Journal of Ethnopharmacology 119 (2008) 501–506

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Validation of smoke inhalation therapy to treat microbial infections

M. Braithwaite [a], S.F. Van Vuuren [a], A.M. Viljoen [b,*]

[a] Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa
[b] Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa

ARTICLE INFO

Article history:
Received 2 July 2008
Received in revised form 28 July 2008
Accepted 30 July 2008
Available online 19 August 2008

Keywords:
Antimicrobial
Artemisia afra
Heteropyxis natalensis
Inhalation
Myrothamnus flabellifolius
Pellaea calomelanos
Smoke
Tarchonanthus camphorates

ABSTRACT

Aim of the study: In traditional healing, the burning of selected indigenous medicinal plants and the inhalation of the liberated smoke are widely accepted and a practiced route of administration. This study elucidated the rationale behind this commonly practiced treatment by examining the antimicrobial activity for five indigenous South African medicinal plants commonly administered through inhalation (*Artemisia afra*, *Heteropyxis natalensis*, *Myrothamnus flabellifolius*, *Pellaea calomelanos* and *Tarchonanthus camphoratus*).
Material and Methods: An apparatus was designed to simulate the burning process that occurs in a traditional setting and the smoke fraction was captured for analysis and bioassay. Methanol and acetone extracts as well as the essential oil (for the aromatic species) were prepared and assayed in parallel with the smoke fraction.
Results: Antimicrobial data revealed that in most cases, the 'smoke-extract' obtained after burning had lower minimum inhibitory concentration (MIC) values than the corresponding solvent extracts and essential oils. The combustion, acetone and methanol extracts produced different chromatographic profiles as demonstrated for *Pellaea calomelanos* where several compounds noted in the smoke fraction were not present in the other extracts.
Conclusion: These results suggest that the combustion process produces an 'extract' with superior antimicrobial activity and provides *in vitro* evidence for inhalation of medicinal smoke as an efficient mode of administration in traditional healing.

1. Introduction

Medicinal plants have been used for their curative properties by traditional healers for centuries (Hutchings et al., 1996; Van Wyk et al., 1997). There are several routes through which medicinal plants may be administered. One of these is through inhalation, which has various advantages in both allopathic and traditional practices. The delivery of drugs directly to the respiratory tract has become an increasingly important therapeutic regimen especially in the treatment of a variety of pulmonary disorders including asthma, bronchitis, pneumonia and cystic fibrosis (Suarez and Hickey, 2000). The inhalation route of administration is used for the treatment and prophylaxis of airway diseases or for delivery of drugs with a systemic effect. The rapid and efficient absorption of drugs in the lungs is facilitated by the large surface area, high blood perfusion with abundance of capillaries and the thin air–blood barrier. The avoidance of first pass metabolism in the liver is also advantageous (Taylor, 2007). Several studies have shown the clinical efficacy of inhalation therapy for the treatment of lung disorders (Clarke, 1972; Neville et al., 1977). The delivery of active compounds directly to the lungs provides a local treatment and effectively delivers the drug to the site of action. In this way, smaller doses are able to achieve a maximal therapeutic effect and have less risk of side-effects than those associated with larger doses. Secondly, the lower doses provide considerable cost savings and reduce systemic exposure to patients. In addition, it has also been noted that the lungs may provide a perfect organ system for systemic delivery of drugs to the body (Suarez and Hickey, 2000). Suarez and Hickey (2000) have cited studies that show that this mode of administration provides substantially greater bioavailability for macromolecules than any other site of entry to the human body.

It is common practice in African traditional healing to burn plants and inhale the emitted smoke (Fig. 1) to treat respiratory complaints including coughs, colds, infections, influenza and asthma (Hutchings et al., 1996; Van Wyk et al., 1997).

Abbreviations: ATCC, American type culture collection; CFU, colony forming units; HPLC, high performance liquid chromatography; INT, *p*-iodonitrotetrazolium violet; MIC, minimum inhibitory concentration; PDA, photodiode array detector; TMD, thermabeam mass selective detector; TSA, tryptone soya agar.

