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South African Journal of Botany 76 (2010) 567–571
SOUTH AFRICAN JOURNAL OF BOTANY
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# South African *Lippia* herbal infusions: Total phenolic content, antioxidant and antibacterial activities

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Received 1 February 2010; received in revised form 14 April 2010; accepted 19 April 2010

**Abstract**

*Lippia javanica* and *Lippia scaberrima* are used as herbal remedies and are commercially traded as health teas in southern Africa under the brands "Mosukujane" and "Musukudu", respectively. This study evaluates the relationship between the presence of phenolic compounds and the antioxidant activities of infusions prepared from four *Lippia* species (*L. javanica*, *L. scaberrima*, *L. rehmannii* and *L. wilmsii*) indigenous to South Africa. The antioxidant activities of the infusions, determined by the 2,2-diphenylpycrylhydrazyl (DPPH) method, were also compared to those of popular black, green and herbal tea brands. Of the four indigenous species, infusions of *L. javanica* and *L. wilmsii* exhibited the highest antioxidant activities ($EC_{50}$: 358 and 525 μg/ml, respectively) and contained the most phenolic compounds (14.8 and 14.5 mg/ml of dry weight gallic acid equivalent, respectively). Antibacterial activities of methanolic extracts of the four *Lippia* species were determined against four human pathogens (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*). The extract of *L. javanica* was the most active against all the pathogens tested. Those *Lippia* species (*L. javanica* and *L. wilmsii*) previously reported to produce higher levels of the pharmacologically active phenylethanoid glycosides verbascoside and isoverbascoside, portrayed stronger antioxidant and antibacterial activities. This study gives credence to the use of infusions of these *Lippia* species for their general health benefits.


*Keywords:* Antibacterial activity; Antioxidant; *Lippia*; Phenolics; Tea

## 1. Introduction

While tea specifically refers to infusions prepared from the leaves of *Camellia sinensis* (Du Toit et al., 2001), herbal teas known as *tisane*, are infusions made from roots, flowers, leaves, seeds or twigs of other plants. Tea is the most popular non-alcoholic beverage in the world, due to the multitude of associated health benefits. Epidemiological studies have linked the consumption of tea (*C. sinensis*) to a lower risk of several types of cancer including gastric, skin, ovarian, stomach, oral cavity, oesophageal and lung cancers (Vinson et al., 1995; Vanessa and Williamson, 2004). Polyphenols present in tea reportedly prevent blood clotting and lower cholesterol levels, thereby reducing the risk of developing cardiovascular diseases (Vinson et al., 1995). The beverage has also been found to exhibit anti-allergenic (Yamamoto et al., 2004) and antimicrobial activities (Di Paola et al., 2005). Rooibos tea is a herbal tea prepared from the fermented aerial parts of *Aspalathus linearis*, a shrub that naturally occurs in the Cederberg area of the Western Cape, South Africa. Several *in vitro* studies using a variety of assays have evaluated the antioxidant activities of infusions of rooibos (Joubert et al., 2008). Findings indicated that fermentation generally decreases the antioxidant activity of rooibos tea.

Many phenolic compounds originating from plants are more powerful antioxidants than vitamins E and C (Vinson et al., 1995). This ability to scavenge free radicals is essential in the prevention of oxidative damage to cells in the human body, caused by exposure to certain chemicals in the environment. There has been an upsurge in interest in plants as sources of natural antioxidants, because the intake of commercial synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and gallic acid esters have been associated with negative effects on health (Karori et al., 2007).

Five *Lippia* species are listed as indigenous to South Africa (Germishuizen et al., 2006). *Lippia javanica* and *L. scaberrima*

