



## Phenylethanoid glycosides from *Lippia javanica*

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### Abstract

*Lippia javanica* (N.L.Burm.) Spreng. is an aromatic, multipurpose medicinal plant from which a number of volatile compounds have been identified, together with toxic triterpenoids and iridoid glycosides. Two additional phenylethanoid glycosides, verbascoside and isoverbascoside, were isolated from *L. javanica* and characterized. High performance liquid chromatography analyses of polar extracts of three other *Lippia* species (*L. scaberrima*, *L. rehmannii* and *L. wilmsii*), indigenous to South Africa, revealed the presence of both isomers. When compared to the other indigenous *Lippia* species, the leaves of *L. javanica* were found to contain the highest concentrations of both isomers. In addition, the intraspecies variation of the verbascoside/isoverbascoside content of *L. javanica*, harvested from the same and different localities, was investigated. The concentrations of the two phenylethanoids remained fairly consistent within and between different populations, even when geographically separated. While these compounds are produced by many genera, they may now be added to the list of iridoid glucosides employed as chemotaxonomic markers for *Lippia* species.

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**Keywords:** Chemotaxonomic marker; Isoverbascoside; *Lippia*; Phenylethanoids; Verbascoside; Verbenaceae

### 1. Introduction

*Lippia javanica* (Verbenaceae), locally known as “fever tea” or “koorsbossie”, is an aromatic woody shrub that grows up to 5 m in height (Retief, 2006). It is widely distributed throughout southern Africa (Van Wyk et al., 2000), and is particularly abundant in Swaziland and in the northern provinces of South Africa (Retief, 2006). The shrub is claimed to have fever and pain-relieving properties and is used mainly as an infusion to treat a variety of ailments, ranging from bronchial infections to skin disorders (Pascual et al., 2001; Van Wyk, 2008). It was recently reported that three compounds from *L. javanica* are able to inhibit the HIV-1 reverse transcriptase enzyme (Mujovo et al., 2008). Traditional medicine practitioners prescribe the synergistic use of *L. javanica* and *Artemisia afra* as a remedy against malaria, as a prophylactic against dysentery and diarrhoea, and for the treatment of fever and influenza (Viljoen et al., 2005).

The essential oils of *L. javanica* have been studied extensively and the variation in the essential oil profiles was used to classify five chemotypes within the species, based on the mono- and sesquiterpenes contents (Viljoen et al., 2005). Triterpenoid saponins such as the toxic metabolites lantadene A (also known as rehmannic acid) and icterogenin have been identified in a few *Lippia* species, with icterogenin also reported from *L. javanica* (Pascual et al., 2001). Some iridoid glycosides including theveridoside were isolated from *Lippia turbinata* and *L. javanica* and were consequently considered as chemotaxonomic markers for the genus *Lippia* (Rimpler and Sauerbier, 1986). *Lippia alba* produces three of these iridoids, together with an additional five flavonoid glycosides and eight phenylethanoid glycosides (Hennebelle et al., 2008). Phenylethanoid glycosides commonly occur in the order Lamiales, to which the Verbenaceae family belongs. Verbascoside (1) and/or isoverbascoside (2) have previously been isolated from *L. alba* (Hennebelle et al., 2008), *L. dulcis* (Kaneda et al., 1992), *L. citriodora* (Bilia et al., 2008) and *L. multiflora* (Taoubi et al., 1997).

Apart from *L. javanica*, four other *Lippia* species (*L. scaberrima* Sonder, *L. rehmannii* H.Pearson, *L. wilmsii*

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H. Pearson and *L. pretoriensis* H. Pearson) are officially recognised as indigenous to South Africa (Retief, 2006). Of these, only *L. javanica* and *L. scaberrima* are sold as caffeine free medicinal teas under the brands “Mosukudu” and “Mosukujane”, respectively (Van Wyk and Gericke, 2000). Although the essential oil profiles of some of these species have been studied (Terblanché and Kornelius, 1996; Viljoen et al., 2005; Combrinck et al., 2006), the phenolic secondary metabolites of *L. javanica* and the other indigenous *Lippia* species are still unexplored. Here we report on the isolation and characterization of Compounds **1** and **2** (Fig. 1) from *L. javanica*. Furthermore, the variation of the compounds within the species, as well as the occurrence of the compounds in three other South African *Lippia* species, was established.