* Corresponding author. Tel.: +27 12 3826360; fax: +27 12 3826243.
E-mail address: viljoenam@tut.ac.za (A.M. Viljoen).

0378-8741/$ – see front matter © 2008 Published by Elsevier Ireland Ltd.
doi:10.1016/j.jep.2008.07.050

Fig. 1. The burning of plant material and inhalation of the smoke is a common route of administration in African traditional healing (photo: Viljoen).

Mohagheghzadeh et al. (2006) sourced ethnobotanical information from 50 countries on the use of medicinal smokes and noted that the most common use relates to treating pulmonary conditions. This comprehensive review further emphasises that despite the extensive use of smoke in healing rites this mode of administration, together with the chemistry and pharmacological properties of the smoke fraction remains poorly explored.

Using two sources (Hutchings et al., 1996; Van Wyk et al., 1997) a number of South African plant species were identified which are burnt and the smoke inhaled for medicinal purposes. This investigation focused on five such plants used to treat respiratory disorders, namely, *Artemisia afra*, *Heteropyxis natalensis*, *Myrothamnus flabellifolius*, *Pellaea calomelanos* and *Tarchonanthus camphoratus*.

Artemisia afra (Asteraceae) commonly known as African Wormwood is an aromatic, erect, multi-stemmed, perennial shrub and is one of the most widely used traditional medicines in South Africa (Van Wyk et al., 1997) where it is extensively used to treat colds, coughs and influenza (Watt and Breyer-Brandwijk, 1962). It is also common practice for the leaves to be heated and the smoke inhaled for therapeutic purposes (Bhat and Jacobs, 1995). Bruneton (1995) confirmed the decongestant and antibacterial effects of the oils of *Artemisia afra*. Antimicrobial activity of *Artemisia afra* was reported in previous studies by Graven et al. (1992), which was later validated through time-kill studies of the essential oil, using various respiratory pathogens (Viljoen et al., 2006).

Heteropyxis natalensis (Heteropyxidaceae) is a small tree of not more than 10 m in height consisting of a branched trunk, dense leafy branches and highly aromatic foliage, hence the vernacular name Lavender tree (Palmer and Pitman, 1972; Coates Palgrave et al., 1973). Infusions of the leaves are mainly used to treat colds. The essential oil of the plant is highly aromatic and previous studies have shown antimicrobial activity against bacteria and fungi (Gundidza et al., 1993; Van Vuuren et al., 2007).

Myrothamnus flabellifolius (Myrothamnaceae), also known as the resurrection plant is a small woody shrub not more than 0.4 m tall, with tough, rigid branches. A remarkable feature of the plant is its ability to transform its seemingly dead and brown leaves to bright green when placed in water. Infusions are usually taken orally or the leaves are burnt and smoke inhaled to treat colds and respiratory ailments (Hutchings et al., 1996). Chest pains and asthma have been treated by inhaling smoke from burning leaves (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996). The essential oil exhibited antibacterial and antifungal activity against 9 out of 11 pathogens tested in a previous study (Viljoen et al., 2002).

Pellaea calomelanos (Adiantaceae) is a common and distinctive fern with an underground rootstock covered in small brown scales and about 6 mm in diameter. The leaves are burnt and the smoke is inhaled to treat chest colds, head colds and asthma (Watt and Breyer-Brandwijk, 1962; Hutchings and Van Staden, 1994; Van Wyk et al., 1997).

Tarchonanthus camphoratus (Asteraceae) referred to as the wild camphor bush is a small tree or shrub of up to 6 m tall with a greyish appearance (Van Wyk et al., 1997). Fresh or dried leaves and branches are crushed and burnt and the smoke is inhaled to treat asthma, rheumatism, and headaches (Watt and Breyer-Brandwijk, 1962; Hutchings and Van Staden, 1994). According to Watt and Breyer-Brandwijk (1962), the Koi and San smoked the dry leaves in the way tobacco is smoked and experience a type of narcotic effect.

