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Buchu (*Agathosma betulina* and *A. crenulata*, Rutaceae) essential oils: their pharmacological action on guinea-pig ileum and antimicrobial activity on microorganisms

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Abstract

The mode of action of essential oils from two buchu species (*Agathosma betulina* and *A. crenulata*, Rutaceae) from the Cape region of South Africa has been studied on smooth muscle in-vitro using guinea-pig ileum. At high concentration, the oils had an initial spasmogenic activity followed by spasmolysis. The spasmolytic action was post-synaptic, not atropine-like and did not involve adrenoceptor or guanylyl cyclase activation. In the presence of the phosphodiesterase inhibitor rolipram, the spasmolytic action of *A. betulina* was significantly increased whilst that due to *A. crenulata* was also increased but not to a significant level. These results suggested a mode of action for the oils involving cyclic adenosine monophosphate. In addition, *A. betulina* appeared to block calcium channels but this was not seen with *A. crenulata*, possibly because the initial spasmogenic activity complicated the study of its spasmolytic action. Neither essential oil (10 μL, undiluted) demonstrated antimicrobial action against *Enterococcus hirae* and *Pseudomonas aeruginosa* but very low activity was observed against *Escherichia coli*, *Saccharomyces cerevisiae* and *Staphylococcus aureus*, suggesting little potential for these oils as antimicrobial agents/preservatives.

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Introduction

The two species of buchu (*Agathosma betulina* and *A. crenulata*, Rutaceae) originate from the Cape area of South Africa; the Nama introduced the herbs to the European settlers and the plants were taken to Europe and used medicinally, especially as a diuretic (*British Pharmacopoeia* 1932; Grieve 1937). Buchu species, used as buchu brandy, tincture, vinegar or a tea, were used as antiseptics, for gravel, inflammation, stomach complaints and "catarrh" of the bladder. Currently they are used for stimulating perspiration in rheumatism and gout, treating cholera, kidney disease, haematuria, calculus, infections of the bladder, urethra and prostate, and as a digestive tonic. Externally the embrocation or buchu vinegar is used for local application to treat rheumatism, bruises, contusions, sprains and fractures, and for cleaning wounds (Grieve 1937; Watt & Breyer-Brandwijk 1976; Simpson 1998).

There is virtually no pharmacological or antimicrobial knowledge about these herbs and so due to their avid usage we have studied the mode of action of the essential oils on smooth muscle in-vitro using guinea-pig ileum, and have tested the oils against five microorganisms in-vitro.

Materials and Methods

Plant material

The leaves and flowers of *Agathosma betulina* and *A. crenulata* were steam-distilled and the essential oils donated by Grassroots Natural Products, South Africa.

Pharmacological studies

The activity of the oils was investigated on the guinea-pig ileum in-vitro, using methodology and reagents as described by Lis-Balchin & Hart (1998). All experiments were repeated at least five times on different strips of ileum. The results, where appropriate, were expressed as mean percentage inhibition $\pm$ s.e.m. For experiments determining calcium channel involvement, the calcium-free buffer included EGTA, 0.5%. The essential oils were diluted in methanol (AR) to give 4×10^{-6} to 8×10^{-5} v/v solutions and were added to the organ bath in a volume no greater than 0.2 mL, at which volume there was nil solvent effect on the tissue.

Antimicrobial studies

Antimicrobial action was studied against *Enterococcus hirae* ATCC 10541, *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 6538, and *Saccharomyces cerevisiae* ATCC 9763, using the agar diffusion method (Lis-Balchin et al 1998). Each essential oil was tested in duplicate against all five microorganisms, using 10 μL undiluted oil pipetted into 4-mm wells in agar, which had been previously inoculated with one of the micro-organisms, and incubated for 48 h at 25°C. The zone of inhibition, over 4 mm, denoted the level of antimicrobial activity.

Results

Both oils had an initial spasmogenic action followed by a spasmolytic action. At the lowest dose (4×10^{-6}), there was only a rise in the base-line (tone), but as the dose was increased there was a distinct contraction followed by dose-related spasmolysis. The IC50 for the spasmolytic action of both oils was 8×10^{-6}. A concentration of essential oil which produced a significant inhibition of the field stimulated contraction of the ileum (i.e. spasmolysis) produced a similar inhibition of the contraction due to exogenous acetylcholine or histamine (Table 1). Concentrations of phentolamine and propranolol, which blocked the antispasmodic effects of noradrenaline, had no effect on the spasmolytic action of the essential oils. Similarly a concentration of ODQ (a selective inhibitor of guanylyl cyclase), which blocked relaxation produced by sodium nitroprusside, had no effect on the activity of the two oils. In contrast, a concentration of the phosphodiesterase inhibitor

Table 1 Summary of the results of studies of the mode of action of buchu essential oils on guinea-pig ileum using different agents (% inhibition of electrically-stimulated contractions).

