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# Tabebuia bahamensis: A Major Source of Pharmacologically Important Ursolic Acid

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***Tabebuia bahamensis*: A Major Source of Pharmacologically Important Ursolic Acid**Samon Shrestha<sup>a</sup>, Mary C. Setzer<sup>1</sup>, Debra M. Moriarity<sup>2</sup>, Bhuwan K. Chhetri<sup>1</sup>, and William N. Setzer<sup>1\*</sup><sup>1</sup>Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA<sup>2</sup>Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, AL 36899, USA

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**Keywords:** *Tabebuia bahamensis*, ursolic acid, cytotoxicity, antimicrobial.**ABSTRACT**

Leaves of *Tabebuia bahamensis*, collected on Abaco Island, Bahamas, were dried and extracted with dichloromethane to yield a crude extract. Using preparative flash column chromatography on silica gel, enabled the isolation of ursolic acid with an excellent yield of 2.06 percent based on the mass of dry leaves. Both the crude leaf extract and ursolic acid produced selective *in-vitro* cytotoxic activity on tumor cells from human mammary and bladder tissue. Ursolic acid also demonstrated antibacterial activity against *Staphylococcus aureus*.

**INTRODUCTION**

Commonly known as “five fingers,” *Tabebuia bahamensis* (North.) Britton, is a small tree in the Bignoniaceae family along with 110 other genera consisting of 650 species of flowering plants of which about 100 species belong to the *Tabebuia* genus (Rahmatullah et al., 2010; Ospina et al., 2013). The Five Fingers tree, which is endemic to the Bahamas, is now commonly observed in tropical and subtropical habitats, has small leaves with 3-5 leaflets that are slender, green on the upper surface and strongly whitish on the lower surface. The tree is slim with straight upright branches and grows well in properly drained, alkaline or acidic soils. Flowers vary in color from white to pink and the tree blooms throughout the year (Popenoe, 1980).

Several species of the genus *Tabebuia* hold ethnopharmacological and ethnobotanical importance. In the Bahamas, the leaves of *T. bahamensis*

are used as a tea for “bodily strain” and for relief of backache (Higgs, 1969). The leaf decoction of *T. bahamensis* has been used as a sex stimulant and aphrodisiac (Halberstein, 2005), and the plant has been an essential component of a “love potion” prepared on Andros Island of the Bahamas (Eshbaugh, 2014).

In the current study, the biological activity of crude leaf extract from *T. bahamensis* and the isolation of ursolic acid in large quantities are detailed. To our knowledge, this is the first examination of the phytochemistry of *T. bahamensis*.

**MATERIALS AND METHODS**

**Plant material.** Leaves of *T. bahamensis* were collected December 16, 2000, on Abaco Island, Bahamas (26° 31.092' N, 77° 4.258 W, 13 m asl). The plant was identified in the field by a qualified local ethnobotanical informant (Dolly Davis), and verified with a field guide (Nickrent et al., 1991). A voucher specimen (TABA2000) has been deposited in the herbarium of the University of Alabama in Huntsville. The leaves were cleaned of debris, chopped, air dried, and 594 g of chopped dry leaves were extracted by refluxing with dichloromethane for four hours using a Soxhlet extractor. The solvent was subsequently evaporated to yield 50.1 g of crude leaf extract.

**Preparative flash chromatography.** The crude, dichloromethane leaf extract (25.0 g) was subjected to flash chromatography (Figure 1) using silica gel (230-400 mesh), in a 90 cm long × 5 cm diameter glass column. A hexane/ethyl acetate step

gradient (hexane, 9:1 hexane/EtOAc, 4:1 hexane/EtOAc, 1:1 hexane/EtOAc, EtOAc) in 200-mL fractions, was used to separate the constituents with detection of eluates by TLC. Fractions F51-F90 had similar TLC eluates and were thus combined and recrystallized in EtOAc/pentane to yield 6.10 g ursolic acid as a colorless crystalline solid.

**NMR spectroscopic characterization.** NMR spectra were measured at 500 MHz on a Varian INOVA spectrometer in DMSO- $d_6$ .  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  11.93 (s, 1H), 5.13 (t,  $J$  = 3.7 Hz, 1H), 4.29 (d,  $J$  = 5.1 Hz, 1H), 3.00 (dt,  $J$  = 10.1, 5.0 Hz, 1H), 2.10 (dd,  $J$  = 11.4, 1.6 Hz, 1H), 1.04 (s, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H), 0.81 (d,  $J$  = 6.4 Hz, 3H), 0.75 (s, 3H), 0.67 (s, 3H).  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  178.24, 138.17, 124.55, 76.81, 54.76, 52.36, 47.00, 46.81, 41.63, 39.09, 38.49, 38.42, 38.37, 38.21, 36.52, 36.30, 32.69, 30.17, 28.25, 27.53, 26.98, 23.79, 23.26, 22.84, 21.07, 17.99, 17.01, 16.91, 16.07, 15.22. The NMR spectra were consistent with ursolic acid (Moghaddam et al., 2007; Silva et al., 2008; Keat et al., 2010).

