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Tabebuia bahamensis: A Major Source of Pharmacologically Important Ursolic Acid

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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}H NMR (500 MHz, DMSO-d_6) δ 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}C NMR (126 MHz, DMSO-d_6) δ 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 anti-microbial activity against the Gram-positive bacteria *Bacillus cereus* (ATCC No. 14579), and *Staphylococcus aureus* (ATCC No. 29213). Anti-microbial 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 adeno-carcinoma (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).

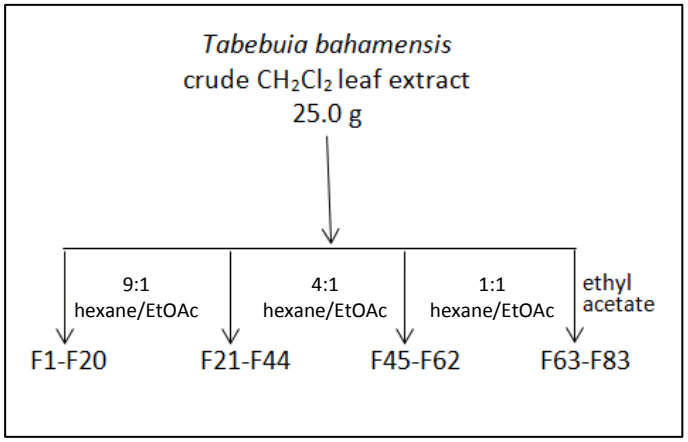

Figure 1. Chromatographic separation scheme for *Tabebuia bahamensis* CH_2Cl_2 crude leaf extract.

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 ± 7.3 % kill at 100 μg/mL), and notably toxic to 5637 bladder tumor cells (93.0 ± 6.9 % kill at 100 μg/mL). The crude extract, however, showed no antimicrobial activity (MIC ≥ 1250 μg/mL against the organisms tested).

The major component in the crude CH_2Cl_2 leaf extract of *Tabebuia bahamensis* was identified by NMR to be ursolic acid (Figure 2). The ^{1}H and ^{13}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).

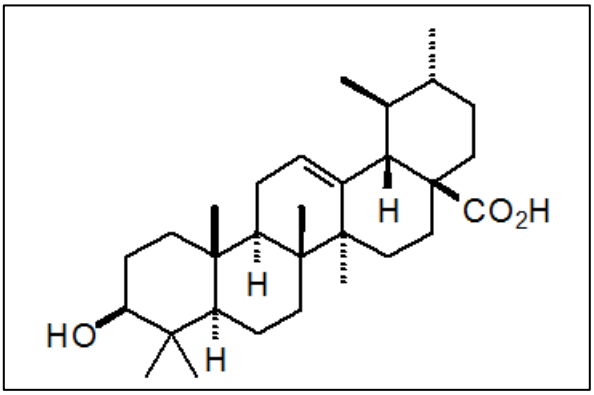

Figure 2. Ursolic acid

Table 1. NMR assignments of ursolic acid.

Position	Carbon δ ^{13}C	Proton δ ^{1}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±1.0, 14.9±0.5, and 18.8±0.4 µg/mL, respectively. In addition, the ursolic acid also showed antibacterial activity against *S. aureus*, with an MIC equal to 156 µg/mL, but was inactive against the other bacteria and fungi that were screened.

DISCUSSION

Ursolic acid (3β-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_{17} and a hydrogen-bond-donor group at C_3 and/or C_{28} 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 anti-depressant (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} = 9.26g/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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REFERENCES

Abe, F., T. Yamauchi, T. Nagao, J. Kinjo, H. Okabe, H. Higo, and H. Akahane. 2002. Ursolic acid as a trypanocidal constituent in rosemary. Biological & Pharmaceutical Bull 25:1485-1487.

Adebayo, O.L., D. Suleman, and A.A. Samson. 2013. Natural products in antileishmanial drug discovery: A review. Journal of Asian Scientific Research 3:157-173.

Balamurugan, G., P. Muralidharan, and S. Polapala. 2010. Aphrodisiac activity and curative effects of *Pedalium murex* (L.) against ethanol-induced infertility in male rats. Turkish Journal of Biology 34:153-163.

Eshbaugh, W.H. 2014. The Flora of the Bahamas, Donovan Correll, and the Miami University Connection. The Botanical Review 80:184-203.

Fontanay, S., M., Grare, J. Mayer, C. Finance, and R.E. Duval. 2008. Ursolic, oleanolic and betulinic acids: Antibacterial spectra and selectivity indexes. Journal of Ethnopharmacology 120:272-276.

Halberstein, R.A. 2005. Medicinal plants: Historical and cross-cultural usage patterns. Annals of Epidemiology 15:686-699.

Higgs, L. 1969. *Bush Medicine in the Bahamas*. Nassau Guardian. Nassau, Bahamas.

Ikeda, Y., A. Murakami, and H. Ohigashi. 2008. Ursolic acid: An anti- and pro-inflammatory triterpenoid. Molecular Nutrition and Food Research 52:26-42.

Kassi, E., Z. Papoutsi, H., Pratsinis N. Aligiannis, M. Manoussakis, and P. Moutsatsou. 2007. Ursolic acid, a naturally occurring triterpenoid, demonstrates anticancer activity on human prostate cancer cells. Journal of Cancer Research and Clinical Oncology 133:493-500.

Keat, N., R. Umar, N. Lajis, and T. Chen. 2010. Chemical constituents from two weed species of *Spermacoce* (Rubiaceae). The Malaysian Journal of Analytical Sciences 14:6-11.