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doi:10.1016/j.sajb.2010.04.010

infusions are used in the region for medicinal purposes and are both sold as natural caffeine-free teas under the names "Mosukujane" and "Mosukudu", respectively (www.exporters.bw/catalogue/herbal/thusano_lefatsheng/index.html). Infusions of *L. rehmannii* are not generally used, possibly due to the presence of the hepatotoxic compounds icterogenin and rehmannic acids (Heikel et al., 1960). This species has been implicated in outbreaks of liver toxicity in sheep (Watt and Breyer-Brandwijk, 1962). Many previous studies have focussed on the compositions of the essential oils obtained from the indigenous *Lippia* species (Viljoen et al., 2005; Linde et al., 2010). However, despite the use of infusions of *Lippia* for medicinal purposes, scientific reports concerning the activities of their polar extracts (Muchuweti et al., 2006; Mujovo et al., 2008; Shikanga et al., 2009) are sparse. Pharmacologically active phenylethanoid glycosides, verbascoside and isoverbascoside, were recently reported to occur in large amounts in the aerial parts of four of the indigenous *Lippia* species (Olivier et al., 2010). These metabolites are well known antioxidants (Frum et al., 2007), yet the antioxidant activities of *Lippia* infusions have not been evaluated. In this investigation, infusions of four *Lippia* species (*L. javanica*, *L. scaberrima*, *L. rehmannii* and *L. wilmsii*) were screened for their antioxidant properties using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Du Toit et al., 2001). The free radical scavenging capacity (RSC) values obtained were compared to those of ten commercial black, green and herbal teas, as well as to those of verbascoside and isoverbascoside. In addition, the total soluble phenolic contents and antibacterial activities of the *Lippia* infusions against four important human pathogens were determined to evaluate the health benefits of the teas.

## 2. Materials and methods

### 2.1. Sampling

Aerial parts of four *Lippia* species were harvested in January 2004 from various regions of South Africa. *L. javanica* was collected from Nelspruit (Mpumalanga), *L. wilmsii* from Petra Mountain Inn (Mpumalanga), *L. rehmannii* from Pretoria West (Gauteng) and *L. scaberrima* from Wolmaransstad (North West Province). Voucher specimens (Bosman&Combrinck 15, 16, 21 and 31, respectively) were deposited in the herbarium of the South African Biodiversity Institute, Pretoria. The collected plant material was air-dried at room temperature (25 °C).

### 2.2. Analysis of total phenolic compounds in indigenous Lippia species

Dried plant material of each of the four *Lippia* species was separated into leaves, flowers and twigs, ground into powder and sieved to a uniform size (<500 μm). Extraction of the soluble phenolic compounds was done on microscale as described by Regnier and Macheix (1996). The combined methanol:acetone:water (7:7:1 v/v/v) supernatants from each sample were dried under a stream of nitrogen and stored at 4 °C.

The concentrations of the soluble phenolic compounds in the extracts were determined using the Folin–Ciocalteau technique as described by Singleton et al. (1999) and adapted by Sivakumar et al. (2005) for microtitre plates. An ELISA microplate reader (Multiskan Ascent V1.24) in continuous mode was used to determine the absorbance of each solution at 720 nm. Spectrophotometric measurements of the phenolic concentrations in the extracts were calculated from a gallic acid (AR grade) standard curve ($y=0.0013x+0.0177$; $R^2=0.9982$) ranging from 0.200 to1.00 mg/ml in methanol. The phenolic content was expressed as mg of gallic acid equivalent per gram of dry plant weight. Single Factor ANOVA was used to determine if differences in the mean values of gallic acid equivalents of the four species and different plant parts were significant.

### 2.3. Antioxidant activities of selected commercial teas and Lippia infusions

Ten commercial black and green teas including Joko™, Five Roses™, Glen®, Rooibos Fresh Pack™, Rooibos Chai Spiced™, Lipton Yellow label™, Lipton Green™, Lipton Herbal™, Tetley® and English Breakfast Tea™ were purchased from a local supermarket (Shoprite Checkers, Pretoria). Teas were prepared from 10 mg of dry powder (<500 μm particle size) per ml of boiling deionised water, while *Lippia* infusions were prepared using 100 mg of dry leaf powder per ml of water. Plant material was steeped for 5 min before filtering through Whatman no. 4 filter paper. The concentration of each extract was calculated after drying 1.00 ml of the extract and obtaining the mass. A series of dilutions of each extract (1.0; 2.0; 3.0; 4.0 and 5.0 mg/ml) was prepared in deionised water (four replicates). In addition, verbascoside (99% pure) and isoverbascoside (99% pure; Industrial Analytical (Pty) Ltd) were prepared as 1.0 mg/ml aqueous solutions. A solution of 0.10 mM 2,2-diphenylpycrylhydrazyl (DPPH; Merck, Germany) in methanol was used for evaluation of the antioxidant activities according to the method used by Du Toit et al. (2001). A freshly prepared dilution series of ascorbic acid (AR grade; Merck, Germany) ranging from 0.20 to 1.0 mg/ml was used as reference. Radical scavenging capacity was expressed in terms of the concentrations of the compounds and teas/infusions required to decrease the initial absorbance of DPPH at 492 nm (instead of 515 nm) by 50% ($EC_{50}$). The $EC_{50}$ value of each sample was graphically determined by plotting the percentage discolouration of DPPH as a function of the reference standard and sample concentrations.

