An appropriate method for extracting the insect repellent citronellol from an indigenous plant (*Pelargonium graveolens* L'Her) for potential use by resource-limited animal owners

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ABSTRACT

Veterinary needs appraisals in rural, peri-urban and urban areas have indicated a need for affordable and accessible veterinary health care. It was also found that farmers and animal owners used indigenous plants for treating animals. In Africa, insects such as *Culex, Culicoides* and *Stomoxys* may transmit diseases, cause irritation to animals or prevent wound healing. Insect repellents used topically are generally safer and cheaper than insecticides. Using readily available commercial sources of ethanol 43 % v/v (brandy and cane spirits), it was shown that citronellol could be extracted from uncrushed leaves of the indigenous shrub *Pelargonium graveolens* L'Hér. Efficacy of extraction was compared to that using reagent grade absolute ethanol. The peak concentration of citronellol was achieved within 7 days of extraction and thereafter remained constant for 4 months. Extraction methods using tap water and cooking oil were not successful. The extraction was also less successful when the leaves were crushed or macerated before being placed into ethanol. Gas chromatography was used to monitor the concentration of citronellol in the different extracts.

Key words: citronellol, indigenous plants, insect repellents, *Pelargonium graveolens*, primary animal health care.

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INTRODUCTION

Veterinary needs appraisals (VNA) carried out in urban, peri-urban and rural areas in South Africa have indicated a need for affordable and accessible remedies for preventing and treating diseases in domestic animals[4,8,9–11]. It has previously been reported and was also found during appraisals that farmers and animal owners use aqueous extractions of different indigenous plants to treat observed symptoms in animals[9,10,14,15–18]. This poses the risk of adverse reactions, as the volume and concentration of the dose are very variable, and many of the plants used contain toxic substances[7,10,18]. A similar problem with herbal extracts has been encountered in human medicine[12,20]. In Africa, insects may transmit diseases, interrupt feeding patterns and prevent wound healing[3]. Citronellol has insect-repellent properties and is present in indigenous *Pelargonium* spp. in southern Africa[1,2,5,17,19]. This genus, probably owing to its pretty flowers and hardiness, was often observed in gardens during VNA in rural, peri-urban and urban communities.

Citronellol (3,7-dimethyl-6-octen-8-ol) is an unsaturated aldehyde (terpene) and is a constituent of rose, geranium and citronella oil. It is an oily liquid that is very slightly soluble in water and miscible with ethanol and ether, and is used as an insect repellent and in perfume. It is volatile and has a very strong, sweet smell[5,19].

The aim of this investigation was to ascertain whether citronellol could be extracted from the leaves of *Pelargonium graveolens* L'Hér using an inexpensive and readily available solvent. Animal owners and farmers situated in areas distant from commercial sources of insect repellents and/or whose available income restricts their access to commercial insect repellents are termed 'resource-limited animal owners and farmers', as they experience physical and financial contraints to the access of resources required for optimal management of their animals.

MATERIALS AND METHODS

Twenty young plants of *P. graveolens*, were acquired commercially and kept in containers for the duration of the experiment. The plants were in the pre-flowering stage and originated from a nursery in the Pretoria area. These plants are very easily propagated, and the plants used were from the same vegetative stock and of the same age. Leaves used for extraction were removed from the plants by snipping them off midway through the leaf stalk. The remainder of the leaf stalk was removed before evaluation. Leaf mass was determined using an analytical balance (Sartorius BP 2105). Three methods were used for preparing leaf material for extraction. Leaves were cut into 0.5 cm squares using a pair of sharp scissors, weighed, divided into 5 g portions and then macerated before extraction with a hand-held blender (Safeway Model TS-370) for 10 minutes. Cut leaves were weighed into 5 g portions and extracted directly. The mean mass of intact leaves, with leaf stalk removed, was determined and 10 small leaves placed in 5 mℓ of extraction fluid. Extraction fluids used were analytical grade absolute ethanol (Merck), tap water, commercial sunflower oil (Epic Oil Mills), commercially available brandy (Chateau-VO, Stellenbosch Farmers' Winery) and cane spirits (Cape to Rio, Snell Pty Ltd).

