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Research Article

Presence of the Indole Alkaloid Reserpine in Bignonia Capreolata L.

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ABSTRACT

*Bignonia capreolata*is a perennial semi-evergreen vine from the Southeast United States that was used as a medicine by the Native Americans but has since fallen out of use. A preliminary screen of *B. capreolata* suggested the presence of the indole alkaloid reserpine. This analysis was undertaken to 1) verify the presence reserpine using LC-MS referenced with an analytical standard of reserpine; and 2) if verified, quantitate the level of reserpine in *B. capreolata* leaf. LC-MS analysis has confirmed the presence of reserpine in *B. capreolata*, which makes this the only known plant outside the Apocynaceae family to contain this indole alkaloid.

INTRODUCTION

Bignonia capreolata(crossvine)is a perennial semi-evergreen vine native to the Eastern United States. It is a member of the Bignoniaceae family, a plant family predominately found in tropical and subtropical regions. It is known by the common name crossvine and has become a popular ornamental plant due to its showy clusters of orange to red trumpet flowers[1].Ethnobotanical use in North Americahas been documented for theAppalachian, Cherokee, Choctaw, Creek, Houma and Koasati tribes. It was primarily used as a blood cleanser, anti-rheumatic, analgesic, alterative, and as a kidney aid for dropsy[1, 2]. North American herbalist Tommie Bass claimed to use*B. capreolata*leaves as a tonic for fatigued farm animals and as a general adaptogen, kidney tonic and blood cleanser[3]. Contemporary use of this plantis minimal except for the students of Mr. Bass. There are no published constituent assays of this plant.A preliminary LC-MSscreen of *B. capreolata*by LC-MS resulted in two probable matches for constituents in the database – a 98.4% match forcarminic acid and 93% match for reserpine. Reserpine, an indole alkaloid, is primarily known as a constituent of *Rauvolfiaserpentina*and other Apocynaceae family members[4, 5]. Reserpine is an irreversible inhibitor of the vesicular monoamine transporter and results in catecholamine depletion and a decrease in sympathetic tone. Reserpine is of medical importance worldwide as a hypotensive and antipsychotic drug; however, currently it is only used as a second-line therapy due to the risk of side effects including depression and bradycardia[4, 5].

The purpose of this study was to verify the presence of reserpine in *B. capreolata* using a commercial standard of reserpine. The presence of reserpine in *B. capreolata*, if verified, would be significant because it has not been shown in this genus to date and in the context of whole botanical medicines may extend the therapeutic potential of this plant.

MATERIALS AND METHODS

Plant Material: Leaf and stem of *Bignonia capreolata*L. were collected in near Shelby, Alabama (USA). A sample of the plant material used for testing was authenticated by a botanist (George Yatskievych, PhD) and submitted to the Missouri Botanical Gardens herbarium (voucher #6257878).

Sample Preparation: Plant material was dried whole and the leaves removed for processing. Powdered and sieved leaves (5.0 g) were extracted in 50 mL extraction buffer (methanol:water:acetic acid (75:20:5)) for 48 hours with shaking. The solution was filtered and the extraction was repeated using the same plant material. The two extracts were then combined and freeze-dried to produce the final extract. A 1% solution of the extract in extraction buffer was used for analysis.

A 1 mg/mL stock solution of reserpine standard (Sigma-Aldrich) was prepared in 10% acetic acid and subsequently diluted with extraction buffer to create 100, 50, 25, 10 and 1 µg/mL standards.

HPLC-MS Analysis

B. capreolata extract and reserpine standard were analyzed using anAcquity UPLC system and MicromassSynapt Ion Mobility TOF mass spectrometer (Waters Corporation). Chromatography was accomplished using a Hypersil Gold 100 x 2.1 mm, 1.9 µm column (Thermo Scientific) and water:acetonitrile (A:B) as the mobile phase. Column conditions were the following: 10% B (0-1 min), 10-100% B (1-12 min), 10% B (12-15 min).Mass spectrometry was run under positive-ion electrospray ionization with a cone voltage of 35 volts and collision energy of either 6 or 30 eV.