	Noradrenaline	Sodium nitroprusside	Isoprenaline	*A. betulina*	*A. crenulata*
Pre-/post-synaptic					
Histamine (% decrease)				59.0 $\pm$ 1.8	62.1 $\pm$ 1.5
Acetylcholine (% decrease)				62.3 $\pm$ 0.9	59.7 $\pm$ 1.2
Adrenoceptors					
Agent alone	45.7 $\pm$ 2.3			66.7 $\pm$ 59.3	73.5 $\pm$ 6.7
Blockade (phentolamine + propranolol)	blocked			68.8 $\pm$ 7.1	80.4 $\pm$ 14.9
Guanylyl cyclase inhibitor					
Agent alone		31.6 $\pm$ 5.8		58.5 $\pm$ 9.6	81.5 $\pm$ 4.8
ODQ		blocked		62.8 $\pm$ 6.3	82.5 $\pm$ 7.3
Agent alone			23.8 $\pm$ 2.4	30.7 $\pm$ 6.5	35.7 $\pm$ 5.4
Rolipram			37.5 $\pm$ 3.1**	50.9 $\pm$ 6.6**	48.9 $\pm$ 8.6
Agent alone			32.3 $\pm$ 3.6	30.7 $\pm$ 6.1	35.0 $\pm$ 11.6
CDP			67.8 $\pm$ 3.3**	45.0 $\pm$ 5.8	36.3 $\pm$ 11.9

