrometry (GC–MS) analyses that allowed the identification of a mixture of (**3**) 6,10,14-trimethyl-2-pentadecanone; phytol and lupeol, along with two unidentified triterpenes (MWs 426 and 440).

The hexane extract of *S. concinnus* (6.0 g) was purified over silica gel CC eluted with mixtures of increasing polarities of hexane, EtOAc and MeOH, affording 62 fractions (SCH-1–SCH-62). Fractions SCH-16 and SCH-17 were submitted to recrystallization affording pure compounds that were analyzed by ^{1}H and ^{13}C NMR. Taraxerol (**4**) (Mahato and Kundu, 1994) and obtusifoliol (**5**) (Teresa et al., 1987) were characterized in fractions SCH-16 and SCH-17, respectively. Other fractions were submitted to GC–MS allowing the identification of (**3**) 6,10,14-trimethyl-2-pentadecanone (SCH-18, SCH-19 and SCH-20); phytol (SCH-15); (**6**) taraxasterol, β-amyrin, (**7**) α-amyrenone and (**8**) 24-methylenecycloartanol (SCH-15); and γ-sitosterol (SCH-18) (Fig. 1).

Compounds structures were elucidated using GC–MS by comparison of mass fragmentation pattern with those from computer databank (Wiley/NIST libraries), considering similarity indices over 90% and with those from the literature records.

4. Chemotaxonomic significance

The present work describes, for the first time, the chemistry of two species of *Struthanthus* and, apart from the report on the occurrence of rutin in *S. subtilis* (Cordero et al., 2003), it is also the second report on the chemistry of the genus. Phytochemical examination of the hexane extract from both plants lead to the isolation/identification of the ubiquitous diterpene phytol, as well as some very common steroids (sitosterol and stigmasterol) and triterpenes (lupeol, taraxasterol, taraxerol and β-amyrin). Lupeol is a pentacyclic lupane-type triterpenoid, widely found in edible fruits and vegetables (Gauthier et al., 2011; Siddique and Saleem, 2011) and occurs in a multitude of taxonomically diverse genera (Wal et al., 2011). Lupeol has a potential to act as an anti-inflammatory, anti-bacterial, anti-viral, anti-protozoal, antiproliferative, anti-angiogenic and cholesterol lowering agent (Gauthier et al., 2011; Siddique and Saleem, 2011; Wal et al., 2011). This compound has also been tested for its therapeutic efficiency against conditions including wound healing, cancer, diabetes, cardiovascular disease, renal toxicity and hepatic toxicity (Siddique and Saleem, 2011; Wal et al., 2011). Taraxerol (**4**) is also widely found in nature and has been extensively investigated for its biological activities which include anti-inflammatory activity by the reduction of the