Quantitation of Reserpine by HPLC: Quantitation of reserpine in *B. capreolata* extract was accomplished using an Alliance HPLC system with 2998 photodiode array (Waters Corporation) and a Waters Xbridge C18 column (150 x 4.6 mm, 3.5 µm) using water and

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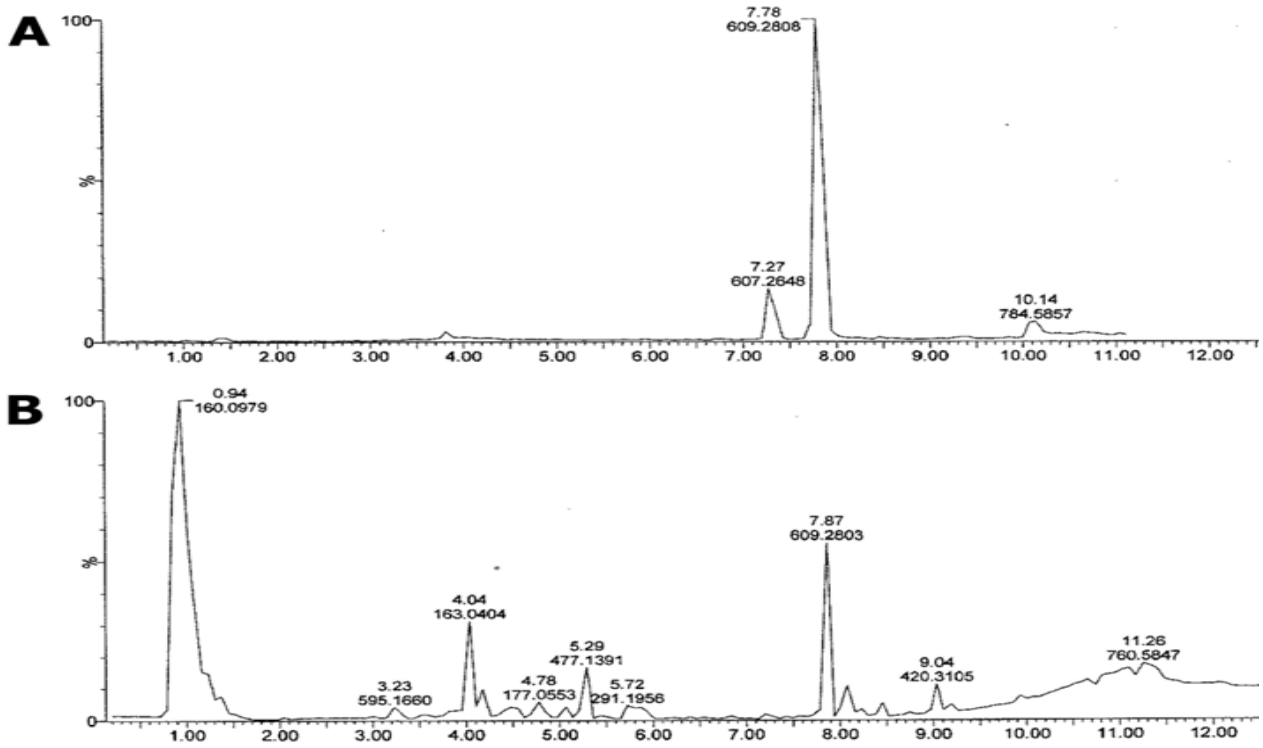

Figure 1: UPLC chromatographs of (A) commercial reserpine standard and (B) *Bignonia capreolata* extract demonstrating the presence of reserpine at 7.8 minutes. Detection was by mass spectrometry; see methods for chromatographic conditions.