Although phenylethanoid glycosides possess variable antioxidant properties, caffeoyl containing compounds such as **1** and **2** have a specifically high potency (Es-Safi et al., 2007; Bilia et al., 2008). Verbascoside (**1**) is reported to exhibit antimicrobial, anti-inflammatory, immunosuppressive, antitumour (Pieroni et al., 2000), diuretic (Herbert et al., 1991) and antibacterial activities (Pennacchio, 2005). Both these structural isomers are known to exhibit analgesic properties (Nakumura et al., 1997).

## 2. Materials and methods

### 2.1. Plant material

Plant material of *L. javanica* was collected for compound isolation purposes from the Hhohho region, Swaziland in 2005. A voucher specimen (TD 57) was deposited in the JRAU Herbarium, Department of Botany and Plant Biotechnology at the University of Johannesburg. Aerial parts of *L. javanica* and three other *Lippia* species used for variation studies were collected from specimens growing in nine localities in South Africa in January 2004, and are listed in Table 1. Voucher specimens were deposited at the herbarium of the South African National Biodiversity Institute (Pretoria).

### 2.2. General

<sup>1</sup>H NMR and <sup>13</sup>C NMR experiments were performed on a Bruker Avance DRX 400, operating at 400.132 MHz for <sup>1</sup>H spectra and 100.625 MHz for <sup>13</sup>C spectra for 1D NMR spectra. The 2D NMR spectra were recorded at 300 MHz for <sup>1</sup>H and at 75 MHz for <sup>13</sup>C spectra using a Bruker Avance 300 spectrometer. IR spectra were obtained from a Bruker Tensor 27 FTIR spectrometer.

Isolation was accomplished by means of column chromatography on silica gel (Kieselgel GF<sub>254</sub> 15 μm, Merck, Johannesburg, South Africa) and preparative thin-layer chromatography (TLC) on glass-backed silica gel F<sub>254</sub> (20 × 20 cm, 0.25 mm) plates from Merck. For monitoring of column fractions, TLC was done on aluminium-backed silica gel 60 F<sub>254</sub> sheets (Merck). Plates were sprayed with 1% ethanolic vanillin followed by 5% ethanolic H<sub>2</sub>SO<sub>4</sub> and heated for 5 min at 100 °C. All solvents, unless otherwise stated, were AR grade and obtained from Merck.

A Labcon platform shaker (Laboratory Marketing Services, Maraisburg, South Africa) was used during extraction of samples used for variation determination purposes. Caffeine (AR grade) used as internal standard for HPLC analysis was obtained from Merck, Germany. Reference standards of verbascoside and isoverbascoside were purchased from Chromadex Incorporated (99% pure; Daimler St, Santa Ana, USA). 0.45 μm nylon Millipore filters (Microsep, Johannesburg, South Africa) were used for sample preparation before injection into the HPLC.

### 2.3. Extraction and isolation of compounds from *L. javanica*

Dried, crushed aerial parts (75.0 g) of *L. javanica* were extracted with 750 ml MeOH (methanol) for 23 h. The mixture was filtered and the solvent evaporated in a fume hood for 48 h to yield a green residue (7.64 g). This residue was partitioned between hexane and 90% aqueous MeOH to allow for the