Previous studies have investigated the antimicrobial activity of essential oil vapour obtained from aromatic plants (Inouye et al., 2001) and the preservative properties of wood smoke for perishable foods have been reported (Holley and Patel, 2005). In this study, we investigated for the first time, the antimicrobial activity and chromatographic profiles of the smoke-derived fractions from ethnomedicinally selected plants.

2. Materials and methods

2.1. Preparation of samples for combustion experiment

Fresh plant material was collected from natural populations and allowed to air dry. The aerial plant parts were broken up into smaller pieces and a standard dry weight of 20 g of plant material was used in each of the combustion experiments. Voucher specimens (Table 1) were prepared and deposited at the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

The combustion apparatus (Fig. 2) consisted of a modified pressure cooker with an entry pipe and an exit pipe, a heating plate, a condenser surrounding the exit pipe, a water bath, a round-bottomed flask and a cryostat. Plant material (20 g) was placed in a foil dish and inserted into the vessel and allowed to burn to produce smoke. A small aquarium pump was attached to the entry pipe to maintain a positive pressure which directed the smoke through the exit pipe into a round-bottomed flask containing solvent (the pump was later found to be unnecessary). A 350 ml mixture of hexane and methanol (1:1) was used to capture polar and non-polar compounds from the smoke fraction. The plant material was burnt for a standard time of 20–30 min to simulate the average time a patient would be exposed to inhala-

Table 1
The MIC values (mg/ml) for inhalation, methanol extract, acetone extract and essential oils

Sample	*Staphylococcus aureus* (ATCC 25923)	*Bacillus cereus* (ATCC 11778)	*Klebsiella pneumoniae* (ATCC 9633)	*Cryptococcus neoformans* (ATCC 90112)
Heteropyxis natalensis Harv. SVV 954				
Inhalation	1.86	0.35	0.70	0.93
Methanol	0.38	0.25	1.00	0.83
Acetone	0.38	0.25	2.00	1.50
Essential oil	32.00	4.00	8.00	8.00
Myrothamnus flabellifolius Welw. SVV 934				
Inhalation	0.72	0.27	0.36	0.36
Methanol	4.00	3.00	3.00	1.00
Acetone	1.00	0.50	1.50	0.25
Essential oil	6.00	2.00	3.00	3.30
Artemisia afra Jacq. ex Willd SVV 935				
Inhalation	0.52	0.26	0.52	0.52
Methanol	2.00	1.00	3.00	2.00
Acetone	0.25	0.25	2.00	0.75
Essential oil	16.00	12.00	8.00	32.00
Pellaea calomelanos (Sw.) Link AV 1280				
Inhalation	0.53	1.00	0.53	0.53
Methanol	>16.00	>16.00	6.00	4.00
Acetone	2.00	4.00	1.50	>32.00
Tarchonanthus camphoratus L. SVV 961				
Inhalation	0.62	0.23	0.93	0.47
Methanol	2.00	1.00	3.00	1.00
Acetone	0.50	<0.13	1.50	0.38
Essential oil	4.00	3.50	6.00	6.60
Positive controls[a]	0.50×10^{-3}	2.50×10^{-3}	0.80×10^{-3}	1.25×10^{-3}
Blank controls (solvent)				
Methanol	16.00	16.00	8.00	8.00
Acetone	>16.00	>16.00	>16.00	>16.00

[a] Ciprofloxacin was used as the control for the bacterial pathogens and amphotericin B was used as the control for *Cryptococcus neoformans*.