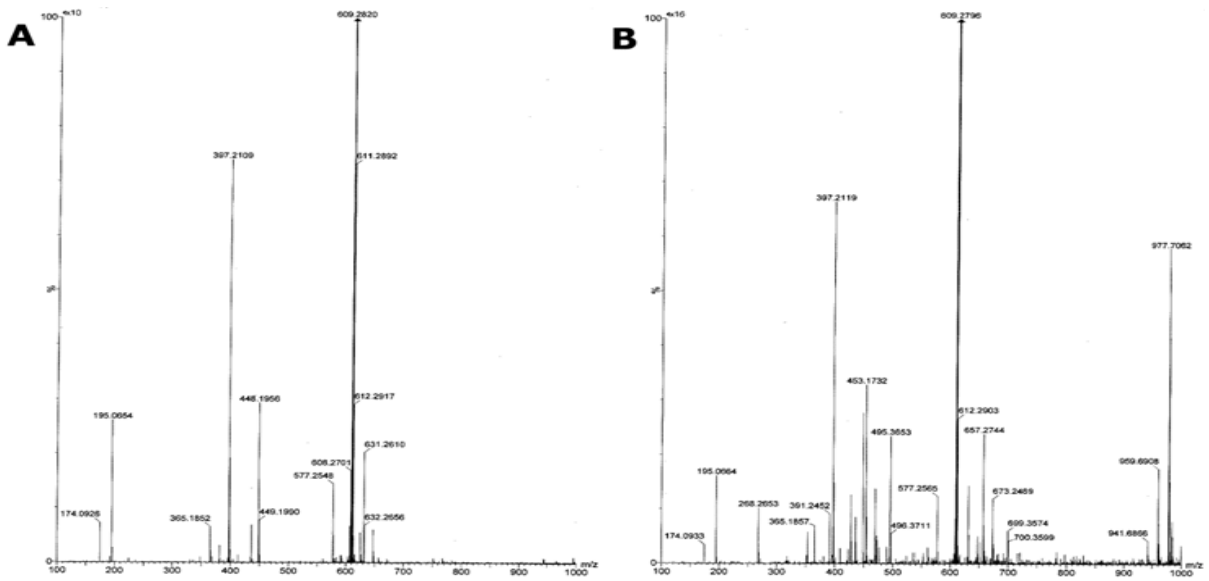

Figure 2: Mass spectra of (A) commercial reserpine standard and (B) the peak at 7.87 minutes of a *Bignonia capreolata* extract. Mass spectra were generated using a MicromassSynapt Ion Mobility TOF mass spectrometer with positive-ion electrospray ionization at a cone voltage of 35 volts and collision energy of 30 eV.

acetonitrile as the mobile phase. Separation was accomplished using a simple gradient from 20-100% acetonitrile over 90 minutes; detection of reserpine was at 267 nm.

RESULTS

Under the LC-MS analysis conditions used reserpine had a retention time of 7.78 minutes and a molecular ion of 609.2808*m/z*. *B. capreolata* extract presented a peak with the same *m/z* (609.2803) at 7.87 minutes (figure 1). The 5 second shift in retention time is most likely due to slight differences in sample pH caused by extra acetic acid in the reserpine standard.

Mass spectra of the two peaks show a high degree of concordance. Fragmentation was minimized in order to improve the accuracy of the mass of the parent

ion;however when the collision energy was increased to 30 eV similar fragmentation was observed between the reserpine standard and the peak at 7.87 minutes of the *B. capreolata* extract (figure 2).

Quantitation of reserpine showed that it was linear in the range tested (1-100 µg/mL) and had a retention time of 34.4 minutes (data not shown).Evaluation of the extract suggests that reserpine is present in *B. capreolata* dried leaf at a concentration of 233 ± 4.7 µg/g.

DISCUSSION

The indole alkaloid reserpine was positively identified in *Bignonia capreolata* L. leaf using LC-MS. This represents the first plant outside of the Apocynaceae family to contain this constituent. Although the reserpine levels in *B. capreolata* were significantly lower than those reported for Rauwolfia species (reported up to 33 mg/g dried root)[6, 7], it is still at a level that could provide therapeutic levels of reserpine - typical adjunctive dose of reserpine is 0.2 mg/day which could be achieved with about 1 gram of dried leaf[8].

Subjective accounts of the effects of ingesting an infusion of three dried leaves of*B. capreolata*daily over three days describe it as energizing (this dose represents about 0.35 g of dried leaf containing approximately 85 µg reserpine)[3]. While this is not necessarily a reported effect of reserpine administration, it is possible that alterations in sympathetic tone caused by reserpine could promote restfulness and feelings of rejuvenation. Still, it seems more likely that the unique effects of *B. capreolata* are due to a synergistic effect of reserpine and other, unidentified constituents.

The presence of reserpine in *B. capreolata* has the potential of widening the therapeutic value of this plant; however,it also suggests caution and provides guidance for future administration of this botanical. Based on this observation, the ingestion of *B. capreolata* should be discouraged in individuals with low blood pressure or a history of depression. Symptoms of toxic exposure may mimic that of reserpine, i.e. dizziness, lethargy, fainting, sinus congestion, slow/abnormal heart rhythm, nausea, vomiting and diarrhea.

Contemporary use of the plant is restricted to the leaf harvested in the summer; however, there are some ethnobotanical reports of using the bark and root[2, 3]. Additionally, the presence of reserpine may also indicate other alkaloids present in this plant - future pharmacognostical evaluations of the whole plant are warranted.

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