Liu, J. 1995. Pharmacology of oleanolic acid and ursolic acid. Journal of Ethnopharmacology 49:7-68.

Liu, J. 2005. Oleanolic acid and ursolic acid: Research perspectives. Journal of Ethnopharmacology 100:92994.

Lu, J., S. Guan, and J. Liu. 2009. Acute and genetic toxicity of ursolic acid extract from *Ledum pulastre* L. Food Science 13:059.

Ma, C.M., S.Q, Cai, J.R. Cui, R.Q. Wang, P.F. Tu, M. Hattori, and M. Daneshtalab. 2005. The cytotoxic activity of ursolic acid derivatives. European Journal of Medicinal Chemistry 40:582-589.

Machado, D.G., V.B. Neis, G.O. Balen, A. Colla, M.P. Cunha, J.B. Dalmarco, M.G. Pizzolatti, R.D. Prediger, and A.L.S. Rodrigues. 2012. Antidepressant-like effect of ursolic acid from *Rosmarinus officinalis* L. in mice: Evidence for the involvement of the dopaminergic system. Pharmacology, Biochemistry and Behavior 103:204-211.

Malviya, N., S. Jain, V.B. Gupta, and S. Vyas. 2011. Recent studies on aphrodisiac herbs for the management of male sexual dysfunction-a review. Acta Poloniae Pharmaceutica 68:3-8.

Moghaddam, F.M., M.M. Farimani, S. Salahvarzi, and G. Amin. 2007. Chemical constituents of dichloromethane extract of cultivated *Satureja khuzistanica*. Evidence-Based Complementary

and Alternative Medicine 4:95-98.

Nickrent, D.L., W.H. Eshbaugh, and T.K. Wilson. 1991. *The Vascular Flora of Andros Island, Bahamas*. Willard Sherman Turrell Herbarium, Miami University, Ohio, USA.

Novotný, L., A. Vachálková, and D. Biggs, 2001. Ursolic acid: An anti-tumorigenic and chemopreventive activity. Neoplasma 48:241-246.

Ospina, L.A.F., Y.C.O. Buendia, and R.G. Ibarra. 2013. Nitric oxide inhibitory activity of hydrogenated synthetic analogues of furanonaphthoquinones isolated from *Tabebuia* spp. Revista Cubana de Farmacia 47:502-516.

Ovesná, Z., A. Vachálková, K. Horváthová, and D. Tóthová. 2004. Pentacyclic triterpenoic acids: New chemoprotective compounds. Minireview. Neoplasma 51:327-333.

Peixoto, J.A., M.L.A. Silva, A.E.M. Crotti, M. R.C.S. Veneziana, V.M.M. Gimenez, A.H. Januário, M. Groppo, L.G. Magalhães, F.F..dos Santos, S. Albuquerque, A.A.d.S. Filho, and W.R. Cunha. 2011. Antileishmanial activity of the hydroalcoholic extract of *Miconia langsdorffii*, isolated compounds, and semi-synthetic derivatives. Molecules 16:1825-1833.

Pemminati, S., H.N. Gopalakrishna, V. Venkatesh, A. Rai, S. Shetty, A. Vinod, P.G. Pai, and A. K. Shenoy, 2011. Anxiolytic effect of chronic administration of ursolic acid in rats. Journal of Applied Pharmaceutical Science 1:68-71.

Popenoe, J. 1980. Bahamian trees from the South Florida landscape. Proceedings of Florida State Horticulture Society 93:86-87.

Rahmatullah, M., W. Samarrai, R. Jahan, S. Rahman, N. Sharmin, Z.U.M. Emdad Ullah Miajee, M.H. Chowdhury, S. Bari, F. Jamal, A.B.M. Anwarul Bashar, A.K. Azad, and A. Ahsan. 2010. An ethnomedical, pharmacological and phytochemical review of some Bignoniaceae family plants and a description of Bignoniaceae plants in folk medicinal uses in Bangladesh. Advances in Natural and Applied Sciences 4:236-253.

Schmidt, J.M., J.A. Noletto, B. Vogler, and W.N. Setzer. 2006. Abaco bush medicine: Chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. Journal of Herbs, Spices & Medicinal Plants 12(3):43-65.

Setzer, M.C., D.M. Moriarity, R.O. Lawton, W.N. Setzer, G.A. Gentry, and W.A. Haber. 2003. Phytomedicinal potential of tropical cloudforest plants from Monteverde, Costa Rica. Revista de Biología Tropical 51:647-674.

Silva, M.G.V, I.G.P. Vieira, F.N.P. Mendes, I.L. Albuquerque, R.N. Dos Santos, F.O. Silva, and S.M. Morais. 2008. Variation of ursolic acid content in eight *Ocimum* species from northeastern Brazil. Molecules 13:2482-2487.

Somova, L.O., A. Nadar, P. Rammanan, and F.O. Shode. 2003. Cardiovascular, antihyper-lipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. Phytomedicine 10:115-121.

van Baren, C., I. Anao, P.D.I. Lira, S. Debenedetti, P. Houghton, S. Croft, and V, Martino. 2006. Triterpenic acids and flavonoids from *Satureja parvifolia.* Evaluation of their antiprotozoal activity. Zeitschrift für Naturforschung 61c:189-192.

Wolska, K.I., A.M. Grudniak, B. Fiecek, A. Kraczkiewicz-Dowjat, and A. Kurek. 2010. Antibacterial activity of oleanolic and ursolic acids and their derivatives. Central European Journal of Biology 5:543–553.