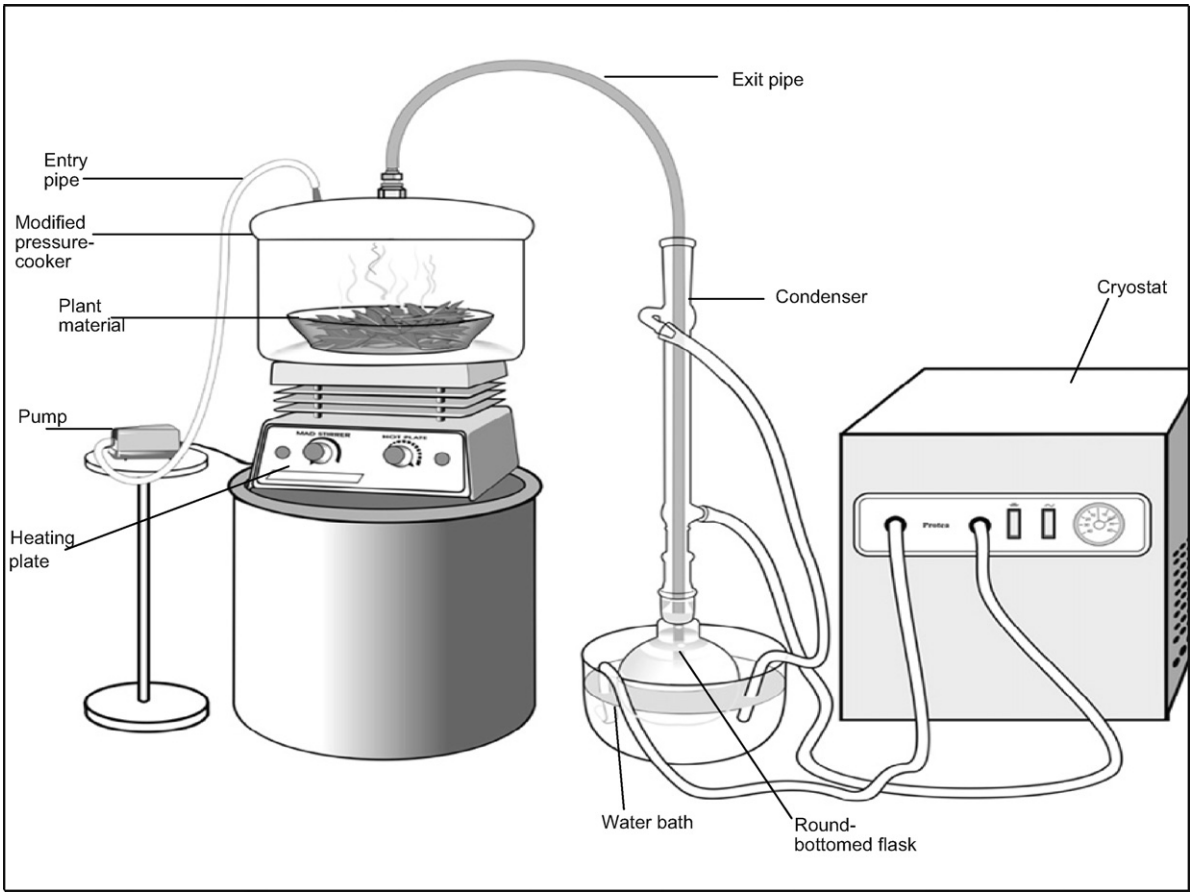

Fig. 2. The combustion apparatus used to mimic the inhalation process.

tion therapy as specified by the traditional healer. The heating plate was maintained at 150 °C. The smoke bubbled through the hexane–methanol mixture in the bottom of the flask. The round-bottomed flask was placed in a water bath containing water and polyethylene glycol. The water–polyethylene glycol mixture was pumped through the water bath in which the round-bottomed flask was placed and also through the jacket of the condenser. The water bath–condenser–cryostat circuit cooled the round-bottomed flask and minimised evaporation of the smoke fraction. The cryostat effectively maintained a temperature of −8 °C. The hexane–methanol smoke fraction was concentrated using a rotary evaporator.

2.2. Preparation of solvent extracts (methanol and acetone) and essential oils

Dried plant material (20 g) was ground and extracted with 50 ml of either methanol or acetone. The macerated plant material was extracted overnight, then filtered and allowed to dry in a fume hood. Essential oil samples of all four aromatic species (excluding *Pellaea calomelanos* which is not aromatic) were made available from previous studies (Van Vuuren, 2007).