### 2.4. Antibacterial activities of Lippia species indigenous to South Africa

Antibacterial activities were determined using the serial microdilution method described by Eloff (1998). Methanolic plant extracts were used to test for antibacterial activities, since the solvent was much easier to dry and the extract compositions were very similar to those of the aqueous extracts. Verbascoside, isoverbascoside and infusions of the four *Lippia* species

were tested against *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The pure compounds were prepared as 1.0 mg/ml solutions in acetone, whereas the plant extracts were prepared as 10.0 mg/ml solutions in acetone. Gentamycin (Virbac®) and acetone were used as positive and negative controls, respectively. The minimum inhibitory concentration (MIC) in each dilution series was determined by recording the concentration of the extract/compound in the first well where no discolouration (no bacterial growth) was observed.

## 3. Results and discussion

Results obtained indicated that the total phenolic contents (Table 1) were higher in the leaf extracts (8.7–14.8 mg/ml) than in the flowers (5.5–9.9 mg/ml) of all the *Lippia* species, while the lowest concentrations were found in twigs (4.6–8.3 mg/ml). A lower phenolic content was expected for twigs, since it is well established that phenolics in twigs are primarily transformed for the manufacture of lignin in order to protect and strengthen cell walls (Passardi et al., 2004).

The highest concentrations of total soluble phenolic compounds were present in the leaf extracts of *L. javanica* (14.8 mg gallic acid equivalent/g) and *L. wilmsii* (14.5 mg gallic acid equivalent/g) (Table 1). Leaf extracts of *L. scaberrima* contained the least phenolic compounds and the value obtained (8.7 mg/g) was significantly lower than those obtained for the other species. A tannic acid equivalent of only 0.64 μg/g was reported by Muchuweti et al. (2006) for an ethanolic extract of *L. javanica*. However, these researchers were unable to correlate total phenolic content and antioxidant activities of their plant extracts. Mujovo et al. (2008) isolated the phenolic compounds apigenin, 5,7-dimethoxy-6-methylflavone and two luteolin derivatives from *L. javanica*, but the metabolites were not quantified.

An infusion prepared from *L. javanica* proved to have the highest antioxidant activity ($EC_{50}$=358 μg/ml) of the four *Lippia* infusions, followed by that of *L. wilmsii*, with an $EC_{50}$ value of 525 μg/ml (Table 2). The least potent infusion was obtained from *L. scaberrima*, as reflected by the high concentration (1150 μg/ml) required to discolour the DPPH. The trend observed for the antioxidant activities of the *Lippia* infusions was the same as that for the total phenolic contents, suggesting a link between the two. The high antioxidant activities displayed by *L. javanica* and *L. wilmsii* infusions can be at least partially attributed to the high levels of verbascoside reported (Olivier et al., 2010) in leaf extracts of these species (1.5 mg/g dw and 1.2 mg/g, respectively). Similarly, the poor antioxidant activities and lower verbascoside levels reported for *L. rehmannii* (0.83 mg/g dw) and *L scaberrima* (0.63 mg/g dw) strengthen this conclusion. The $EC_{50}$ values of verbascoside (89 μg/ml) and isoverbascoside (101 μg/ml) were very low and close to that of ascorbic acid (75 μg/ml), confirming reports that caffeoyl-containing compounds such as verbascoside and isoverbascoside are particularly powerful antioxidant agents (Bilia et al., 2008). These two phenylpropanoid glycosides are highly soluble in water due to their polar nature, arising from the presence of two sugar moieties. The influence of verbascoside on the antioxidant activities of the infusions was presumed to be greater than that of its isomer, since verbascoside occurs in approximately ten-fold higher concentrations in the leaves of all four species (Olivier et al., 2010). Our results support the finding by Muchuweti et al. (2006) who reported a 75% inhibition of the DPPH radical by an ethanolic extract of *L. javanica*.