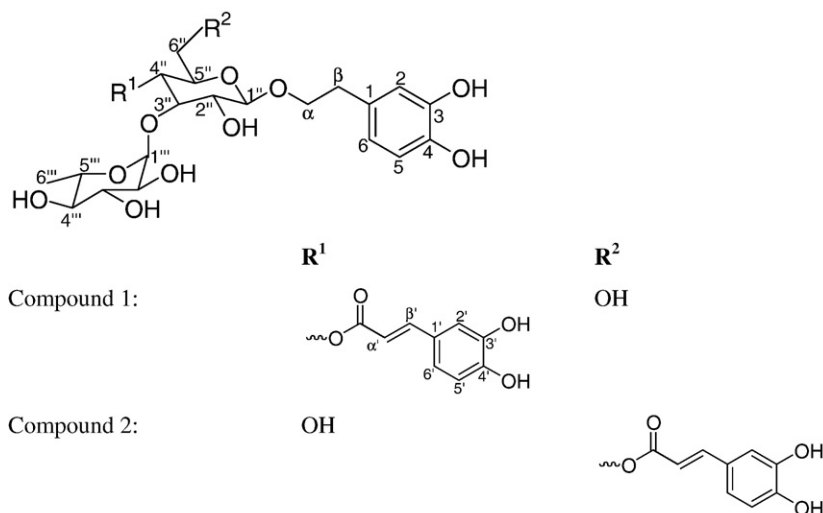


Fig. 1. Structures of phenylethanoid glycosides: **1**, verbascoside; **2**, isoverbascoside.

Table 1  
Localities and voucher specimen numbers of plants harvested for analyses.

| Species              | Locality number | Province   | Locality                                    | Voucher specimen no.  |
|----------------------|-----------------|------------|---|-----------------------|
| <i>L. scaberrima</i> | 1               | North West | Mooibank, (Potchefstroom-Orkney)            | Bosman & Combrinck 29 |
| <i>L. rehmannii</i>  | 2               | Gauteng    | Zandfontein Cemetery (Pretoria)             | Bosman & Combrinck 21 |
| <i>L. wilmsii</i>    | 3               | Mpumalanga | Petra Mountain Inn                          | Bosman & Combrinck 16 |
| <i>L. javanica</i>   | 4               | Mpumalanga | Nelspruit Botanical Gardens                 | Bosman & Combrinck 15 |
| <i>L. javanica</i>   | 5               | Mpumalanga | Ferreira Street, Nelspruit                  | Bosman & Combrinck 13 |
| <i>L. javanica</i>   | 6               | Gauteng    | Tshwane University Of Technology (Pretoria) | Bosman & Combrinck 23 |
| <i>L. javanica</i>   | 7               | North West | Losberg, Potchefstroom                      | Bosman & Combrinck 28 |
| <i>L. javanica</i>   | 8               | Mpumalanga | Long Tom Pass                               | Bosman & Combrinck 14 |
| <i>L. javanica</i>   | 9               | Mpumalanga | Lydenburg road (10 km from Nelspruit)       | Bosman & Combrinck 01 |

partial separation of the polar (4.61 g) and non-polar (3.01 g) fractions. The polar extract was subjected to column chromatography applying gradient elution. Initially, an eluent consisting of  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (50:30:3) was used, followed by  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (40:30:3), and finally  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (50:20:2), to obtain two fractions: A (0.92 g) and B (1.62 g).

Fraction A was subjected to repeated silica gel column separations using gradient solvent systems, starting with  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (50:30:3), and gradually increasing the polarity to  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (40:30:3). A column fraction, containing Compound **1**, was finally purified by means of preparative TLC. The plate was developed twice using  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (50:30:2) as eluent. Small strips on the sides of the plates were sprayed, followed by heating only the sprayed strips to develop bands of compounds. These bands were marked on the unsprayed portion, scraped off, and the compounds eluted from the silica gel with warmed acetone to yield 15 mg of Compound **1**, after filtering. Fraction B was subjected to column chromatography elution using  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (70:30:0.5), before increasing the polarity of the solvent to  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (70:30:3), to yield impure Compound **2**. Further purification was done by means of column chromatography, eluting with  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  (70:30:1), to yield 11 mg of the pure compound.