2.3. Antimicrobial assays/minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of test substance that prevents visible growth of an organism after incubation in a specified growth medium and was used to quantitatively measure the *in vitro* antimicrobial activity of test extracts before and after burning. In this study, the MIC was determined by using the microtitre plate technique (Eloff, 1998a).

Antimicrobial assays were performed on the 19 samples against four micro-organisms; *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Klebsiella pneumoniae* (ATCC 9633) and *Cryptococcus neoformans* (ATCC 90112). The selection of test organisms was based on respiratory pathogenesis. The MICs were performed at least in duplicate and in some instances triplicate, where MIC values were not congruent between repetitions.

The MIC microtitre plate method was employed, containing a standard inoculum of bacteria (1×10^6 colony forming units), liquid growth media (Tryptone Soya broth) and doubling serial dilutions of the antimicrobial substance of interest (inhalation extracts, solvent extracts and essential oils). The plates were then incubated for 24 h (bacterial pathogens) and for 48 h (*Cryptococcus neoformans*) at 37 °C. After incubation, 40 μl of a 0.02 mg/ml *p*-iodonitrotetrazolium violet (INT) solution was prepared and added to each well. Microtitre plates were viewed after 6 h and yeast plates were viewed after 24 h. The INT solution was used to indicate biological activity and showed a colour change in relation to the concentration of microbial growth. The positive controls consisted of wells containing commercially available antibiotics, either ciprofloxacin (for bacteria) or amphotericin B (for the yeast *Cryptococcus neoformans*). The positive controls gave an indication of the sensitivity of the micro-organisms tested. Negative controls consisted of broths that were incubated under the same conditions as the plates so that sterility of the nutrient media was ensured. Blank controls (methanol or acetone) were also included to ascertain whether the diluents had any antimicrobial activity.

2.4. High performance liquid chromatography (HPLC)

The methanol and acetone extracts and the samples obtained from the combustion experiment were analysed by high performance liquid chromatography using a Waters 2690 HPLC system (Phenomenex Aqua C_{18} column, 250 mm × 2.1 mm at 40 °C) and equipped with a 996 photodiode array detector (PDA) and thermabeam mass selective detector (TMD). The samples were dissolved in methanol at a concentration of 50 mg/ml and 10 μl injected under the following conditions: mobile phase flow rate, 0.2 ml/min; nebulizer gas flow, 30 L/h; nebulizer temperature, 80 °C; expansion region, 90 °C; and source temperature, 225 °C. The initial mobile phase was acetonitrile, 90% water containing 100 mM formic acid and the solvent ratio was changed through a linear gradient to 90% acetonitrile, 10% water with 100 mM formic acid at 40 min. This ratio was maintained for 10 min after which the solvent ratio was converted to that of the initial starting conditions. Data analysis was performed using the Empower® software program.

3. Results and discussion

The MIC was used to facilitate a comparison of the antimicrobial activities of inhalation extracts compared to those of the conventional solvent extracts and oils (where applicable) of the same plants (Table 1).

The inhalation extract of *Heteropyxis natalensis* exhibited MIC values ranging between 0.35 and 1.86 mg/ml for all four pathogens tested. Comparatively, the inhalation extract of *Heteropyxis natalensis* indicated the most significant activity against *Klebsiella pneumoniae* when compared to the essentials oil, methanol and acetone extracts. For *Myrothamnus flabellifolius* the inhalation extract had lower MICs (0.27–0.72 mg/ml) compared to the methanol (1.00–4.00 mg/ml), acetone (0.25–1.50 mg/ml) and essential oil (2.00–6.00 mg/ml) when tested against all the pathogens, with the exception of *Cryptococcus neoformans* for which the acetone extract displayed slightly better activity. The inhalation extract of *Artemisia afra* also displayed lower MIC values (0.26–0.52 mg/ml) compared to the methanol extracts (1.00–3.00 mg/ml) and oil (8.00–16.00 mg/ml). The most promising result was obtained for the inhalation extract of *Pellaea calomelanos* which was more active (0.53–1.00 mg/ml) than the methanol (4.00 to 16.00 mg/ml) and acetone extracts (1.50 to 32.00 mg/ml) for all pathogens tested. For *Tarchonanthus camphoratus* the inhalation extracts had lower MIC values (0.23–0.93 mg/ml) compared to the methanol extracts (1.00–3.00 mg/ml) and oil (3.50–6.60 mg/ml).