The $EC_{50}$ value obtained for *L. javanica* (358 μg/ml) compared well to those of many commercial teas with high antioxidant capacities such as Glen (300 μg/ml), Lipton Green (320 μg/ml) and Lipton Yellow (365 μg/ml) teas. However, teas prepared from Five Roses (225 μg/ml) and Joko (213 μg/ml) leaves exhibited higher antioxidant activities than the infusion of *L. javanica*. The $EC_{50}$ values obtained in this study compared favourably with values reported by Du Toit et al. (2001): 230 μg/ml for Five Roses, 350 μg/ml for Glen and 400 μg/ml for Lipton Yellow label tea. Concerning the herbal teas, Rooibos Fresh Pack displayed the best antioxidant activity (333 μg/ml) compared to the rather poor activities of Rooibos Chai Spiced (740) and Lipton Herbal

Table 1
Concentrations of total soluble phenolic compounds in different aerial parts of four *Lippia* species.

| Species | Gallic acid equivalent (mg/g of dry weight)[a] | | |
|---|---|---|---|
| | Leaves | Flowers | Twigs |
| *Lippia javanica* | 14.8a | 9.9a | 8.3a |
| *Lippia scaberrima* | 8.7c | 5.5d | 4.6d |
| *Lippia rehmannii* | 12.5b | 7.0c | 6.2c |
| *Lippia wilmsii* | 14.5a | 8.6b | 7.4b |

[a] Each value represents the mean of four replicate determinations. Within each column, means followed by different lower-case letters differed significantly ($p>0.05$).

Table 2
Antioxidant activities of *Lippia* infusions and popular tea brands expressed as $EC_{50}$ values, ascorbic acid equivalents and the number of cups of tea equivalent in RSC to a single capsule (200 mg) of ascorbic acid. All values are the mean of four replicates.

| Tea/infusion | $EC_{50}$ (μg/ml) | Ascorbic acid equiv. (mg/g dw.) | Cup equiv. |
|---|---|---|---|
| *L. javanica* | 358 | 209 | 0.40 |
| *L. wilmsii* | 525 | 144 | 0.60 |
| *L. rehmannii* | 720 | 104 | 0.90 |
| *L. scaberrima* | 1150 | 65 | 1.4 |
| Joko™ | 213 | 353 | 0.30 |
| Five Roses™ | 225 | 333 | 0.30 |
| Tetley® | 275 | 273 | 0.30 |
| Glen® | 300 | 250 | 0.40 |
| Lipton Green™ | 320 | 234 | 0.40 |
| English Breakfast™ | 325 | 231 | 0.40 |
| Lipton Yellow label™ | 365 | 205 | 0.40 |
| Rooibos Fresh Pack™ | 333 | 225 | 0.40 |
| Lipton Herbal™ | 575 | 130 | 0.70 |
| Rooibos Chai Spiced™ | 740 | 101 | 0.90 |
| Ascorbic acid | 75.0 | – | – |
| Verbascoside | 89.0 | – | – |
| Isoverbascoside | 101 | – | – |

(575 μg/ml). These results are in agreement with the very low antioxidant activities (>620 μg/ml) for four fermented Rooibos brands reported by Du Toit et al. (2001).

The RSC of the teas were calculated as ascorbic acid equivalents based on 200 mg, or one capsule, of ascorbic acid. One capsule ensures an intake of 120 mg ascorbic acid, which is the recommended daily intake (RDI) required for reduced susceptibility to chronic diseases such as cancer and cardiovascular diseases (Carr and Frei, 1999). The results were also expressed as the number of cups of tea required to obtain the same RSC as that provided by a 200 mg capsule of ascorbic acid (Table 2). With the exception of *L. scaberrima*, there were no significant differences between the numbers of cups of each *Lippia* infusion required. One cup of each of these infusions is theoretically sufficient to supply the equivalent RDI of ascorbic acid. However, it must be strongly emphasised that these are only *in vitro* results that do not take the bioavailability of the compounds into account. Moreover, intraspecies variations may result in variable activities when different batches of plant material are used for the preparation of teas and infusions. Actively growing tea (*C. sinensis*) plants are known to respond to their environment by accumulating different amounts of secondary metabolites (Chen et al., 2010).