In order to accurately monitor whether extraction of citronellol was successful, analysis was performed using a Varian 6000 Gas Chromatograph (GC) with a Supelco wax-10 column of 30 m by 0.75 mm. The carrier gas was helium and a flame ionisation detector was used. Retention times were used for peak identification. Citronella oil and geranium oil (Burgess and Finch) were used as standards. Two micro-litre samples were injected immediately after preparation. Thereafter, 3 samples were monitored daily for 10 days. All GC analyses were performed in triplicate to ensure repeatability. This procedure was repeated 4 months later to see whether the concentration had changed. The temperature profile for the GC analysis is shown in Table 1.

RESULTS

Large leaves had a mean mass of 0.6497 g and small leaves, a mean mass of 0.2756 g. When leaves were cut up into

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Table 1: **Temperature profile for gas chromatograph analysis of citronellol.**

Temperature (°C)	Heating rate (°C/min)	Hold time (min)
65	20.0	2.0
125	17.0	2.0
250	15.0	0

0.5 cm squares, or macerated before being immersed in ethanol, peaks corresponding to citronellol could not be determined.

Alternative solvents, water and cooking oil at room temperature, were used with the same lack of success. It was therefore decided to soak the intact leaves in ethanol using 10 small leaves per teaspoon of ethanol. The mass of these samples was 2.0511 g in 5 mℓ cane spirits and 2.1784 g in 5 mℓ brandy. Extraction was successful and the results from whole leaves using analytical grade absolute ethanol, brandy and cane spirits compared to the standard (citronella oil) are shown in Table 2.

Figure 1 shows the citronellol peak (detector response) in cane spirits after 7 days. Table 3 shows how the amount extracted increases with time, reaching a plateau at 7 days. It then remained constant for 4 months.

Analytical grade absolute ethanol (99 %v/v) extracted the citronellol more rapidly (within 4 days) and reached a higher final relative amount (percentage peak area 0.24). However, analytical grade chemicals are expensive and only available in chemical laboratories.

DISCUSSION

According to Watt and Breyer-Brandwijk[18], extracts and concoctions of *Pelargonium* spp. are generally used to treat diarrhoea and dysentery in calves and horses. The southern Sotho also boil the leaf of *P. reniforme* to produce a mucilage that is used to cover wounds and protect against infestation by maggots. A decoction or infusion of the leaves of *Pelargonium* spp. is also considered to be a good remedy for skin inflammations and is said to be cleansing and soothing. It is not recognised in traditional medicine as an insect repellent, but the presence of citronellol in extracts of the plant is well recognised in scientific textbooks[5,19]. The insect repellent effect may result from its strong sweet smell, that masks body odours and carbon dioxide, which are considered to attract biting insects to humans and animals[2,6,14].

Insect repellents used topically are less likely to induce acute or fatal toxic reactions in animals than concoctions administered orally. Extracts from *Pelargonium* spp. have also been described as 'soothing' to the skin[18]. The plant is a widespread perennial in southern Africa. We therefore attempted to extract the insect repellent chemical citronellol from the leaves of this plant using solvents known to be available to animal owners in resource-limited communities. Ravid *et al.*[13] successfully used GC analysis to determine the presence of citronellol, so this method was chosen to monitor whether extraction was successful.

Citronellol was successfully extracted from the intact leaves of *P. graveolens,* by soaking them in absolute ethanol, cane spirits or brandy for 7 days. The efficacy of the extraction was confirmed by GC analysis. The 43 % v/v ethanol products (brandy and cane spirits) are commercially available in all the areas visited during VNA. Owners could buy a single 'tot' of brandy or cane spirits at a relatively low cost (less than R10.00) and use 1 teaspoonful to extract citronellol from 10 small leaves. Once the extract has been made it can be used for 120 days or longer, as it was shown that the extract is stable at room temperature. This extraction method is simple and affordable and could be performed by resource-limited animal owners and stock farmers. The extract can be applied to wounds or skin to repel insects.