**Antimicrobial screening.** The crude leaf extract and ursolic acid were screened for antimicrobial activity against the Gram-positive bacteria *Bacillus cereus* (ATCC No. 14579), and *Staphylococcus aureus* (ATCC No. 29213). Antimicrobial activity of the crude leaf extract and ursolic acid against Gram-negative bacteria were tested with *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 10798), and the fungi *Candida albicans* (ATCC No. 10231) and *Aspergillus niger* (ATCC No. 16888). Minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique as previously described (Setzer et al., 2003; Schmidt et al., 2006).

**Cytotoxicity screening.** *In-vitro* cytotoxicity screening was tested against human MCF-7 breast adenocarcinoma (ATCC No. HTB-22), human SK-Mel-28 malignant melanoma (ATCC No. HTB-72), human Hep G2 hepatocellular carcinoma (ATCC No. HB-8065), human MDA-MB-231 breast adenocarcinoma (ATCC No. HTB-26), and human 5637 bladder carcinoma (ATCC No. HTB-9) cells as

previously described (Setzer et al., 2003; Schmidt et al., 2006).

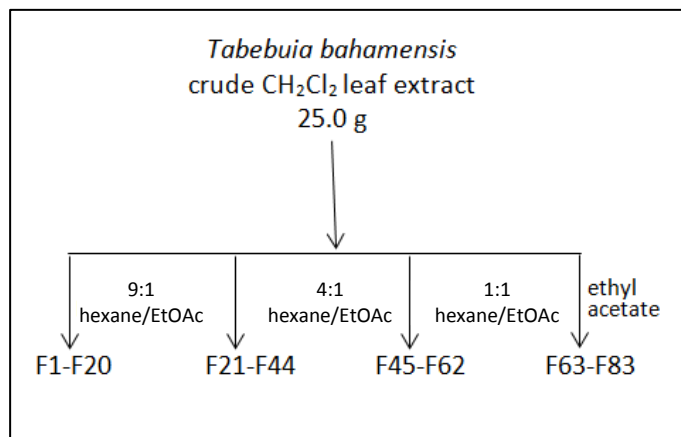


Figure 1. Chromatographic separation scheme for *Tabebuia bahamensis*  $\text{CH}_2\text{Cl}_2$  crude leaf extract.

## RESULTS

Crude leaf extract of *Tabebuia bahamensis* demonstrated selective cytotoxic activity against several human tumor cell lines. The extract was non-toxic to SK-Mel-38 and Hep-G2 cells, marginally toxic to MDA-MB-231 cells ( $58.8 \pm 7.3$  % kill at 100  $\mu\text{g/mL}$ ), and notably toxic to 5637 bladder tumor cells ( $93.0 \pm 6.9$  % kill at 100  $\mu\text{g/mL}$ ). The crude extract, however, showed no antimicrobial activity ( $\text{MIC} \geq 1250$   $\mu\text{g/mL}$  against the organisms tested).

The major component in the crude  $\text{CH}_2\text{Cl}_2$  leaf extract of *Tabebuia bahamensis* was identified by NMR to be ursolic acid (Figure 2). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were consistent with those previously reported for this compound (Moghaddam et al., 2007; Silva et al., 2008; Keat et al., 2010) (Table 1).

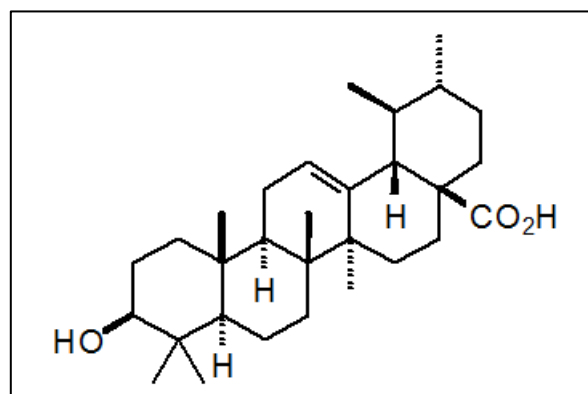


Figure 2. Ursolic acid

Table 1. NMR assignments of ursolic acid.

