African Journal of Biotechnology Vol. 5 (13), pp. 1289-1293, 3 July 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

*Full Length Research Paper*

# The scientific merit of *Carpobrotus mellei* L. based on antimicrobial activity and chemical profiling

**E. P. Springfield[1]* and F. Weitz[2]**

[1]South African Traditional Medicines Research Group, School of Pharmacy, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa.
[2]Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa.

Accepted 31 March, 2006

**Species of the genus *Carpobrotus* are similar in appearance and have been used for medicinal purposes over many generations. *Carpobrotus mellei* is endemic to the south-Western Cape of South Africa, and also used for various ailments. To date no scientific validation and information has been reported on *C. mellei*. This study investigated the antimicrobial potential of *C. mellei* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Mycobacterium smegmatis*, and determined the minimum inhibitory concentrations (MIC) by two-fold serial dilution. *C. mellei* showed antimicrobial activity against *S. aureus* and *M. smegmatis* in the disc diffusion method. Eight chemical compounds showed clear zones of inhibition in the bioautograms, seven against *S. aureus* and three compounds were active against *M. smegmatis*. The ethyl acetate extracts have MIC values of 7.5 mg/ml and 15 mg/ml against *S. aureus* and *M. smegmatis*, respectively. Phytochemical tests indicated the presence of flavonoids, hydrolysable tannins, phytosterols and aromatic acids. High performance liquid chromatography (HPLC) showed a species-specific spectrum at a wavelength of 280 nm. The results confirm that *C. mellei* has scientific merit, and can substitute one of the other *Carpobrotus* species for antimicrobial usage within the south-Western Cape of South Africa.**

**Key words:** *Carpobrotus mellei*, antimicrobial activity, chemical profile, scientific validation.

## INTRODUCTION

The genus *Carpobrotus* (Aizoaceae) is immediately recognizable both in the field and in the herbarium by its trailing habit and long, robust internodes. Correct identification of species within the genus *Carpobrotus* must be done by a taxonomist because of their overall similarity. *Carpobrotus mellei* has relatively small flowers and differs from all the other species by the extremely long stigmas overtopping the stamens. This species is restricted to the mountains of the south-Western Cape (Wisura et al., 1993). Species within the *Carpobrotus* genus are used to treat infections of the mouth and throat, and also said to be effective against toothache, earache, and oral and vaginal thrush (Watt and Breyer-Brandwijk, 1962; van Wyk et al., 1997).

Active antimicrobial flavonoids were purified and identified from *Carpobrotus edulis* by Van der Watt et al. (2001). A publication by Springfield et al. (2003) validated medicinal claims, and added scientific information concerning antimicrobial analysis and chemical profiling of *Carpobrotus muirii* and *Carpobrotus quadrifidus*, respectively.

In the Montagu magisterial district of the Western Cape of South Africa, species of *Carpobrotus* are being used for “dropsy, sprue, mouth sores, sore throat, and St. Anthony’s fire” (Montagu museum 1998). The similar appearance in this genus and the occurrence of *C. mellei* in the south-Western Cape, together with the widespread distribution of *C. edulis* through most parts of South Africa (Wisura et al., 1993), cause speculation that *C.*

*Corresponding authors E-mail: evan@puris.co.za; Telephone: +27 (0)21 865 2342; Puris Natural Aroma Chemicals, P.O. Box 12127, Die Boord, 7613

*edulis* and *C. mellei* are being used interchangeably in this area (W. Plaaitjies, South Africa, oral communication). No scientific information is available on *C. mellei*. The current investigation followed the methods used by Springfield et al. (2003) to generate scientific information on *C. mellei*, which include antimicrobial findings and a detailed chemical profile to illustrate the major chemical class of components.

## MATERIALS AND METHODS

### Plant material

Specimens of *C. mellei* were collected at Montague in the south-Western Cape, South Africa (33° 40' 15 South; 20° 07' 52 East) at an altitude of 600 m and were authenticated by the curator of the University of the Western Cape Herbarium. Voucher specimens (Springfield003) were prepared and deposited in the UWC Herbarium. The collected plant material were cut in smaller pieces and dried at 40°C in an oven for five days, until dry. The dried material was milled, to shorten the extraction period (Eloff, 1998a) and passed through a 850 microns sieve.