#### 2.4. Quantitative analysis of verbascoside and isoverbascoside

##### 2.4.1. Sample preparation

For the interspecies study, leaves, flowerheads and twigs of the four species, harvested from localities 1 to 4 (Table 1), were individually analysed to establish the concentrations of Compounds **1** and **2** present in each sample. The intraspecies variation of the compounds present in leaves was investigated in *L. javanica*, collected from localities 4 to 9 (Table 1). To determine the variation within a single population, six individual specimens were collected from locality 4. Portions (5.00 g) of the dried, milled material were individually defatted thrice with  $\text{CHCl}_3$  (20 ml), where after 80% aqueous MeOH (3 × 20 ml) was used for extraction of the target compounds. The aqueous methanol extracts were shaken for 30 min using a platform shaker, before filtering. Combined MeOH extracts of each sample were separately concentrated to 20 ml using a Büchi rotary evaporator at 50 °C. After adding 20 ml of deionised water to each extract, the solutions were partitioned

with 50 ml of  $\text{CHCl}_3$ , and this layer put aside. The aqueous layer was re-extracted twice with additional  $\text{CHCl}_3$  portions, before diluting to 50.0 ml with deionised water. A dilution series was prepared from purchased reference standards of verbascoside and isoverbascoside, ranging from 100.0 to 10.0 mg/l, incorporating caffeine as an internal standard, in MeOH, for use as calibration standards. Standards and extracts were filtered through 0.45 µm nylon filters prior to analysis.

The limit of detection (LOD) and limit of quantification (LOQ) were determined for each analyte using data obtained from regression functions (Excel software) as described by Miller and Miller (2000). Concentrations of the compounds were calculated and expressed as mg/g of dry plant material. Thereafter One-way Anova (single factor without replication) and the Least Significant Difference (LSD) tests were applied to the data. Analysis results with  $p \leq 0.05$  were considered to be significantly different.

##### 2.4.2. HPLC analyses

The HPLC system used consisted of a Varian Prostar HPLC (Model 230; Varian, SMM, Midrand, South Africa) coupled to a Varian UV/Vis detector (Model 310) at 280 nm, and fitted with a manual injection valve with a 20 µl loop. Separation was achieved on a Varian C18 reversed phase column (4.6 × 250 mm; 5 µm particle diameter) at room temperature (25 °C). The Star Chromatography Workstation (Version 6.3) was used for data collection. The initial mobile phase (1 ml/min) was 10% acetonitrile (HiperSolv<sup>®</sup>; HPLC grade) in MilliQ<sup>®</sup> water. Thereafter a linear gradient was applied to reach 40% aqueous acetonitrile after 30 min. The column was then cleaned, by increasing the acetonitrile concentration to 100%, before allowing the column to equilibrate at the initial mobile phase concentration.

### 3. Results and discussion

#### 3.1. Identification of verbascoside (**1**) and isoverbascoside (**2**)

Chemical structures of **1** and **2** (see Fig. 1) were determined by utilizing 1D <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra as well as 2D COSY, HMBC and HSQC spectra. The spectroscopic data are summarized in Table 2.

Significant features visible from the NMR spectra for these structural isomers were two anomeric proton signals in the <sup>1</sup>H NMR spectrum observed at  $\delta_{\text{H}}=4.39$  (*d*, *J*=7.9 Hz), 5.20 (*d*,

Table 2

<sup>1</sup>H (400 MHz) and <sup>13</sup>C NMR (100 MHz) data for verbascoside (**1**) and isoverbascoside (**2**) (CD<sub>3</sub>OD).

