ABSTRACT

Plant-derived secondary metabolites are a source of promising bioactive molecules in the search for safer more selective cancer drugs. Mexico’s flora is extremely diverse and many species, such as Phoradendron wattii, form part of traditional medicine. Compounds with notable cytotoxic activity have been isolated from P. wattii, but their concentrations may vary seasonally. The aim was to identify any variation in active metabolite concentrations in Phoradendron wattii methanol extracts in response to season. Betulin exhibited the most evident seasonal variations, being most abundant during the midsummer drought. Cytotoxic activity was highest (29 ± 1 μg/mL) in the rainy season methanol extract. Though not the most abundant metabolite in the extracts, 3α,24-dihydroxy-lup-20(29)-en-28-oic acid is apparently one of the most active among them and is a promising chemotaxonomic biomarker for this species. In summary, secondary metabolite concentrations in P. wattii methanol extracts varied in response to season, and these variations influenced cytotoxic activity.

1. Introduction

Mexico harbors an immensely diverse flora, including many species employed in traditional medicine for centuries to treat several illnesses exhibiting cancer-like symptoms. A study by our group was the first to evaluate the cytotoxic effect of several plants...
forming part of traditional medicine practices in Yucatan, Mexico in cancer cell lines (Caamal-Fuentes et al. 2011). One of the most promising species in this study was *Phoradendron wattii* Krug & Urb. (class: Magnoliophyta; order: Santalales; family: Santalaceae) (synonym: *Phoradendron vernicosum* Greenm.) (Phoradendron wattii Krug & Urb. 1897), from which eleven compounds were isolated, some with notable cytotoxic activity (i.e., 5.22 \( \mu \)g/mL) (Valencia-Chan et al. 2017) (Table S1 and Figure S1). In order to perform further biotechnological evaluation, seasonal variation in *P. wattii* secondary metabolite production, and consequent differences in extract cytotoxic activity, was hypothesised. The Yucatan Peninsula is distinguished by its tropical climate represented by two periods: dry season (November to April) and rainy season (May to October). This rainy season is characterised by a short period of drought (midsummer drought) between June and August (Estrada-Medina et al. 2016). The present study objective was to identify any seasonal variation in the content of previously characterised secondary metabolites in *P. wattii* methanol extracts and evaluate if this variation affects extract cytotoxic activity.

2. Results and discussion

Biological activity against the HeLa cell line was 29 ± 1 \( \mu \)g/mL CC50 in the rainy season, 1,023.3 ± 1.5 \( \mu \)g/mL CC50 in the dry season extract and 100.7 ± 1.5 \( \mu \)g/mL CC50 in the midsummer drought extract (Table 1). This coincides with reports of bioactivity in other hemiparasitic plants such as *Loranthus micranthus* (eastern Nigeria African mistletoe) in which antidiabetic activity was higher in extracts from material collected during the rainy season (Osadebe et al. 2010).

Composition of the *P. wattii* extracts was evaluated using eleven previously isolated and characterised metabolites (Table 1) (Valencia-Chan et al. 2017). The composition of the evaluated extracts was similar in this prior study (Table 1 and Figure S2). However, a quantitative and qualitative seasonal variation was observed in extract metabolite composition in the present study. The midsummer drought extract exhibited the lowest metabolite diversity, although its betulin (2) peak was quite high, representing 72% of the peaks in this extract. This was followed by the dry season extract, with 3–20% variation, and the rainy season extract, with 2–20% variation.

Cytotoxic activity differed between the three extracts (Table 1). The main constituent of the midsummer drought season extract was betulin (2), a lupane-type pentacyclic triterpenoid found as a secondary metabolite in over two hundred plant species (Jäger et al. 2009; Amiri et al. 2020). Betulin (2) anticancer properties have been evaluated previously (see (Amiri et al. 2020) for a detailed list of studies), and it has a reported CC50 >20 \( \mu \)g/mL against HeLa cells. This CC50 is much lower than the >200 \( \mu \)g/mL for the betulin standard (2) and still lower than the 100.7 \( \mu \)g/mL of the midsummer drought extract. Interaction with other more active compounds, such as 3\( \alpha,24\)-Dihydroxylup-20(29)-en-28-oic acid (4) and 3\( \alpha,23\)-O-Isopropylidenyl-3\( \alpha,23\)-dihydroxylup-20(29)-en-28-oic acid (10), is therefore probably occurring.