### Phytochemical analysis

Phytochemical analyses were carried out according to the methods of Latté (1999). The plant extracts prepared according to Latté (1999) were screened for the following biologically active secondary compounds: coumarins, flavonoids, hydrolysable tannins, condensed tannins, alkaloids, phytosterols, and aromatic acids.

### Solvent extraction

Several extraction methods was considered, which included not only well-known techniques associated with major phytochemical groups such as flavonoids, but also other approaches, based on solubilities in water and various solvent combinations (to determine hydrophylicity or lipophilicity), and on molecular weights, polarity, and degree of ionizability. The milled plant material (100 g) was shaken in 200 ml of acetone : water (2:1) and further extracted according to Latté (1999) to obtain four fractions of different polarities. The extract, from lowest to highest polarity, were petrol ether (PE), chloroform (CF), ethyl acetate (EA) and water (WT). Each extract was evaporated to dryness on a Buchi Rotavapor (Labortechnik, Switzerland). A part of the ethyl acetate and water fractions respectively, were redissolved in 80% ethanol and the tannins were removed according to Van der Watt et al. (2001).

### Antimicrobial screening

The microorganisms used were *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231) and *Mycobacterium smegmatis*. *M. smegmatis* was a gift from Professor Paul van Helden of the Department of Biochemistry and Physiology at the University of Stellenbosch Medical School. The *S. aureus*, *P. aeruginosa* and *C. albicans* were obtained from the Medical Biosciences Department at the University of the Western Cape.

The PE, CF, EA and WT extracts were dissolved in 100% methanol (MeOH) to a final concentration of 40 mg/ml. All solutions were sterilized by filtration through a 0.45 µm membrane filter (MSI, Westboro, MA). Sterile 9 mm discs were impregnated with 50 µl of extract and placed on the surface of agar plates inoculated with a microbial culture. Each extract was tested in triplicate. Ciprofloxacin (40 µg/disc) served as positive control for *S. aureus, P. aeruginosa* and *M. smegmatis*, whereas amphotericin B (25 µg/disc) was the control for *C. albicans*. Agar plates containing the fungi and bacteria were incubated at 37°C for 24, while those of mycobacteria were incubated at 37°C for 48. After incubation, inhibition zones were recorded as the diameter of the growth free zones around the disc.

For direct bioassay on thin layer chromatography (TLC) plates, 21 µl EA extract (95 mg/ml) was applied to 5 x 20 cm silica F254 (Merck) glass plate. Best separations were achieved with ethyl acetate : methanol : water (100:13.5:10) according to Van der Watt et al (2001). The developed TLC plates were dried overnight, and agar, which was inoculated with *S. aureus*, and *M. smegmatis* respectively, was poured over the plates. Agar plates containing the bacteria and mycobacteria were incubated at 24 and 48 h, respectively. After incubation, the plates were sprayed with a 2 mg/ml solution of *p*-iodonitrotetrazolium violet (Sigma). Clear zones on the chromatogram indicated inhibition of growth (Beugue et al., 1972).

A stock solution of (100 mg/ml) was prepared for the tannin-free ethyl acetate and aqueous residues in distilled water. Minimum inhibitory concentration (MIC) was determined by two-fold serial dilution of extracts beyond the concentration where no inhibition of growth of *S. aureus* (ATCC 29213) and *M. smegmatis* was observed. The wells in the dilution series were inoculated with the relevant cultures, incubated overnight at 37°C and 48 h for *M. smegmatis*. Then 0.2 mg/ml *p*-iodonitrotetrazolium violet [INT] (Sigma) was added. After further incubation bacterial growth was indicated by the red colour of the INT formazan produced. The minimum bactericidal/mycobactericidal concentration (MBC/MMC) was determined by examining the microplates at different times (Eloff, 1998b).