| Carbon no.          | 1                    |                                      | 2                    |                                      |
|---------------------|----------------------|--------------------------------------|----------------------|--------------------------------------|
|                     | δ <sub>C</sub> (ppm) | δ <sub>H</sub> (ppm), <i>J</i> (MHz) | δ <sub>C</sub> (ppm) | δ <sub>H</sub> (ppm), <i>J</i> (MHz) |
| <i>Aglycone</i>     |                      |                                      |                      |                                      |
| 1                   | 131.5                |                                      | 131.5                |                                      |
| 2                   | 116.3                | 6.69 <i>d</i> (2.2)                  | 116.3                | 6.64 <i>m</i>                        |
| 3                   | 144.7                |                                      | 144.6                |                                      |
| 4                   | 146.1                |                                      | 146.1                |                                      |
| 5                   | 117.1                | 6.69 <i>d</i> (8.6)                  | 117.1                | 6.64 <i>m</i>                        |
| 6                   | 121.3                | 6.57 <i>dd</i> (8.0, 1.7)            | 121.3                | 6.53 <i>dd</i> (8.1; 2.0)            |
| α                   | 72.3                 | 4.15 <i>m</i> ; 3.80 <i>m</i>        | 72.3                 | 4.05 <i>m</i> ; 3.67 <i>m</i>        |
| β                   | 36.6                 | 2.81 <i>t</i> (7.2)                  | 36.5                 | 2.75 <i>t</i> (7.0)                  |
| <i>Ester moiety</i> |                      |                                      |                      |                                      |
| 1'                  | 127.7                |                                      | 127.6                |                                      |
| 2'                  | 115.2                | 7.07 <i>d</i> (1.8)                  | 115.2                | 7.01 <i>d</i> (1.9)                  |
| 3'                  | 148.0                |                                      | 148.0                |                                      |
| 4'                  | 149.8                |                                      | 149.7                |                                      |
| 5'                  | 116.5                | 6.79 <i>d</i> (8.2)                  | 116.5                | 6.72 <i>d</i> (8.2)                  |
| 6'                  | 123.2                | 6.97 <i>dd</i> (8.2, 1.6)            | 123.2                | 6.92 <i>dd</i> (8.3, 2.0)            |
| α'                  | 114.7                | 6.27 <i>d</i> (15.9)                 | 114.7                | 6.20 <i>d</i> (15.9)                 |
| β'                  | 146.8                | 7.54 <i>d</i> (15.9)                 | 146.8                | 7.52 <i>d</i> (15.9)                 |
| C=O                 | 168.3                |                                      | 168.3                |                                      |
| <i>Glucose</i>      |                      |                                      |                      |                                      |
| 1''                 | 104.2                | 4.39 <i>d</i> (7.9)                  | 104.1                | 4.32 <i>d</i> (7.9)                  |
| 2''                 | 76.0                 | 3.41 <sup>a</sup>                    | 76.0                 | 3.57 <sup>a</sup>                    |
| 3''                 | 81.7                 | 3.54 <i>m</i>                        | 81.7                 | 3.54 <sup>a</sup>                    |
| 4''                 | 70.6                 | 4.97 <i>m</i>                        | 70.4                 | 4.87 <i>m</i>                        |
| 5''                 | 76.2                 | 3.54 <i>m</i>                        | 76.2                 | 3.65 <i>m</i>                        |
| 6''                 | 62.4                 | 3.84 <i>m</i> ; 3.63 <i>m</i>        | 62.3                 | 3.90 <i>m</i> ; 3.65 <i>m</i>        |
| <i>Rhamnose</i>     |                      |                                      |                      |                                      |
| 1'''                | 103.0                | 5.20 <i>d</i> (1.1)                  | 103.0                | 5.14 <i>d</i> (1.5)                  |
| 2'''                | 72.3                 | 3.94 <i>br s</i>                     | 72.2                 | 3.77 <i>m</i>                        |
| 3'''                | 72.0                 | 3.67 <sup>a</sup>                    | 72.0                 | 3.39 <sup>a</sup>                    |
| 4'''                | 73.8                 | 3.28 <sup>a</sup>                    | 73.8                 | 3.26 <sup>a</sup>                    |
| 5'''                | 70.4                 | 3.58 <sup>a</sup>                    | 70.6                 | 3.63 <sup>a</sup>                    |
| 6'''                | 18.4                 | 1.10 <i>d</i> (6.1)                  | 18.4                 | 1.04 <i>d</i> (6.2)                  |

Assignments confirmed by HSQC, HMBC and COSY experiments.