Although citronellol is reported to be efficacious as an insect repellent, there is little information on its potency and that of citronellol against different insect species[1,19]. Clinical trials using the extract have to be performed to determine the potency of the extract in repelling insects and its safety for external use more accurately.

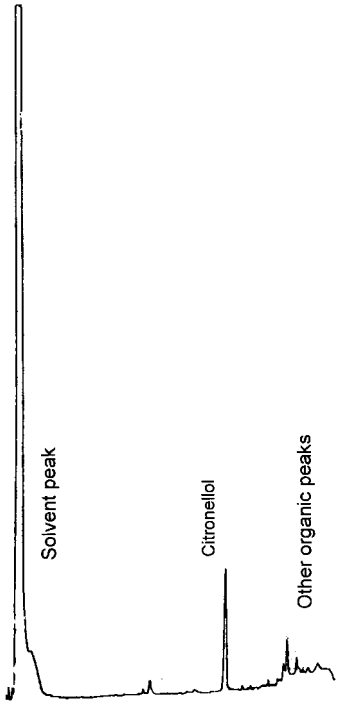

Fig. 1: **Gas chromatogram made after 7 days using cane spirits as solvent, showing the solvent peak, citronellol peak and other organic peaks.**

Table 2: **Retention time of citronellol extracted using different solvents, compared to standard** (citronella oil)**.**

Solvents and standard	Retention time (mins)
Reagent grade absolute ethanol	10.86
Brandy (43 % v/v)	10.85
Cane spirits (43 % v/v)	10.83
Citronella oil	10.86

Table 3: **Relative amounts** (normalised peak area) **of citronellol extracted using brandy and cane spirits over a 10-day period and after 120 days.**

Extraction time (days)	Cane spirits (normalised peak area)	Brandy (normalised peak area)
1	0.06	0.07
4	0.08	0.09
7	0.10	0.12
10	0.11	0.12
120	0.12	0.13

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Book review — Boekresensie

Fasciolosis

Edited by J P Dalton

2000. CABI Publishing, New York, and Oxford University Press, New York, 544 pp., hard cover. US$ 160.00. ISBN 0 85199 260 9.

This book is outstanding and I recommend it without reservation, except for the rather high price. There are many aspects that make it well worth reading. Firstly, the editing by John Dalton was professionally executed. The chapters written by well-known and informed authors follow a logical sequence, each building on what has gone before. And the final chapter does a good job of filling the gaps in the previous chapters in this book and existing text books, which variously ignore *Fasciola gigantica* because of historical research biases towards *F. hepatica.* It is a beautifully balanced, clearly written textbook. It is on good paper; the layout is consistent throughout; there is a complete index; it is well referenced; the variations in illustration styles are not distracting, except perhaps the GIS maps of Ethiopia, which may originally have been in colour but become less instructive in too many shades of grey.

This book is encyclopaedic in its coverage of fasciolosis. The first five chapters have laid the groundwork – life cycle, intermediate development, development in the final host, epidemiology and control, prediction. This lays the foundation for pathology, fasciolicide action and drug resistance, metabolism, neurobiology, immunology, new vaccines, human fasciolosis, immunodiagnosis, and molecular biology. Finally, because all the previous chapters have emphasised *F. hepatica* for historical and geographical reasons, as this is a parasite of temperate regions and most researched, the last chapter revisits all the previous points but in respect to the present knowledge of *F. gigantica,* essentially a tropical fluke. The contributing authors are to be commended.

Reading this textbook, a number of points come to mind and obviously one can only select a few. Anyone bored with *F. hepatica* research should certainly head off into the barely charted waters of *F. gigantica.* On another tack, it is interesting to note that with the understandable exception of molecular biology, each chapter quotes historical references. The historical observations frequently carry the same weight as recent references, which inspires great confidence. I suspect it also reflects the scientific maturity of the writers involved in producing this book

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