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REVIEW

In vitro evaluation of antibacterial and immunomodulatory activities of *Pelargonium reniforme*, *Pelargonium sidoides* and the related herbal drug preparation EPs® 7630

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Abstract

The importance of *Pelargonium* species, most notably *Pelargonium reniforme* and *Pelargonium sidoides*, in traditional medicine in the Southern African region is well documented. Nowadays, a modern aqueous-ethanolic formulation of the roots of *P. sidoides* (EPs® 7630) is successfully employed for the treatment of ear, nose and throat disorders as well as respiratory tract infections. To provide a scientific basis of its present utilization in phytomedicine, EPs® 7630, extracts and isolated constituents of the titled *Pelargoniums* with emphasis on *P. sidoides* were evaluated for antibacterial activity and for their effects on nonspecific immune functions. The samples exhibited merely moderate direct antibacterial capabilities against a spectrum of Gram-positive and Gram-negative bacteria. Functional bioassays including an in vitro model for intracellular diseases, a fibroblast-lysis assay (tumour necrosis factor (TNF) activity), a fibroblast-virus protection assay (IFN activity) and a biochemical assay for nitric oxides revealed significant immunomodulatory properties. Gene expression experiments (iNOS, IFN-α, IFN-γ, TNF-α, Interleukin (IL)-1, IL-10, IL12, IL-18) not only confirmed functional data, they also clearly showed differences in the response of infected macrophages when compared to that of noninfected cells. ELISA confirmed the protein production of TNF-α, IL-1α and IL-12, while FACS analyses reaffirmed the cytokines IL-1α and IL-12 at the singular cell level. The current data provide convincing support for the improvement of immune functions at various levels, hence, validating the medicinal uses of EPs® 7630. Despite considerable efforts, the remedial effects cannot yet be related to a chemically defined principle.

Keywords: *Pelargonium sidoides*; Antibacterial activity; Immunomodulation; Nitric oxides; Cytokines; Gene expressions

Introduction

Pelargonium species indigenous to the Southern African region are highly estimated by traditional healers and the native population for their curative properties (Watt and Breyer-Brandwyk, 1962; Hutchings, 1996). Several ethnic groups including Zulu, Bantu, Xhosa and Miengu are using infusions of the tubers to treat diarrhoea and dysentery. The Afrikaans names ''rabassum'', ''rabas'' or ''rooirabas'' are commonly employed for a number of medicinally used species including *Pelargonium antidysentericum*, *Pelargonium rapaceum*, *Pelargonium triste*, *Pelargonium reniforme* and *Pelargonium sidoides* (Van Wyk et al., 1997). Interest in the latter two species has been heightened by reports of antitubercular activities and their potential

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as a remedy for ear, nose and throat disorders as well as respiratory tract infections (Kolodziej, 2002). Following the well-documented therapeutic benefits in these conditions, a modern formulation, EPs® 7630, has been elaborated from the traditional herbal medicine and successfully introduced in modern phytotherapy (Umckaloabo®, marketed by Spitzner Arzneimittel, Ettlingen, Germany). Although accumulating evidence suggests *P. sidoides* to form the origin of the popular traditional herbal medicine (Kolodziej and Kayser, 1998), commonly termed umckaloabo, it is most likely that some medicinal records apply to mixtures prepared from both species. This and the earlier taxonomic ambiguity prompted a parallel study on the pharmacological profile of botanically defined plant material of the titled *Pelargonium* species in order to provide a rationale for the therapeutic activity, which has been demonstrated in a number of clinical studies (Heil and Reitermann, 1994; Dome and Schuster, 1996; Haidvogl et al., 1996; Blochin et al., 1999; Blochin and Heger, 2000; Heger and Bereznoy, 2002; Golovatiouk and Chuchalin, 2002). This report represents an overview of pharmacological in vitro investigations from the authors' laboratory including recent findings not reported previously.

Traditional use

Since the current utilization of this herbal remedy in phytomedicine is based on its traditional use, a brief note appears appropriate. Etymologically, the name "umckaloabo" was suggested to derive from the Zulu words "umKhulkane" for complaints associated with lung disorders and "uHlabo", which means chest pain. Although commonly accepted, this linguistic origin is questioned, as "umckaloabo" itself is apparently unknown among Zulu tribes (Bladt, 1977). It is assumed that this name is in error with a similar yet unknown one, while the indicated Zulu words came out accidentally as perfect terms expressing the traditional use. Due to detailed information elsewhere (Bladt, 1977; Helmstädter, 1996; Kolodziej et al., 1995), the interesting history of this herbal medicine is not reiterated.

Decoctions and infusions of the roots enjoy a wide reputation by traditional healers and are highly valued by the southern African native population for its curative and palliative effects in the treatment of respiratory tract infections, gastrointestinal disorders, hepatic disorders and menstrual complaints, while the aerial parts are employed in wound healing (Watt and Breyer-Brandwyk, 1962; Hutchings, 1996).

Regarding the treatment of gastrointestinal disorders such as diarrhoea and the application of the aerial parts as wound healing agent in folk medicine, the fairly high concentrations of oligomeric proanthocyanidins (ca 9%) occurring in this indigenous medicine may, at least in part, explain the traditional use (Scholz, 1994; Hör et al., 1995). The claimed curative effects related to hepatic disorders may tentatively be explicable on the basis of the radical scavenging activities of the broad range of phenolic compounds. Flavonoids and hydrolysable tannins isolated from *P. reniforme* showed marked antioxidant effects using a DPPH radical generating system and a luminol-dependent chemiluminescence assay (Latté and Kolodziej, 2004). In both assays, the polyphenols tested showed higher radical scavenging activities than the reference antioxidant, ascorbic acid (IC_{50} 2.6–32.9 μM vs. 40.9 μM in the DPPH test, and 2–25 times stronger effects in the chemiluminescence assay). Regarding structure–activity relationships, the antioxidant potentials generally increased in the order of *C*-glucosyls < *O*-glycosides < aglycones < galloylated *C*-glucosyls in the series of the tested flavonoids, while for tannins the presence of galloyl and HHDP entities were important determinants.

Antibacterial activity

Following the therapeutic use of *P. reniforme*/*P. sidoides* in traditional and modern phytomedicine for the treatment of respiratory tract infections, we initially turned our attention to antibacterial activities including antimycobacterial potentials.

To obtain clues as to whether these botanically defined *Pelargonium* species have antimycobacterial potencies as claimed in earlier records, methanol extracts of the roots and some typical phenol and coumarin isolates were tested for this particular biological activity in collaboration with the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF), Alabama, USA. At 12.5 μg/ml, the crude extract of *P. sidoides* showed some interesting inhibitory activity (96%) against *Mycobacterium tuberculosis* in a primary radiorespirometric bioassay (BACTEC 460 system), while that of *P. reniforme* was inactive. This finding does not necessarily imply lack of antimycobacterial properties of the latter species, possibly detectable at higher sample concentrations. Interestingly, none of the isolated simple phenolic constituents and coumarins exhibited any antimycobacterial activities under these experimental conditions. With the minimum inhibitory concentration (MIC) of 100 μg/ml, determined in a broth microdilution Alamar Blue assay, the same extract was only moderately active against *M. tuberculosis*, when compared with the MIC of 0.06 μg/ml of the clinically used drug, rifampicin, as a reference compound.

Conspicuously, subsequent experiments including aqueous acetone extracts of both root material and aerial parts as well as fractions (CH_2Cl_2, EtOAc, *n*-BuOH, H_2O phase)