<sup>a</sup> Signals unclear due to overlapping.

*J* = 1.1 Hz) for **1** and at δ<sub>H</sub> = 4.32 (*d*, *J* = 7.9 Hz), 5.14 (*d*, *J* = 1.5 Hz) for **2**, indicating the presence of two sugar moieties, confirmed as β-glucopyranose and α-rhamnopyranose from HSQC and HMBC experiments. The connectivity between the sugars was confirmed by the HMBC correlation between H-1'' of the rhamnose and C-3''' of the glucose in both cases indicating an interglycosidic linkage of the C-1'''(*rha*) → C-3'''(*glu*). Both isomers showed a correlation between H-1'' of the glucopyranose unit with C-α of the phenyl ethyl aglycone in the respective HMBCs confirming the position of the aglycone in the structures. While the <sup>13</sup>C spectra of both compounds showed the presence of a carbonyl carbon (δ<sub>C</sub> = 168.3) characteristic of ester carbonyls, differentiation between **1** and **2** was evident from HMBC experiments where an ester linkage was observed from H-4'' of the glucopyranose unit to the carbonyl carbon of the caffeoyl unit of verbascoside (**1**), and from H-6'' of the glucopyranose unit to the carbonyl carbon of the caffeoyl unit of isoverbascoside (**2**). The complete assign-

ment of all proton and carbon resonances was based on the COSY, HSQC, and HMBC experiments and compared well with those from literature (Liu et al., 1998; Kanchanapoom et al., 2002). We can thus confirm that compound **1** was found to be 3,4-dihydroxy-β-phenylethoxy-*O*-[4''-β-caffeoyl-α-rhamnopyranosyl-(1''',3''')-*O*-β-glucopyranoside], known as verbascoside, and compound **2** was 3,4-dihydroxy-β-phenylethoxy-*O*-[6''-β-caffeoyl-α-rhamnopyranosyl-(1''',3''')-*O*-β-glucopyranoside], known as isoverbascoside.

Verbascoside (**1**): yellow amorphous solid, UV (PDA, MeOH): λ<sub>max</sub> = 326 nm, 289 nm. <sup>1</sup>H and <sup>13</sup>C NMR (<sup>1</sup>H: 300 MHz; <sup>13</sup>C: 75 MHz, MeOH-d<sub>4</sub>; Table 2). IR (KBr) ν = 3384 cm<sup>-1</sup> (O-H), 2855 and 2925 cm<sup>-1</sup> (C-H), 1717 cm<sup>-1</sup> (C=O), 1635 cm<sup>-1</sup> (C=C), 1521 and 1385 cm<sup>-1</sup> (aromatic rings).

Isoverbascoside (**2**): yellow amorphous solid, UV (PDA, MeOH): λ<sub>max</sub> = 322 nm, 288 nm. <sup>1</sup>H and <sup>13</sup>C NMR (<sup>1</sup>H: 300 MHz; <sup>13</sup>C: 75 MHz, MeOH-d<sub>4</sub>; Table 2). IR (KBr) ν = 3405 cm<sup>-1</sup> (O-H), 2927 cm<sup>-1</sup> (C-H), 1698 cm<sup>-1</sup> (C=O), 1630 cm<sup>-1</sup> (C=C), 1523 and 1383 cm<sup>-1</sup> (aromatic rings).

### 3.2. Variation determination by quantitative HPLC analyses

The production of verbascoside (**1**) and isoverbascoside (**2**) by *L. javanica* is most probably linked to the medicinal use of the plant, as these metabolites are well known for their biological activities (Herbert et al., 1991; Pieroni et al., 2000; Pennacchio, 2005). The traditional medicinal use, which involves the preparation of herbal infusions of aerial parts, should result in the extraction of both compounds, since the presence of the sugar moiety renders them water-soluble. Verbascoside and isoverbascoside analyses were done to determine the occurrence and variation of these metabolites in *L. javanica* and the other indigenous *Lippia* species, and to identify the plant part in which the compounds are most abundant.