** Significantly enhanced $P > 0.005$.

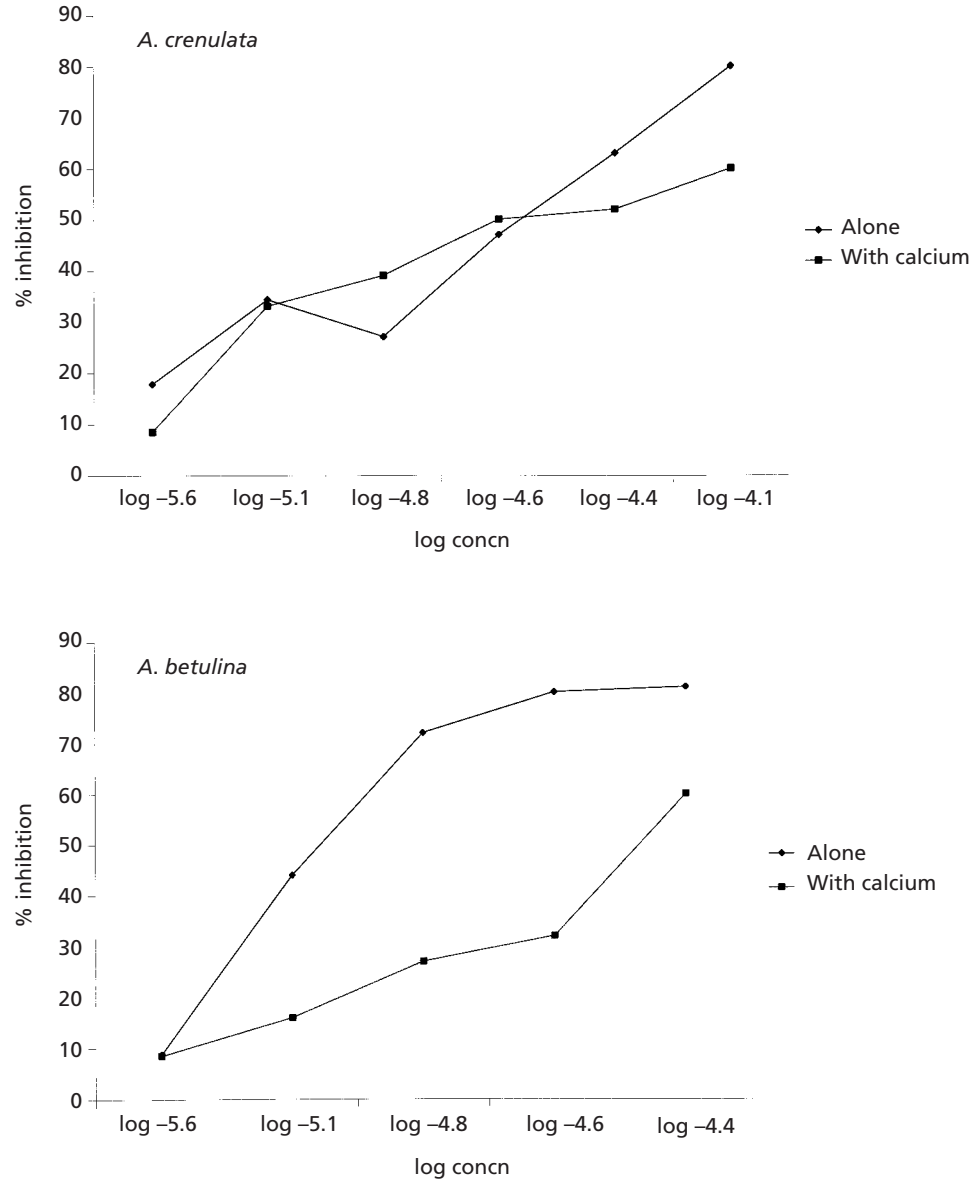

Figure 1 The involvement of calcium channels in the mode of action of buchu.

rolipram, which increased the spasmolytic action of isoprenaline, also increased the spasmolytic action of the two oils, with that due to *A. betulina* reaching statistical significance (Table 1).

The dose–response curve for the spasmolytic action of verapamil was not significantly different from the curve relating dose to the ability of verapamil to block a calcium-induced contraction of the ileum in depolarizing Krebs solution. A similar relationship was found for *A. betulina* at higher concentrations whereas with *A. crenulata* there was a clear disparity between the two curves (Figure 1).

There was virtually no antimicrobial activity shown by the buchu oils against *E. hirae* and *P. aeruginosa* and very low activity, compared with the potent antimicrobial thyme oil, against *E. coli*, *S. cerevisiae* and *S. aureus* (Table 2).

Discussion

Both essential oils demonstrated a spasmogenic and spasmolytic activity emphasizing the varied pharmacological activity of their constituents. The investigation

Table 2 Antimicrobial activity of buchu essential oils (zone of inhibition (mm)).

Essential oil (10 µL undiluted)	*Escherichia coli*	*Enterococcus hirae*	*Pseudomonas aeruginosa*	*Saccharomyces cerevisiae*	*Staphylococcus aureus*
A. betulina	6	4	4	6.3	5.8
A. crenulata	5.1	4	4	6.1	5.4
Thyme	39.1	11.7	23.2	17.9	24.0

of the spasmolytic activity, observed as an inhibition of the contraction induced by the release of acetylcholine from the stimulated post-synaptic parasympathetic nerve, showed it to be via the muscle rather than the nerve. The activity did not mimic that of atropine, because exogenous acetylcholine and histamine were affected to the same extent, and was not mediated via the stimulation of adrenoceptors. Guanylyl cyclase did not appear to be involved in the spasmolytic action of either oil but the increased activity observed in the presence of rolipram indicated that a component of both oils was probably capable of stimulating adenylyl cyclase. The results suggest that both essential oils contain components capable of blocking calcium channels; in the case of *A. betulina* this would appear to make an important contribution to the overall spasmolytic effect whereas with *A. crenulata* it is of much less significance. The results for *A. betulina* were in keeping with the widespread involvement of calcium channels in the mechanism of action for a wide range of natural components including some essential oils and their components (Neuhaus-Carlisle et al 1997; Vuorela et al 1997) and also in the case of valerian essential oil (unpublished data). The results for *A. crenulata* were, however, in direct agreement with those for essential oils such as lavender and geranium oils, where calcium channels were seemingly not important in the mechanism of action, except at high concentrations (Lis-Balchin & Hart 1999).

The very poor or even absent antimicrobial activity suggested that, as the species used represented a wide spectrum of microorganisms, the likelihood of potent antimicrobial action against other organisms seemed remote. The use of buchu as a "catty" odour in perfumery and as a replacement for blackcurrant bud flavour in food (Kaiser et al 1975; Nijssen & Maarse 1986) seemed more appropriate, based on their essential oil content. However, the oils should be used in very low dosages for any purpose. This is because the two main components of the oils are ρ-diosphenol and diosphenol, which have an unknown toxicity. In fact *A. crenulata* contains approximately 50% pulegone (Posthumus et al 1996) which makes it potentially as toxic as pennyroyal (which has a folk medicinal role as an abortifacient).

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