Most of the inhalation extracts had low MIC values and in many cases exhibited superior antimicrobial potency when compared to the solvent extracts and essential oils. The inhalation extract was more potent than the methanol extracts and essential oils in 85% of the samples tested. The antimicrobial effect of the inhalation extract compared to the solvent extracts and oils were dependent on both plant species and pathogen studied. Efficacy ranged from minimal antimicrobial activity (*Heteropyxis natalensis* with *Bacillus cereus*) to enhanced activity where the antimicrobial effect is more than 60 times greater (*Pellaea calomelanos* with *Cryptococcus neoformans*) in the inhalation extract. A study undertaken by Nautiyal et al. (2007) also showed the variation in antimicrobial activity when pathogens were exposed to the medicinal smoke from a mixture of odoriferous and medicinal herbs. In this study 17 of the 28 pathogens were completely eliminated after 12 h exposure to medicinal smoke. Previous studies on various wood smoke extracts (*Avicennia nitida*, *Rhizophora racemosa*, *Mitragyna ciliate*, *Alstonia boonei*, *Terminalia ivorensis* and *Khaya* spp.) have shown to inhibit microbial growth with *Khaya* spp. exhibiting the most pronounced antimicrobial effect (Asita and Campbell, 1990).

The acetone extract however, did indicate higher antimicrobial activity for selected samples. Superior antimicrobial activity of acetone extracts have been previously shown (Eloff, 1998b) and this

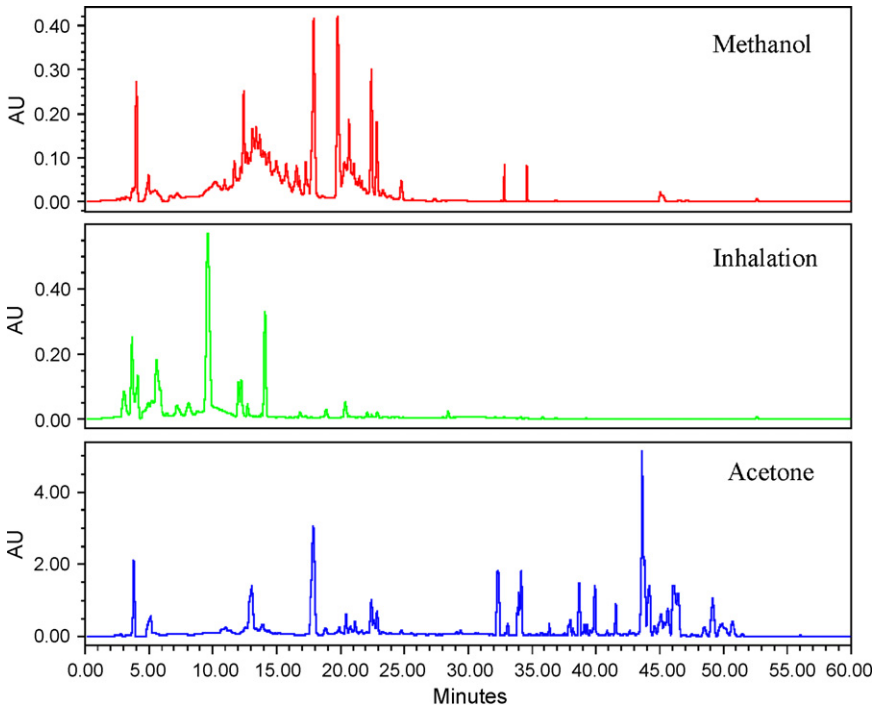

Fig. 3. HPLC–UV chromatograms of the acetone, methanol and inhalation extracts of *Pellaea calomelanos*.

present study confirms that acetone may be the preferred solvent for extraction of bioactive components.