The LOD and LOQ for verbascoside (**1**) were determined as 4.94 mg/l and 14.4 mg/l, respectively, while the values for isoverbascoside (**2**) were 4.66 mg/l and 15.5 mg/l, respectively. It was established that the two isomers were present, above the LOQs, in the aerial parts of all four species. Concentrations of both compounds were higher in the leaves than in the flowers and twigs for all species. These differences were significant, with the exception of verbascoside present in *L. rehmannii* leaves (Table 3). *L. javanica* leaves contained the highest concentration of verbascoside (1.5 mg/g), while leaves of *L. scaberrima* contained only 0.63 mg/g. The lowest levels of verbascoside were found in the twigs of *L. scaberrima* (0.16 mg/g). The concentrations of isoverbascoside ranged from 0.18 mg/g in *L. rehmannii* leaves to 0.019 mg/g in *L. scaberrima* twigs, but for all species the concentrations were highest in the leaf samples. These findings serve to justify the traditional use of infusing a combination of the aerial plant parts for phytomedicinal purposes, since both compounds occur in all plant parts, although the concentrations vary.

*L. javanica* was selected for the intraspecies investigations, since this species produced the highest levels of the two



Table 3  
Concentrations of compounds in the aerial parts of the four *Lippia* species.

| Plant part                   | Average mass in mg g <sup>-1</sup> of dry weight |                 |
|------------------------------|--|-----------------|
|                              | Verbascoside                                     | Isoverbascoside |
| <i>L. javanica</i> leaves    | 1.5 a  | 0.15 a          |
| <i>L. javanica</i> flowers   | 1.2 b  | 0.089 b         |
| <i>L. javanica</i> twigs     | 0.61c  | 0.051 c         |
| <i>L. scaberrima</i> leaves  | 0.63 c   | 0.074 b         |
| <i>L. scaberrima</i> flowers | 0.37 d   | 0.029 d         |
| <i>L. scaberrima</i> twigs   | 0.16 e   | 0.019 d         |
| <i>L. rehmannii</i> leaves   | 0.83 f   | 0.18 e          |
| <i>L. rehmannii</i> flowers  | 0.68 f   | 0.067 b         |
| <i>L. rehmannii</i> twigs    | 0.43 d   | 0.028 d         |
| <i>L. wilmsii</i> leaves     | 1.2 b  | 0.13 f          |
| <i>L. wilmsii</i> flowers    | 0.86 f   | 0.041 c         |
| <i>L. wilmsii</i> twigs      | 0.50 c   | 0.035 d         |

Each value represents the mean of three replicates. The values are ordered from highest to lowest in each column by means of the lower-case letters where “a” indicates highest and “f” lowest. Averages followed by the same lower-case letter within each column did not differ significantly ( $p \leq 0.05$ ).

compounds. Determination of verbascoside (**1**) in leaves from six individual specimens within a single population showed that there were no significant differences in the concentrations (Table 4). With regard to the isoverbascoside (**2**) content, only one specimen differed significantly from the rest. Although plants growing in the same area are exposed to the same climate and average precipitation, they could experience small differences in their micro-environments (soil nutrients, run-off and sunlight). These factors have been shown to affect secondary metabolite profiles (Burbott and Loomis, 1957; Galambosi and Peura, 1996). The lack of variation displayed in the population investigated suggests that the genetic traits for verbascoside/isoverbascoside production of these individuals are similar.