After inspecting Table 1, we note that the majority compounds were 2, 3, 4 and 10. The level of betulin (2) is the highest in the three extracts but it regains its peak during rainy and dry (midsummer drought) seasons. Betulin (2) is present in *P. wattii* at low levels year-round, but humidity and temperature environmental apparently promote
its synthesis and accumulation (Yin et al. 2015). The midsummer drought extract did have the highest betulin (2) level, but CC$_{50}$ was three-fold lower in the rainy season extract (Tables 1 and S1). This suggests that other, less abundant, compounds such as 3, 4 and 10 may be responsible for extract biological activity, rather than just betulin (2) (see below).

As mentioned, above, compound 4 was notably present in the rainy season extract (13.8 ± 0.3%). This compound’s cytotoxic activity is reported as 11.38 ± 2.6 μg/mL and it is not harmful to normal cells (SI = 4) (Valencia-Chan et al. 2017). The reduced presence of this metabolite in the dry season and midsummer drought extracts may be due to factors such as increased carbohydrate synthesis during dry conditions to improve the probability of survival. Under dry conditions hemiparasitic plant species such as P. wattii may prioritise synthesis of glucose and fructose, rather than of 4. This result suggests that 4 is one of the most active compounds in P. wattii, and that seasonal variations in levels of this metabolite influence extract cytotoxic activity.

### 3. Conclusion

Secondary metabolite composition in P. wattii varies between seasons. Cytotoxic activity versus HeLa cells was higher in the rainy season extract. This may be linked to levels of 3α,24-dihydroxyup-20(29)-en-28-oic acid (4), a novel compound proposed as one of the most active molecules in P. wattii and a potential chemotaxonomic biomarker for the species.

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**Table 1. Abundance of peaks and cytotoxic effect (CC$_{50}$) on HeLa cell line from the evaluated extracts.**

| Compound                          | Rainy season abundance of the compound (%) | Dry season | Midsummer drought | p-value$^*$ |
|-----------------------------------|-------------------------------------------|------------|-------------------|-------------|
| 1                                 | 4 ± 0.2                                   | 4.5 ± 0.3  | 2 ± 0.2           | Rainy and dry <0.001 vs. MSD |
| 2                                 | 20.7 ± 1.4                               | 18.6 ± 0.5 | 71.8 ± 1.6       | Rainy and dry <0.001 vs. MSD |
| 3                                 | 17.9 ± 0.3                               | 20.3 ± 0.7 | 3 ± 0.3           | Rainy and dry <0.001 vs. MSD; rainy < 0.001 vs. dry; dry > 0.001 vs. MSD |
| 4                                 | 13.8 ± 0.3                               | 7.7 ± 0.4  | 4.6 ± 0.5         | Rainy <0.001 vs. dry and MSD; dry > 0.001 vs. MSD |
| 5                                 | 6.7 ± 0.4                                | 7.6 ± 0.5  | 3.8 ± 0.5         | Rainy and dry <0.001 vs. MSD |
| 6                                 | 2.7 ± 0.3                                | 3.1 ± 0.3  | 2.6 ± 0.4         | NS |
| 7                                 | 2.7 ± 0.3                                | 3.1 ± 0.2  | 2.7 ± 0.3         | NS |
| 8                                 | 6.9 ± 0.6                                | 6.3 ± 2.7  | 2.7 ± 0.3         | Rainy and dry <0.001 vs. MSD |
| 9                                 | 6.8 ± 0.3                                | 7.8 ± 0.5  | 2.7 ± 0.5         | Rainy and dry <0.001 vs. MSD |
| 10                                | 13.7 ± 0.5                               | 15.6 ± 0.7 | 2.3 ± 0.3         | Rainy= 0.007 vs. dry; rainy and dry <0.001 vs. MSD |
| 11                                | 2.7 ± 0.4                                | 3.3 ± 0.4  | 2.2 ± 0.3         | Dry = 0.02 vs. MSD |
| MeOH extract (CC$_{50}$ in μg/mL) | 29 ± 1                                    | 1,023.3 ± 1.5 | 100.7 ± 1.5   | Rainy and dry <0.001 vs. MSD; rainy <0.001 vs. dry |