Researchers investigating the biological activity of traditional medicines are often criticised for not mimicking the traditional methods of preparation. As a matter of convenience, solvent extracts are preferred in the laboratory, while aqueous extracts are obviously the method of preparation in a traditional setting. This study aimed to more closely simulate the traditional process by producing the smoke fraction through a combustion process. Jäger (2003) raised concerns about the traditional way of preparing plant extracts and it was concluded that the traditional means of preparation may not lead to very active extracts. In this study, however, the inhalation extracts proved to be more active in several cases when compared to solvent extracts.

Various factors may also influence the biological activity of inhaled substances as a viable therapy. Time of exposure to the inhaled smoke, the concentration of 'smoke' produced, the heat of combustion and the mode of burning (open fire, electric stove and gas stove) may all influence the effect achieved. Some studies on the inhalation of essential oils concluded that the antimicrobial action by gaseous contact is most efficient when the pathogen is exposed at high vapour concentration for a short time (Inouye et al., 2001). Various temperatures may produce different compounds with changing activities. For example, in a study by Jäger et al. (1996), the effect of heating plant material at different temperatures between 160 and 220 °C resulted in extracts with differing activity in a germination bioassay.

The various extracts show different chromatographic profiles, and for the sake of brevity only HPLC data for *Pellaea calomelanos* is shown (Fig. 3), as similar trends were observed for the other four species investigated (Braithwaite, 2007). Comparison of the HPLC–UV data (retention times and spectral data) showed few common compounds in all three of the extracts. The combustion process clearly produces compounds not present in any of the solvent extracts and the formation of many 'new' compounds during the combustion process is likely. The review paper by Simoneit (2002) indicates the complexity in composition of smoke derived from biomass burning and the variation in composition for various plant taxa. It has also been noted that the degradation of certain essential oil (volatile) components (e.g. limonene and α-pinene) by oxidation may produce oxygenated products that exhibit better activity than the parent hydrocarbons (Inouye et al., 2001). It is reasonable to speculate that the superior MIC values obtained for the inhalation extract of *Pellaea calomelanos* may be linked to the presence of unique compounds (and artefacts) in the combustion sample.

4. Conclusions

Despite the extensive past and present day use of smoke in healing rites (Mohagheghzadeh et al., 2006), this route of administration remains poorly explored. The promising antimicrobial activity documented here and the benefits of inhalation therapy (less invasive, site-specific, the dose reaches the bronchioles and the lung area immediately, reduced systemic exposure and thus less adverse effects, etc.) could explain the benefits of inhalation therapy in traditional medicine. It is noteworthy that of all extracts tested in this study, the smoke obtained after combustion exhibited the best activity against *Klebsiella pneumoniae*, a virulent pathogen associated with infections of the respiratory tract. Thus, the use of smoke to inhibit microbial growth extends not only to the well-established preservation of foods (Holley and Patel, 2005) but also gives scientific credibility to the therapeutic administration which has been used for centuries (Mohagheghzadeh et al., 2006). One must, however, take into account that smoke is known to contain several toxic compounds (e.g. benzopyrenes) and future studies should investigate if the antimicrobial activity is ascribed to general cytotoxicity. In addition, it remains a challenge for future research to isolate and identify the compounds in the complex smoke fraction.

References

Asita, A.O., Campbell, I.A., 1990. Anti-microbial activity of smoke from different woods. Letters in Applied Microbiology 10, 93–95.