### HPLC

The chromatographic system used includes a Beckman HPLC system consisting of a double pump Programmable Solvent Module 126, Diode Array detector Module model 168, with 32 Karat Gold software supplied by Beckman, Column C18 Bondapak 5 µm and dimensions (250 x 4.6 mm). The chromatographic conditions were as follows: Mobile phase, solvent A: methanol (MeOH); solvent B: 5% acetic acid ($CH_3COOH$); Mode: gradient, increasing the organic phase (MeOH) from 20% to 90% over 18 min; flow rate: 1ml/min; reference standard: Rutin (2.5 g dissolved in 100 ml MeOH); injected volume: 10 µl. The run time was 25 min. Detection wavelength of 270 nm on channel A and 360 nm on channel B.

## RESULTS

*C. mellei* tested positive for flavonoids, hydrolysable tannins, phytosterols and aromatic acids, and negative for coumarins, condensed tannins and alkaloids. The dried leaves of *C. mellei* showed antibacterial activity against *S. aureus* and *M. smegmatis* for the extracts prepared (Table 1). No activity was detected in the lipophilic extracts (petroleum ether and chloroform). Eight major compounds were present in the tannin-free ethyl acetate extract of *C. mellei*, of which seven showed activity against *S. aureus* and only three compounds active against *M. smegmatis* (Table 2). The TLC separation in the bioautograms (not shown) indicated the

**Table 1.** Growth inhibition activity of *Carpobrotus mellei* against *S. aureus, P. auruginosa, C. albicans* and *M. smegmatis*.

| Plant extracts[a]/drugs | Zone of inhibition of micro-organisms (mm) | | | |
|---|---|---|---|---|
| | ***S. aureus*** | ***P. aeruginosa*** | ***C. albicans*** | ***M. smegmatis*** |
| PE | 0 | 0 | 0 | 0 |
| CF | 0 | 0 | 0 | 0 |
| EA | 16.5 | 0 | 0 | 10 |
| WT | 12.5 | 0 | 0 | 10 |
| Ciprofloxacin | 50 | 50 | 0 | 40 |
| Amphotericin | 0 | 0 | 14 | 0 |

0, no inhibition.
[a]PE - petroleum ether; CF - chloroform; EA - ethyl acetate; WT – water.

**Table 2.** Growth inhibition activity of tannin-free ethyl acetate fraction of *Carpobrotus mellei* against *S. aureus* and *M. smegmatis*.

| Rf | Zone of inhibition of microorganisms (L x B/mm) | | |
|---|---|---|---|
| | ***S. aureus*** | ***M. smegmatis*** | **Colour (UV 366 nm)** |
| 0.05 | 4 x 20 | 0 | Yellow |
| 0.25 | 7 x 20 | 0 | Light Yellow |
| 0.30 | 7 x 20 | 0 | Orange |
| 0.38 | 4 x 20 | 0 | Orange |
| 0.41 | 0 | 4 x 20 | NC |
| 0.48 | 9 x 20 | 0 | Brown |
| 0.51 | 5 x 20 | 7 x 20 | Light brown |
| 0.59 | 10 x 20 | 4 x 20 | Brown |

L x B - Length x Bret.
NC – No Colour visible under UV 366 nm.
0, no inhibition.

**Table 3.** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tannin free ethyl acetate- and aqueous extracts against *S. aureus* and *M. smegmatis*.

| Plant extracts[b] | MIC(mg/ml) - *S. aureus*[a] | MIC (mg/ml) - *M. smegmatis* |
|---|---|---|
| EA | 7.5 | 15 |
| WT | 15 | 30 |
| **Plant extracts[b]** | **MBC (mg/ml) - *S. aureus*[a]** | **MBC (mg/ml) - *M. smegmatis*** |
| EA | 7.5 | 30 |
| WT | 15 | 30 |

[b]EA - ethyl acetate; WT – water.

compounds present in the extract under UV-366 nm and in some cases these compounds were present with $R_f$ values similar to the antibacterial compounds visible on the bioautograms.