A comparison of the antimicrobial activities of methanolic extracts of the four species, revealed that *L. javanica* displayed the highest antimicrobial activities (0.13–0.42 mg/ml) against all four pathogens, followed by the extract of *L. wilmsii* (Table 3). Extracts of *L. scaberrima* were the least active, although in some cases the MIC values obtained corresponded to those of *L. rehmannii*. Verbascoside displayed strong antibacterial activities against *S. aureus*, *E. faecalis* and *E. coli* (MIC ≤ 0.10 mg/ml), thereby indicating the contributory role of the compound in the antimicrobial activities of the extracts. The antibacterial properties of verbascoside have previously been demonstrated (Pennacchio, 2005).

Minimum inhibitory concentrations obtained for the methanolic extracts of *L. javanica* and *L. wilmsii* are promising, since natural products with MIC values below 1 mg/ml are generally considered to be noteworthy findings (Gibbons, 2004). The *in vitro* antibacterial activities of the infusions could possibly account for the effectiveness of *L. javanica* against fever, infections of the respiratory tract and other secondary bacterial infections (Hutchings et al., 1996). The volatile components from the species were previously reported to have strong antibacterial activities against *S. aureus*, *E. coli* and *Bacillus subtilis* (Manenzhe et al., 2004). Some of these volatiles could contribute to the antibacterial activity of the infusions. Although *L. scaberrima* is also used in traditional medicine to treat symptoms similar to those treated with *L. javanica* (Van Wyk et al., 2000), it was found to be a less effective antioxidant and antibacterial agent against the pathogens tested. The role of verbascoside and isoverbasoside in the activity of the extracts are unclear, but a previous report by De Andrade Lima et al. (2003) indicates synergistic activity of these compounds against *S. aureus, E. coli* and *E. faecalis*, *B. subtilis* and *Candida albicans*. The phenolic compound apigenin, isolated by Mujovo et al. (2008) from *L. javanica* is a well known antibacterial agent, which would certainly contribute to the observed inhibitory action of the extract against the bacteria tested. Apigenin was shown to be highly active against *Vibrio cholera* and *E. faecalis* (Martini et al., 2004), while Basile et al. (1999) reported the inhibition of *Salmonella typhi*, *Pseudomonas mirabilis* and *P. aeruginosa* by the compound.

The *in vitro* antioxidant and antibacterial activities of four *Lippia* species indigenous to South Africa were demonstrated in this study. These activities may be attributed to the presence of phenolic compounds and the phenylethanoid glycosides, verbascoside and isoverbascoside, in infusions prepared from the leaves. The use of "Mosukujane" and "Musukudu" as general health teas have been validated by the high total phenolic contents and antioxidant activities of the infusions, and the antibacterial activities of the methanolic extracts.

Table 3
Minimum inhibitory concentrations of *Lippia* extracts and compounds against four human pathogens.

| Extract/compound | Minimum inhibitory concentration (mg/ml) | | | |
|---|---|---|---|---|
| | Microorganism | | | |
| | *Staphylococcus aureus* | *Enterococcus faecalis* | *Escherichia coli* | *Pseudomonas aeruginosa* |
| *Lippia javanica* | 0.13 | 0.14 | 0.31 | 0.42 |
| *Lippia wilmsii* | 0.31 | 0.31 | 0.31 | 0.63 |
| *Lippia rehmannii* | 0.63 | 0.63 | 1.3 | 1.3 |
| *Lippia scaberrima* | 1.3 | 0.63 | 1.3 | 1.3 |
| Verbascoside | 0.060 | 0.10 | 0.10 | 0.25 |
| Isoverbascoside | 0.13 | 0.15 | 0.25 | 0.25 |
| Gentamycin (+ control) | 0.020 | 0.040 | 0.070 | 0.040 |
| Acetone (− control) | >2.5 | >2.5 | >2.5 | >2.5 |

## Acknowledgements

The authors thank the National Research Foundation (South Africa) for financial support and the staff of the Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria for assistance with the antibacterial assays.

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Edited by LJ McGaw