The specimens used for the inter-population comparison were harvested from areas as far apart as North West Province and the Lowveld of Mpumalanga, which have prominent differences in climate and geology. It was therefore expected that the concentrations of secondary metabolites in the individuals could differ substantially (Van Vuuren et al., 2007). However, only small variations in the levels of

Table 4  
Concentrations of verbascoside and isoverbascoside in *L. javanica* within a single population.

| Specimen | Average mass in mg g <sup>-1</sup> of dry weight |                 |
|----------|--|-----------------|
|          | Verbascoside                                     | Isoverbascoside |
| 1        | 1.5 a  | 0.15 a          |
| 2        | 1.4 a  | 0.16 a          |
| 3        | 1.3 b  | 0.15 a          |
| 4        | 1.4 ba   | 0.13 b          |
| 5        | 1.5 a  | 0.14 ba         |
| 6        | 1.5 a  | 0.090 c         |

Each value represents the mean of three replicates. The values are ordered from highest to lowest in each column by means of the lower-case letters where “a” indicates highest. Averages followed by the same lower-case letter within each column did not differ significantly ( $p \leq 0.05$ ).

verbascoside (1.2–1.6 mg/g) and isoverbascoside (0.12–0.16 mg/g) were detected in *L. javanica* plants from the six different localities (Table 5). The specimen from Losberg (North West Province) contained the lowest levels of verbascoside (1.2 mg/g), which differed significantly from the rest. In contrast, the specimen from Lydenburg (Highveld) exhibited the highest concentration of verbascoside (1.6 mg/g), but the lowest level of isoverbascoside (0.12 mg/g). The verbascoside content of specimens from Long Tom Pass, which is also situated on the Highveld, was comparable to those from the Lowveld (Localities no. 4 and 5), indicating that climate and geology did not play a major role in the production of these metabolites. These findings are in agreement with Rodolfo et al. (2006) who established that the concentrations of phenolic compounds, including verbascoside, were consistent in different populations of *Lippia multiflora* from Ghana. The consistency of the phenylethanoid concentrations found in *L. javanica* suggests that the plant can be harvested over a large area for medicinal purposes. This justifies the use of the plant by different ethnic groups (Van Wyk et al., 2000), which are geographically separated.

#### 4. Conclusions

This is the first report on the presence of verbascoside and isoverbascoside in *L. javanica* and three other *Lippia* species indigenous to South Africa. The finding that these pharmacologically important secondary metabolites are present in polar extracts of the aerial plant parts, sheds more light on the medicinal uses of these plants. Furthermore, small variation in concentration of these compounds in widely distributed populations justifies similar effective medicinal use in all areas of distribution. Moreover, these compounds may in future be used as chemotaxonomic markers for *Lippia* species in southern Africa.

While this study focussed mainly on the production of phenylethanoids by South African *Lippia* species, a further investigation concerning the effect of latitude on the metabolite pattern of *L. javanica* from localities across southern Africa will be valuable. *L. javanica* occurs abundantly in South Africa between 22° (Makhado-Messina area) and 33° (Katberg area,

Table 5  
Concentrations of verbascoside and isoverbascoside in *Lippia javanica* from different localities.

| Locality no. | Locality                      | Average mass in mg g <sup>-1</sup> of dry weight |                 |
|--------------|-------------------------------|--|-----------------|
|              |                               | Verbascoside                                     | Isoverbascoside |
| 4            | Botanical Gardens (Nelspruit) | 1.5 a  | 0.14 a          |
| 5            | Ferreira Street               | 1.5 a  | 0.15 ab         |
| 6            | Long Tom Pass                 | 1.4 a  | 0.13 ac         |
| 7            | Losberg                       | 1.2 b  | 0.16 bd         |
| 8            | Lydenburg                     | 1.6 ac   | 0.12 c          |
| 9            | TUT (Pretoria west)           | 1.4 a  | 0.15 ab         |

Each value represents the mean of three replicates. The values are ordered from highest to lowest in each column by means of the lower-case letters where “a” indicates highest. Averages followed by the same lower-case letter within each column did not differ significantly ( $p \leq 0.05$ ).

Eastern Cape) (Retief, 2006), yet collections for this study were only done between 25° (Orkney) and 27° (Lydenburg) in South Africa only. Ecological niches such as *Lippia* species growing in tropical areas in sandy soils (in northern KwaZulu-Natal for example), as well as those from the South African Highveld, should be included in such an extensive variation study.

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