Phytochemistry 151 (2018) 26–31

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Seasonality effect on the composition of oxindole alkaloids from distinct organs of *Uncaria tomentosa* from the Caribbean region of Costa Rica

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A R T I C L E I N F O

Article history:
Received 9 May 2017
Received in revised form
21 March 2018
Accepted 23 March 2018

Keywords:
Uncaria tomentosa
Rubiaceae
Tetracyclic and pentacyclic oxindole alkaloids
Seasonality effect

A B S T R A C T

Uncaria tomentosa (Willd.) D.C. (Rubiaceae), commonly known as "Uña de Gato" or "Cat's Claw", is a tropical vine from the rainforest used in traditional medicine and spread through Central and South America, including Costa Rica. There is an increasing demand for medicinal extracts with biological activity attributed mainly to oxindole alkaloids (OA), where the ratio between tetracyclic (TOA) and pentacyclic oxindole alkaloids (POA) determines its feasibility for medicinal applications. The ratio is affected by distinct factors including the dynamics of environmental conditions during seasons. The purpose of the study was to assess the seasonality effect in oxindole alkaloids content in relation to plant organs from *U. tomentosa* grown in the Caribbean region of Costa Rica. Young leaves followed by mature leaves presented the highest amount of total OA during seasons; for these, isoryncophylline, pteropodine and isomitraphylline, were the predominant OA. The POA/TOA ratio of both leaf materials was nearly 1:1 ($3.2\ mg\ g^{-1}$: $3.1\ mg\ g^{-1}$). Bark and root material showed a pentacyclic chemotype in all seasons with a ratio of 6:1 ($6.7\ mg\ g^{-1}$: $1.3\ mg\ g^{-1}$) with pteropodine and isomitraphylline as the predominant POA. The POA content presented seasonality with a significant increase from rainy to dry season in young leaves, bark and roots. In contrast, TOA amount remained virtually unchanged in all plant parts. Humidity and temperature between the studied seasons were constant except for precipitation, reflecting that differences of water content had an effect in the POA amounts. Further studies of abiotic factors, like water stress, could explain the variation of POA content due to seasonality.

1. Introduction

Uncaria tomentosa is a tropical rainforest vine plant, distributed naturally from Peru to Guatemala (Fazio et al., 2008) and used by Peruvian indigenous groups in traditional medicine (Obregón Vílchez, 1995). In Costa Rica, an *U. tomentosa* cultivar was found in the Caribbean rainforests (Alvarenga Venutolo et al., 2008) and it has been subject to many efforts, allowing the species' domestication, planting, cultivation and reproduction in commercial plantations (Alvarenga Venutolo et al., 2008). Moreover, further research has developed protocols for plant micropropagation (Alvarenga Venutolo, 2010), callus/cell suspension culture (Sánchez-Calvo and Alvarenga-Venutolo, 2015) and scale-up in bioreactor (Alvarenga Venutolo et al., 2014). However, most of the *U. tomentosa* reports are related to the medicinal properties that comprise anti-inflammatory, anti-cancer, contraceptive, anti-asthmatic, anti-arthritic and immunostimulant activity (García Giménez et al., 2010; Heitzman et al., 2005; Keplinger et al., 1999; Obregón Vílchez, 1995; Pero, 2010). The plant's medicinal applications are attributed to different specialized products including oxindole alkaloids (OA), terpene glycosides, sterols, flavonoids, flavons and phenols (Biró-Sándor, 2015). The OA are considered the main active compounds responsible for the medicinal activities and are classified in two chemotypes: tetracyclic (TOA) and pentacylic indole alkaloids (POA) (Reinhard, 1999). Both produce different responses in human health; POA work in the cellular immune system and TOA in the central nervous system and have an

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https://doi.org/10.1016/j.phytochem.2018.03.008
0031-9422/

antagonistic effect when combined (Reinhard, 1999).

Although OA are distributed widely in all plant organs, the root bark is traditionally used for medicinal purposes due to the high amount of POA and formulated as a hydroalcoholic extract with a dry matter in the range of 0.3–1.3% (Alonso, 1998). Thus, diverse cat's claw products are commercialized in different preparations: tea, tablets, capsules and as an aqueous standardized extract (known concentration of OA) such as C-MED-100 and AC-11 (Falkiewicz and Lukasiak, 2001). Consequently, the derived products have been part of diverse *in vitro* and *in vivo* analysis such as Krallendorn (Immodal Pharmaka, Austria), and have been tested in pre-clinical and clinical trials (Falkiewicz and Lukasiak, 2001).

The production of OA is affected during seasons by changes in environmental factors such as water supply, temperature and light as shown in *Tabernaemontana pachysiphon* (Höft et al., 1998). Similarly, *U. tomentosa in vitro* plantlets grown under photoperiod (16 h light) and continuous light showed distinct alkaloid profiles (Luna-Palencia et al., 2013). Laus et al. (1997) observed variation in TOA and POA content in *U. tomentosa* during distinct seasons in Peru, although the reasons remain not understood. Although the POA and TOA content of the Costa Rican cultivar of *U. tomentosa* has been studied (Navarro Hoyos et al., 2015), the seasonality effect still remains unknown though it is highly relevant towards medicinal applications. In this study, we analyzed the effect of seasonal changes in the oxindole alkaloid content (POA and TOA) and their relation to different plant organs (root bark, roots, and young and mature leaves) from the *U. tomentosa* cultivar of Costa Rica.