The MIC values for *S. aureus* in both the ethyl acetate- and aqueous extracts are significantly lower than that obtained for *M. smegmatis* (Table 3). The HPLC 'fingerprint' (Figure 1) of the ethyl acetate extract of *C. mellei* show major peaks at the retention times (min) 5.65, 6.93, 8.90, 11.03, 14.05, 14.52, 14.85, 15.42 and 15.97 at a wavelength of 280 nm. An almost-similar HPLC chromatogram is obtained at 360 nm (chromatogram not shown), but the compounds eluting before time 14.05, does not absorb at 360 nm.

## DISCUSSION

Despite the use of herbal medicines over many centuries, only a relatively small number of plant species has been

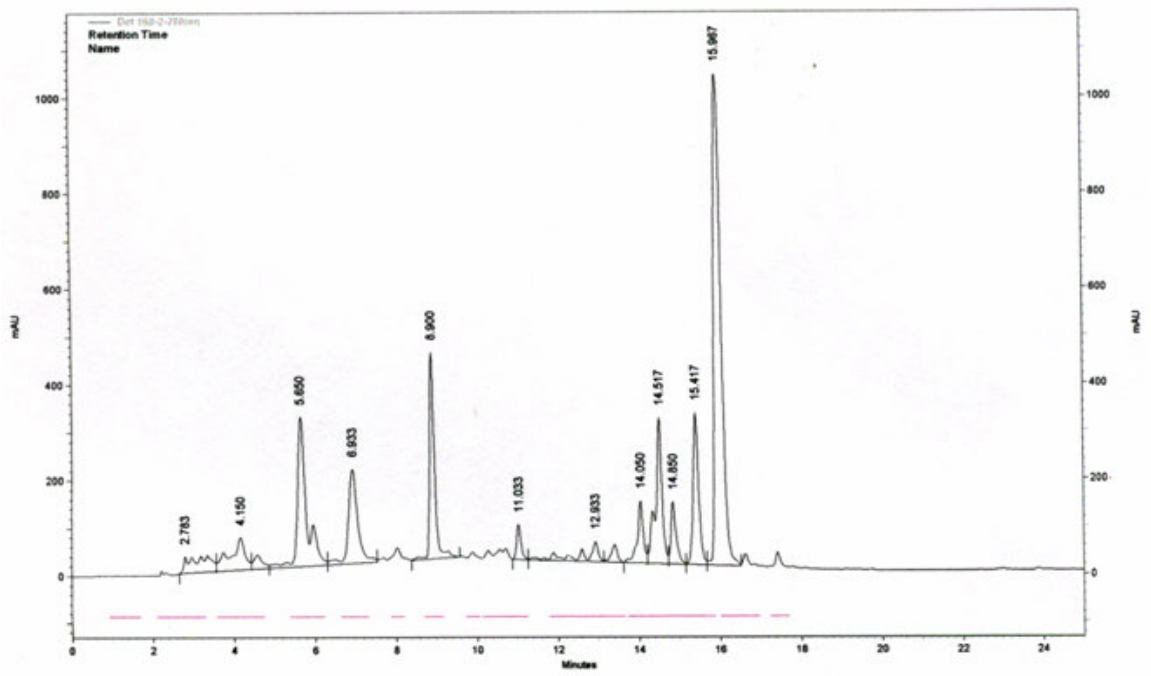


**Figure 1.** HPLC fingerprint of the active ethyl acetate extract of *C. mellei*.

scientifically studied for possible medical applications. Safety and efficacy data are available for an even smaller number of plants, their extracts and active ingredients and preparations containing them. The results from the bioautograms are substantial to give insight on quality of bioactives present in the active extracts. A very high tannin content was observed in the leaves of *C. mellei*, as reported in the studies by Springfield et al. (2003) and Van der Watt et al. (2001), which resulted in the tannins being removed prior to the bioautography analysis. The MIC and MBC values obtained for *C. mellei* indicated more precisely the antibacterial activity of the active extracts, and significance in cases where people depend on the usage of this medicinal herb.