Position	Carbon $\delta$ $^{13}\text{C}$	Proton $\delta$ $^1\text{H}$
1	36.30	1.56
2	26.98	1.44
3	76.81	3.00 (dt, $J = 10.1, 5.0$ Hz, 1H)
4	38.37	-
5	54.76	0.67
6	17.99	1.30, 1.47
7	32.69	1.27
8	39.09	-
9	47.00	1.47
10	36.52	-
11	22.84	1.85
12	124.55	5.13 (t, $J = 3.7$ Hz, 1H)
13	138.17	-
14	41.63	-
15	27.53	1.00, 1.79
16	23.79	1.52, 1.93
17	46.81	-
18	52.36	2.10
19	38.49	0.92
20	38.42	1.30
21	30.17	1.27, 1.42
22	38.21	1.53
23	28.25	0.89 (s, 3H)
24	16.07	0.67 (s, 3H)
25	15.22	0.86 (s, 3H)
26	16.91	0.75 (s, 3H)
27	23.26	1.04 (s, 3H)
28	178.24	-
29	17.01	0.81 (d, $J = 6.4$ Hz, 3H)
30	21.07	0.91 (s, 3H)

Tests with ursolic acid for *in-vitro* cytotoxic activity against MDA-MB-231, MCF-7, and 5637 cells and proved to be extremely cytotoxic with  $IC_{50}$  of  $14.8 \pm 1.0$ ,  $14.9 \pm 0.5$ , and  $18.8 \pm 0.4$   $\mu\text{g/mL}$ , respectively. In addition, the ursolic acid also showed antibacterial activity against *S. aureus*, with an MIC equal to 156  $\mu\text{g/mL}$ , but was inactive against the other bacteria and fungi that were screened.

## DISCUSSION

Ursolic acid (3 $\beta$ -hydroxy-urs-12-en-28-oic acid) is a triterpenoid that is common in many plants and can

be considered pharmacologically important for several biological activities. The cytotoxic effects of ursolic acid observed in this study are consistent with previous studies that have reported ursolic acid as an antitumor agent in several stages of tumor development (Ovesná et al., 2004). Ursolic acid has also induced apoptosis in tumor cells and prevented malignant transformation of normal cells (Novotný et al., 2001).

The cytotoxic effects of ursolic acid are apparently due to the ability of the compound to block the cell cycle progression in the G1 phase and trigger apoptosis (Ma et al., 2005). As a result, the antiproliferative and apoptotic activities of ursolic acid could potentially to be useful for treatment of hormone refractory and androgen-sensitive prostate cancer (Kassi et al., 2007). A structure-activity study by Ma et al., (2005) has revealed that the carbonyl group at C<sub>17</sub> and a hydrogen-bond-donor group at C<sub>3</sub> and/or C<sub>28</sub> are necessary for the cytotoxic effect. The antibacterial activity of ursolic acid against *S. aureus* has been reported in other studies (Fontanay et al., 2008; Wolska et al., 2010).

Ursolic acid has been demonstrated to be active against promastigotes of *Leishmania amazonensis* and *L. donovani* (Peixoto et al., 2011; Adebayo et al., 2013), epimastigotes of *Trypanosoma cruzi* (Abe et al., 2002), and trypomastigotes of *T. brucei rhodesiense* (van Baren et al., 2006). Ursolic acid is also active as a hepatoprotective agent, and is known to be effective against acute, chemically-induced liver injury and chronic liver fibrosis and cirrhosis (Liu, 1995, 2005).

Ursolic acid acts an anti-inflammatory agent by inhibiting histamine release from mast cells and also by inhibiting lipoxygenase and cyclooxygenase activity (Liu, 1995; Ikeda et al., 2008). Pharmacologically, ursolic acid has anti-hyperlipidemia and an antioxidant activity (Liu, 1995; Somova et al., 2003) along with demonstrated anxiolytic and antidepressant (Pemminati et al., 2011; Machado et al., 2012) activities in rodent models. Plant extracts containing ursolic acid have also shown aphrodisiac effects (Balamurugan et al., 2010; Malviya et al., 2011). Ursolic acid is generally considered to be non-toxic, showing neither an acute ( $LD_{50} = 9.26\text{g/kg}$ ) nor chronic genetic toxicity in mice (Lu et al., 2009).

Establishing *Tabebuia bahamensis* as a major source of ursolic acid, with a yield of 2.06 percent based on the mass of dry leaves, would make the ursolic acid readily available. The cytotoxicity of this ursolic acid against MDA-MB-231, MCF-7, and 5637 cell lines reaffirms the potential of ursolic acid to be developed into an efficient anticancer agent. The biological activities of ursolic acid are consistent with some traditional uses of *T. bahamensis*.

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