Bhat, R.B., Jacobs, T.V., 1995. Traditional herbal medicine in Transkei. Journal of Ethnopharmacology 48, 7–12.
Braithwaite, M.C., 2007. A laboratory model for studying inhalation therapy in traditional healing rites. M.Sc. Dissertation. University of the Witwatersrand, South Africa.
Bruneton, J., 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. Springer, Hampshire.
Clarke, T.J., 1972. Effect of beclomethasone diproprionate delivered by aerosol in patients with asthma. Lancet 1, 1361–1364.
Coates Palgrave, K., Coates Palgrave, O.H., Drummond, R.B., 1973. Trees of Southern Africa. Struik, Cape Town.
Eloff, J.N., 1998a. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711–713.
Eloff, J.N., 1998b. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.
Graven, E.H., Deans, S.G., Svoboda, K.P., Mavi, S., Gundidza, M.G., 1992. Antimicrobial and antioxidative properties of the volatile (essential) oil of *Artemisia afra* Jacq. Flavour and Fragrance Journal 7, 121–123.
Gundidza, M., Deans, S.G., Kennedy, A.I., Mavi, S., Waterman, P.G., Gray, A.I., 1993. The essential oil from *Heteropyxis natalensis* Harv.: its antimicrobial activities and phytoconstituents. Journal of Science Food and Agriculture 63, 361–364.
Holley, R.A., Patel, D., 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology 22, 273–292.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg, South Africa.
Hutchings, A., Van Staden, J., 1994. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1: Plants used for headaches. Journal of Ethnopharmacology 48, 7–12.
Inouye, S., Takizawa, T., Yamaguchi, H., 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. Journal of Antimicrobial Chemotherapy 47, 565–573.
Jäger, A.K., Light, M.E., Van Staden, J., 1996. Effects of source of plant material and temperature on production of smoke extracts that promote germination of light-sensitive lettuce seeds. Environmental and Experimental Botany 36, 421–429.
Jäger, A.K., 2003. Evaluation of antibacterial activity of traditionally prepared South African remedies for infections. South African Journal of Botany 69, 595–598.
Mohagheghzadeh, A., Faridi, P., Shams-Ardakani, M., Ghasemi, Y., 2006. Medicinal smokes. Journal of Ethnopharmacology 108, 161–184.
Nautiyal, C.S., Chauhan, P.S., Nene, Y.L., 2007. Medicinal smoke reduces airborne bacteria. Journal of Ethnopharmacology 114, 446–451.
Neville, A., Palmer, J.B., Gaddie, J., May, C.S., Palmer, K.N., Murchison, L.E., 1977. Metabolic effects of salbutamol: comparison of aerosol and intravenous administration. British Medical Journal 1, 413–414.
Palmer, E., Pitman, J., 1972. Trees of Southern Africa. Balkema, Cape Town.
Simoneit, B.R.T., 2002. Biomass burning: a review of organic tracers for smoke from incomplete combustion. Applied Geochemistry 17, 129–162.
Suarez, S., Hickey, A.J., 2000. Drug properties affecting aerosol behaviour. Respiratory Care 45, 652–666.
Taylor, K.M.G., 2007. Pulmonary drug delivery. In: Aulton, M.E. (Ed.), Aulton's Pharmaceutics, The Design and Manufacture of Medicines. Churchill Livingstone, Edinburgh, pp. 1–736.
Van Vuuren, S.F., Viljoen, A.M., Őzek, T., Demirci, B., Başer, K.H.C., 2007. Seasonal and geographical variation of *Heteropyxis natalensis* essential oil and the effect thereof on the antimicrobial activity. South African Journal of Botany 73, 441–448.
Van Vuuren, S.F., 2007. The Antimicrobial activity and essential oil composition of medicinal aromatic plants used in african traditional healing. Ph.D. Thesis. University of the Witwatersrand, South Africa.
Van Wyk, B.E., Van Oudshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa, first ed. Briza Publications, Pretoria.
Viljoen, A.M., Klepser, M.E., Ernst, E.J., Keele, D., Rolling, E., Van Vuuren, S., Demerci, B., Baser, K.H.C., Van Wyk, B.-E., 2002. The composition and antimicrobial activity of the essential oil of the resurrection plant *Myrothamnus flabellifolius*. South African Journal of Botany 68, 100–105.
Viljoen, A.M., Van Vuuren, S.F., Gwebu, T., Demirci, B., Başer, K.H.C., 2006. The geographical variation and antimicrobial activity of African Wormwood (*Artemisia afra* Jacq.) essential oil. Journal of Essential Oil Research 18, 19–25.
Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, second ed. Livingstone, London.