The secondary compounds found in *C. mellei* are similar to those present in *C. muirii* and *C. quadrifidus* (Springfield et al., 2003). Flavonoids have been associated with a range of pharmacological activities including antibacterial properties (Hostettman et al., 1995), modulation of immune cell function, hepatoprotection, anticarcinogenicity, and antiviral activity (Middleton and Kandaswami, 1993). In a publication by Van der Watt et al. (2001), bioactive compounds were isolated from *C. edulis* and identified as flavonoids. It is likely that the phytochemical class, flavonoids, may be the sole contributor to the bioactivity in the genus *Carpobrotus*.

According to Bauer and Tittel (1996) and Springfield et al. (2005), HPLC fingerprinting is the best way for chemical characterization, and therefore this study also established a HPLC fingerprint for the active tannin-free ethyl acetate fraction. The peak for the reference standard, Rutin (Chromatogram not shown), appeared at retention time (min) of 19.66, and is used to monitor the extract. The reference standard is run during a sequence of analyses as an external standard. Because the retention time of the reference standard Rutin are known, a correction factor can be applied to the unknown plant chromatogram if any significant shifts occur in the retention time of the reference standard (Bauer and Tittel, 1996; Gong et al., 2004).

In conclusion, this study provides new scientific information about *C. mellei*, based on its antimicrobial potential and chemical profiling that has never been reported.

## ACKNOWLEDGEMENTS

The authors are grateful to the South African Medical Research Council, the National Research Foundation and the Applied Herbal Sciences Programme for financial support for this research.

## REFERENCES

Bauer R, Tittel G (1996). Quality assessment of herbal preparations as a precondition of pharmacological and clinical studies. Phytomedicine 2: 193-198.

Beugue WJ, Kline RM (1972). The use of tetrazolium salts in

bioautographic procedures. J. Chromatogr. 64: 182-184.
Eloff JN (1998a). Which extractant should be used for screening and isolation of antimicrobial components from plants. J. Ethnopharmacol. 60: 1-8.
Eloff JN (1998b). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64: 711-713.
Emboden W (1997). Narcotic plants. Hallucinogens, stimulants, inebriants, and hynotics, their origins and uses, Macmillan Publishing Co., Inc., Canada. pp. 46-48.
Hostettman K, Marston AJ, Wolfender L, Miallard M (1995). Screening for flavanoids and related compounds in medicinal plants by LC-UV-MS and subsequent isolation of bioactive compounds. Akademiai, Kiaho: Budapest. pp. 35-52.
Gong F, Liang YZ, Fung YS (2004). Correction of retention time shifts for chromatographic fingerprints of herbal medicines. J. Chromatogr. 1029: 173-183.
Latte KP (1999). Phytochemische und pharmakologische untersuctunger an Pelargonium reniforme curt., Ph.D thesis, University of Berlin.
Middleton EJR, Kandaswami C (1993). The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne J.B ed., The Flavonoids: Adv. in Res since 1986. Chapman and Hall, London. pp. 619-652.
Montague Museum (1998). Herbal Remedies of Montague Museum. 2nd edition, Montague, Printmor Printing Press. p. 8.
Springfield EP, Amabeoku G, Weitz F, Mabusela W, Johnson Q (2003). An assessment of two *Carpobrotus* species extracs as potential antimicrobial agents. Phytomedicine 10: 434-439.
Springfield EP, Eagles PKF, Scott G (2005). Quality assessment of South African herbal medicines by means of HPLC fingerprinting. J. Ethnopharmacol 101: 75-83.
Van der Watt E, Pretorius JC (2001), Purification and identification of active antibacterial components in *Carpobrotus edulis* L. J. Ethnopharmacol 76: 87-91.
Van Wyk B, van Oudshoorn B, Gericke N (1997). Medicinal plants of South Africa. Briza Publications, Pretoria, South Africa. p. 70**.**
Watt JM, Breyer-Brandwijk BN (1968). The Medicinal and Poisonous Plants of Southern and Eastern Africa*,* 2nd edition., Livingstone, London. pp. 47-69.
Wisura W, Glen HF (1993). The South African species of Carpobrotus (Mesembryanthema, Aizoaceae) Contribution, Boletim Herbage 15: 76-107.