ABSTRACT

The bioactivity-guided fractionation of the total ethanolic extract of the leaves of *Tabebuia aurea* revealed the cytotoxic and antileishmanial potency of the ethyl acetate fraction, in which its phytochemical investigation resulted in the isolation of five triterpenes; identified as oleanolic acid (1), ursolic acid (2), pomolic acid (3), tormentic acid (4), 3β,6β,19α-trihydroxy-urs-12-en-28-oic acid (5) in addition to one triterpenoid glucoside, spathodic acid 28-O-β-D-glucopyranoside (6). Whereas compound 1 showed cytotoxic activity against three different cell lines; A549, MCF-7 and HepG2 with IC$_{50}$ values of 31.7 ± 1.2, 27.4 ± 1.8 and 28.8 ± 1.1 µg/mL, respectively (etoposide as a positive control: 28.1 ± 4.2, 22.5 ± 4.5, and 20.4 ± 0.8 µg/mL, respectively), while compounds 1 and 2 showed antileishmanial activity with IC$_{50}$ values of 10.2 ± 0.9 µg/mL and 5.1 ± 0.4 µg/mL, respectively (miltefosine: 7.7 ± 2.1 µg/mL).

1. Introduction

Triterpenoids are a large group of natural products with structural diversity; they exhibit over 100 distinct skeletons (Xu et al. 2004; Isah et al. 2016). Pentacyclic...
triterpenes are a member of these wide-ranging isoprene-derived secondary metabolites (Isah et al. 2016). They are derived mainly as a result of the cyclization of oxidosqualene and squalene, involving the formation of carbocations through multiple enzymatic and redox stages (Jäger et al. 2009). Triterpenes are well known for their many promising physiological and pharmacological activities, including anti-inflammatory (Banno et al. 2006), antibacterial (Cunha et al. 2007), besides their role in calming the nerves (Luo and Lin 2002). Additionally, the literature survey revealed their role in the immune regulation (Martín et al. 2012), regulation of blood sugar (Melo et al. 2010) and lowering of blood pressure (Somova et al. 2003). Furthermore, in vivo and in vitro studies revealed many antitumor effects of triterpenes (Kikuchi et al. 2011), such as ursolic acid which can clearly inhibit S180 (murine sarcoma), TSCC-α (human tongue cancer) and HL-60 cell (human promyelocytic leukemia) proliferation, likewise the induction of breast cancer MCF-7 cell apoptosis and blocking of tumor angiogenesis (Wang et al. 2012, Zhang et al. 2014). The oleanolic acid also exhibits a significant tumor inhibitory effect through a cell-cycle arrest on HCT15 (human colon carcinoma cell line) (Li et al. 2002). Therefore, these triterpenes are promising lead compounds for the development of new bioactive agents with low toxicity and high efficiency.

Likewise, many researches revealed the varied potency of the triterpenoids with different skeletons against cutaneous leishmaniasis that occurs 0.7–1.2 million cases each year worldwide (Alvar et al. 2012). On our continuous search to find effective cytotoxic and antileishmanial agents from natural sources, the cytotoxic and antileishmanial activity of the total extract, sub-fractions and isolated compounds from *T. aurea* were investigated.

*Tabebuia* genus (Silva Manso) Benth. & Hook. ex S. Moore (Bignoniaceae) is widely distributed in tropical and subtropical regions of the American continent (Reis et al. 2014). *T. aurea* [Synonym: *T. argentea* Britton (Bureau & K. Schum) and *Bignonia aurea* Silva Manso] (IPNI 2019) is considered an important species for the local population of the Brazilian Cerrado, for timber logging and for homemade recipes for medical purposes (Braga et al. 2006). Additionally, the ethnobotanical studies have revealed that *T. aurea*, known as ‘paratudo’, is widely used in traditional medicine, in the form of infusion or maceration with alcohol or chewing of stem bark during the day, to treat snake bites as an anti-inflammatory medication (Reis et al. 2014). Laboratory studies revealed the effect of *T. aurea* hydroethanolic extract in reducing inflammation, haemorrhage and myotoxic activities of the snake venom in experimental mice (Nocchi et al. 2020). Additionally, flavonoids, phenolic glycosides, lignans, and iridoids are reported in the leaves of *T. aurea* (De Abreu et al. 2014; Hamed et al. 2020; Mahmoud et al. 2021), likewise the exhibition of remarkable antioxidant and nephroprotective activities against carbon tetrachloride-induced nephrotoxicity in rats, as well as the exhibition of a prominent in-vitro antityrpanosomal activity against *Trypanosoma brucei* (Mahmoud et al. 2021). This study aims to evaluate the cytotoxic and antileishmanial activities of the leaves of *T. aurea* as a total extract, sub-fractions and consequently isolated phytoconstituents from the most promising fraction.