2. Results and discussion

The sampled material had an age of 10–12 years with a vine growth reaching a height of 30 m and width in the stems of 8–25 cm (Fig. 1). The square branches had a width of 2–6 cm with oval leaves in the branch axis with curved thorns of 1.5–2 cm in length. The root bark corresponded to the dark red phenotype as described by Laus and Keplinger (1994).

The monthly weather parameters during the time of sampling were obtained from the National Weather Institute of Costa Rica (2016) and described as follows: Collection 1 corresponds to the early rainy season (325 ± 16.9 mm precipitation; 25.63 ± 0.8 °C temperature; 90.33 ± 3.3% humidity), collection 2 to the late rainy season (155.3 ± 11.0 mm precipitation; 25.71 ± 0.7 °C temperature; 88.67 ± 2.6% humidity) and collection 3 to the dry season (100.5 ± 9.8 mm precipitation; 25.46 ± 0.7 °C temperature; 86.63 ± 2.7% humidity). Although the temperature and humidity were similar during seasons, precipitation differed remarkably between the rainy and dry season.

2.1. Seasonality effect in oxindole alkaloids

Both POA and TOA were detected in mature and young leaves. In the latter, all alkaloids were present except for isopteropodine and traces of speciophylline and mitraphylline (Fig. 3). The most abundant alkaloids were isoryncophylline, pteropodine and isomitraphylline. Mature leaves revealed a similar profile with reduced OA content with traces of isopteropodine. As expected, higher amounts of isoryncophylline and pteropodine were found in old leaves, as shown by Laus et al. (1997).

It is known that OA are formed in young leaves and transported to mature leaves and roots and stored as isomers (Shellard and Houghton, 1972). As an example, mitraphylline is synthesized in leaves and further isomerized to isomitraphylline and mostly translocated to roots (Laus et al., 1997). In our case, isomitraphylline contents were higher in leaves than in bark and root material, similar to other investigations (Peñaloza et al., 2015). Consequently, variability in POA profile could be caused by environmental conditions and stage of development of the plants.

The OA content in young and mature leaves changed between seasons. In young leaves, we observed an increase in alkaloid content from the early to late rainy season followed by a reduction in the dry season. On the contrary, mature leaves showed no significant variation within the rainy season and a slight reduction during the dry season. The biosynthesis of OA takes place in young leaves (Keplinger et al., 1999), therefore it is possible that young leaves are more susceptible to environmental conditions than mature leaves, resulting in distinct profiles during seasons. Consequently, accumulation of distinct secondary metabolites over time could explain the differences in POA levels between young and mature leaves (Honorio et al., 2017).

Analogous to leaf samples, root material showed smaller amounts of isopteropodine, speciophylline and mitraphylline (Fig. 3). Pteropodine and isomitraphylline appeared in higher amounts and isoryncophylline in lower content. Seasonal effects could only be observed in pteropodine and isomitraphylline with an increase in content from early rainy season towards the dry season. Bark material showed a similar OA profile but in higher content. Pteropodine was the most abundant OA, similar as shown by Domínguez Torrejón et al. (2010) in bark material from Peru.

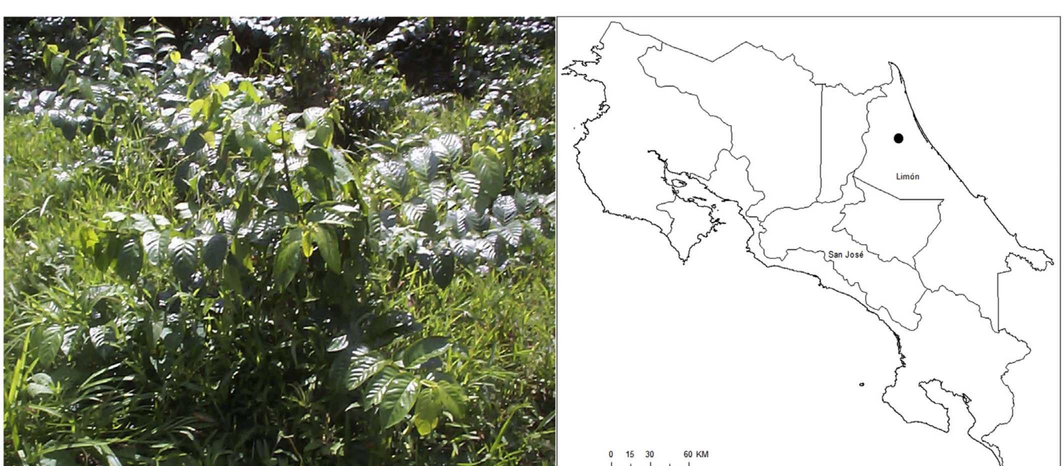

Fig. 1. (A) Domesticated *U. tomentosa* from the organic plantation under agroforestry management. (B) Location of *U. tomentosa* plantation in the Caribbean region of Costa Rica.