1: 3α,23-dihydroxyup-20(29)-en-28-oic acid; 2: betulin; 3: 3α,23-dihydroxy-30-oxolup-20(29)-en-28-oic acid; 4: 3α,24-dihydroxyup-20(29)-en-28-oic acid; 5: 3-epi-betulinic acid; 6: betulone; 7: betulinic acid; 8: oleanolic acid; 9: betulonic acid; 10: 3α,23-O-isopropylidenyl-3α,23-dihydroxyup-20(29)-en-28-oic acid; 11: lupenone. AUC: area under the curve. NS: not significant. MeOH: methanol. MSD: midsummer drought. $^*$One-way ANOVA with a posteriori Tukey’s test.
Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Amiri S, Dastghaib S, Ahmadi M, Mehrbod P, Khadem F, Behrouj H, Aghanoori MR, Machaj F, Ghamsari M, Rosik J, et al. 2020. Betulin and its derivatives as novel compounds with different pharmacological effects. Biotechnol Adv. 38:107409. [accessed 2021 Jan 6] https://linkinghub.elsevier.com/retrieve/pii/S0734975019300990.

Caamal-Fuentes E, Torres-Tapia LW, Simá-Polanco P, Peraza-Sánchez SR, Moo-Puc R. 2011. Screening of plants used in Mayan traditional medicine to treat cancer-like symptoms. J Ethnopharmacol. 135(3):719–724. [accessed 2020 Aug 19] https://pubmed.ncbi.nlm.nih.gov/21501677/.

Estrada-Medina H, Cobos-Gasca V, Acosta-Rodríguez JL, Peña-Fierro S, Castilla-Martínez M, Castillo-Carrillo C, Franco-Brito S, López-Castillo D, López-Díaz M, Luna-Flores W, et al. 2016. The drought of the Yucatan Peninsula. Water Technol Sci. 7(5):151–165.

Jäger S, Trojan H, Kopp T, Laszczyk M, Scheffler A. 2009. Pentacyclic triterpene distribution in various plants – rich sources for a new group of multi-potent plant extracts. Molecules. 14(6):2016–2031. http://www.mdpi.com/1420-3049/14/6/2016.

Osadebe PO, Omeje EO, Uzor PF, David EK, Obiorah DC. 2010. Seasonal variation for the antidiabetic activity of Loranthus micranthus methanol extract. Asian Pac J Trop Med. 3(3):196–199.

Krug & Urb. Phoradendron Wattii Krug. 1897. Bot Jahrb Syst. 24(1):43.

Valencia-Chan LS, García-Cámara I, Torres-Tapia LW, Moo-Puc RE, Peraza-Sánchez SR. 2017. Lupane-type triterpenes of Phoradendron vernicosum. J Nat Prod. 80(11):3038–3042. [accessed 2020 Aug 19] https://pubmed.ncbi.nlm.nih.gov/29120172/.

Yin J, Liang T, Wang S, Zhang M, Xiao J, Zhan Y, Li C. 2015. Effect of drought and nitrogen on betulin and oleanolic acid accumulation and OSC gene expression in white birch saplings. Plant Mol Biol Rep. 33(3):705–715. [accessed 2021 Feb 25] https://link.springer.com/article/10.1007/s11105-014-0778-1.