## 2. Results and discussion

The bioassay-guided fractionation of the total ethanolic extract (TEE) and sub-fractions of the leaves of *T. aurea* revealed the high potency of the ethyl acetate (EtOAc)
fraction as a cytotoxic agent against different cell lines including A549, MCF-7, and HepG2 with IC$_{50}$ 34.2 ± 3.5, 38.2 ± 2.8, and 42.3 ± 2.9 µg/mL, respectively, and showed noticeable antileishmanial activity with IC$_{50}$ value of 48.1 ± 0.6 µg/mL as shown in Table S1. The etoposide, a clinical antitumor drug developed from a plant constituent, was used as a positive control (IC$_{50}$ values: 28.1 ± 4.2, 22.5 ± 4.5, and 20.4 ± 0.8 µg/mL, respectively). Furthermore, the EtOAc fraction was subjected to column chromatography using normal and reversed phase silica gel and final purification using reversed phase high performance liquid chromatography (RP-HPLC) resulting in the isolation of six triterpenoidal compounds, which were identified based on intensive spectroscopic analyses; (Tables S2, S3 and Figures S1–S19) together with comparing their physical and chemical properties with the reported data. The isolated compounds include, a oleanane type compound, oleanolic acid (1) (Onoja and Ndukwe 2013), besides four ursane type compounds identified as ursolic acid (2) (Mahmoud et al. 2019), pomolic acid (3) (Lee et al. 2005; Mahmoud et al. 2019), tormentic acid (4) (Taniguchi et al. 2002; Mahmoud et al. 2019) and 3β, 6β, 19x-trihydroxy-urs-12-en-28-oic acid (5) in addition to one triterpenoidal glucoside, spathecid acid 28-O-β-D-glucopyranoside (6) (Zhang et al. 2005) (Figure 1). Compound 5 was isolated from T. aurea for the first time, while compounds 3 and 4 were obtained for the first time from the genus. In addition, compound 6 was reported in the family Bignoniaceae for the first time.

The identified compounds (1–6) were also investigated for their cytotoxicity against cancer cell lines and antileishmanial activity (Table S1). Whereas compound 1 exhibited noticeable cytotoxic activity against the three different cell lines; A549, MCF-7 and HepG2 with IC$_{50}$ values of 31.7 ± 1.2, 27.4 ± 1.8 and 28.8 ± 1.1 µg/mL, respectively, and antileishmanial activity with IC$_{50}$ value of 10.2 ± 0.9 µg/mL. Miltefosine was used as a control (IC$_{50}$ 7.7 ± 2.1 µg/mL), which is the sole oral clinical agent for leishmaniasis and
listed in the WHO model list of essential medicines which are necessary for a health care system. On the other hand, Compound 2 showed relatively selective activity against HepG2 cell line and leishmania, with IC50 value of 26.7 ± 1.5 and 5.1 ± 0.4 µg/mL, respectively. Compound 4 exhibited cytotoxicity against MCF-7 cell line with IC50 value of 25.3 ± 1.4 µg/ml, but negligible antileishmanial activity.

3. Conclusion

The cytotoxic and antileishmanial assay of the different fractions of T. aurea leaves showed that the ethyl acetate-soluble fraction exhibited the highest potency. The use of different chromatographic techniques led to the isolation of six triterpenes 1–6, which exhibited varied cytotoxic activities against tumor cell lines and anti-leishmania activity. Compound 1 may be non-specific caused by general toxicity, but 2 and 4 showed weak selectivity against HepG2 and leishmania. At the present stage of our research, however, further investigations are needed to clarify their actual mechanism of action and selectivity.

Disclosure statement

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