Biochemical Systematics and Ecology 55 (2014) 156–163

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

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

Chemical profiling and chemometric analysis of South African propolis

Deepak Kasote [a], Tasneem Suleman [b], Weiyang Chen [a], Maxleene Sandasi [a], Alvaro Viljoen [a,*], Sandy van Vuuren [b]

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

ARTICLE INFO

Article history:
Received 10 January 2014
Accepted 15 March 2014
Available online

Keywords:
UPLC-PDA-qTOF-MS/MS
South African propolis
Brazilian propolis
Chemometric analysis

ABSTRACT

UPLC-ESI-MS analysis of 39 South African propolis samples was undertaken to report on the chemical composition and variability of South African propolis and to compare the chemical profiles to Brazilian samples ($n = 3$). Chemo-geographical patterns within South African propolis were further analysed by chemometrics. South African propolis samples displayed typical UPLC-ESI-MS fingerprints, which were different from their Brazilian counterparts. UPLC-PDA-qTOF-MS/MS was used to identify marker compounds from representative groups and 15 major phenolic acids and flavonols from common South African propolis were identified. Chemometric analysis of the UPLC-ESI-MS data revealed two distinct clusters among the South African samples and also confirmed that the South African propolis was chemically distinct from the Brazilian propolis. The majority of the samples were phytochemically congruent with propolis from the temperate regions.

1. Introduction

Propolis is a resinous material prepared by honeybees (*Apis mellifera* L.) using beeswax and plant exudates. Bees generally use propolis as a construction material for sealing openings and cracks within the beehive (Bankova et al., 2002). Propolis has become a popular ingredient in complementary healthcare, and the use of this natural material in pharmaceutical and food preparations is growing due to its wide range of therapeutic properties including; antimicrobial, anti-oxidant, anti-inflammatory, immunomodulatory and anticancer activities (Monzote et al., 2012; Shi et al., 2012; Chan et al., 2013).

The chemical composition of propolis is known to be complex and variable between seasons and regions (Salatino et al., 2011). Numerous factors, such as the floristic composition of the area, location and time of collection impact on the chemical composition of propolis (Tagliacollo and Orsi, 2011). Propolis samples analysed from various parts of the world have been collectively reported to contain over 300 different chemical compounds, including polyphenols, esters of phenolic acids, flavonoids, sesquiterpenes, diterpenes, triterpenes, lignans, prenylated benzophenones, aldehydes, steroids and coumarins (Hernández et al., 2010; Farooqui and Farooqui, 2012). Phenolics (mostly flavonoids) constitute over 50% of the total weight of propolis (Bankova et al., 1996).

Different types of propolis have been reported according to the geographical area of production, botanical source and chemical composition. The most common types of propolis are Temperate, Birch, Tropical, Mediterranean and Pacific (Bankova, 2005). The Temperate Zone propolis is generally referred to as Poplar propolis because it is mainly produced from

* Corresponding author. Tel.: +27 12 382 6373; fax: +27 12 382 6243.
E-mail addresses: viljoenam@tut.ac.za, SandasiM@tut.ac.za (A. Viljoen).

http://dx.doi.org/10.1016/j.bse.2014.03.012
0305-1978/

the bud exudates of *Populus* trees (Popova et al., 2004). Poplar-derived propolis is rich in flavonoids, aromatic acids and the corresponding ester analogues (Sawaya et al., 2010). Birch propolis is found specifically in Russia and is distinctly different from Poplar propolis (Christov et al., 2006). Pacific propolis, characteristically rich in prenylated flavanones (Popova et al., 2010), is another important type of propolis found in Taiwan, Japan and the Solomon Islands. Propolis obtained from the tropical region mainly contains prenylated *p*-coumaric acid derivatives, flavonoids, benzophenones, lignans and terpenes (Popova et al., 2009). Brazilian propolis, a highly valued propolis from the tropical regions, gained tremendous commercial importance due to a wide range of health benefits (Salatino et al., 2011). Green and brown propolis are the most common types of Brazilian propolis. Green propolis is derived from *Baccharis dracunculifolia*. It is rich in prenylated phenylpropanoids, triterpenoids, chlorogenic and benzoic acids (Righi et al., 2011). Brown propolis is derived from *Copaifera* species and contains mainly flavonoids and terpenes (Sawaya et al., 2006). In addition, red Brazilian propolis obtained from *Dalbergia ecastophyllum* (L) Taud contains isoflavonoids, prenylated benzophenones and naphthoquinone epoxide (Trusheva et al., 2006; Piccinelli et al., 2011). As propolis is considered a valuable therapeutic and health-promoting agent and is often incorporated into commercial product formulations for health benefits, chemical standardisation is a prerequisite to ensure quality and efficacy. The standardisation of propolis is challenging due to the inherent chemical complexity and variability (Bankova, 2005), and hence, metabolite profiling of propolis is a valuable tool to standardise as well as provide a chemical signature for a chemotype that may have superior health benefits.

Although South Africa has an active bee-keeping community, there is a paucity of scientific research on the biological properties and chemistry of locally produced propolis. This is surprising, as numerous websites claim that South African propolis is a widely valued health product. Some studies on the anti-oxidant, antimicrobial and anti-inflammatory activities of propolis have been documented (Garedew et al., 2004; Kumazawa et al., 2004; Du Toit et al., 2009). Kumazawa et al. (2004) reported that South African propolis contains different types of phenolic compounds, hinting at some similarities with Poplar propolis. Recently, Zhang et al. (2014) reported the chemical profiles of five samples obtained from KwaZulu-Natal in South Africa. Three of these samples showed chemical profiles similar to temperate region Poplar propolis while the other two samples were rich in diterpenoid acids, characteristic of propolis from the eastern Mediterranean regions. Most of these studies are based on a few random samples, and at present, there is no comprehensive study available on the chemistry of South African propolis from various provenances. Hence, the objective of this study was to investigate the composition of 39 propolis samples collected from various regions in the country by UPLC-ESI-MS. The secondary objective was to use chemometric modelling to observe possible geographical patterns and to compare South African propolis to Brazilian propolis which is generally used as the “golden standard” of all commercially produced propolis.

2. Materials and methods

2.1. Chemicals

Phenolic and flavonoid standards, such as caffeic acid, ferulic acid, gallic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, rutin hydrolyte and quercetin, were purchased from Sigma–Aldrich Chemie GmbH, Germany. Ultra-pure

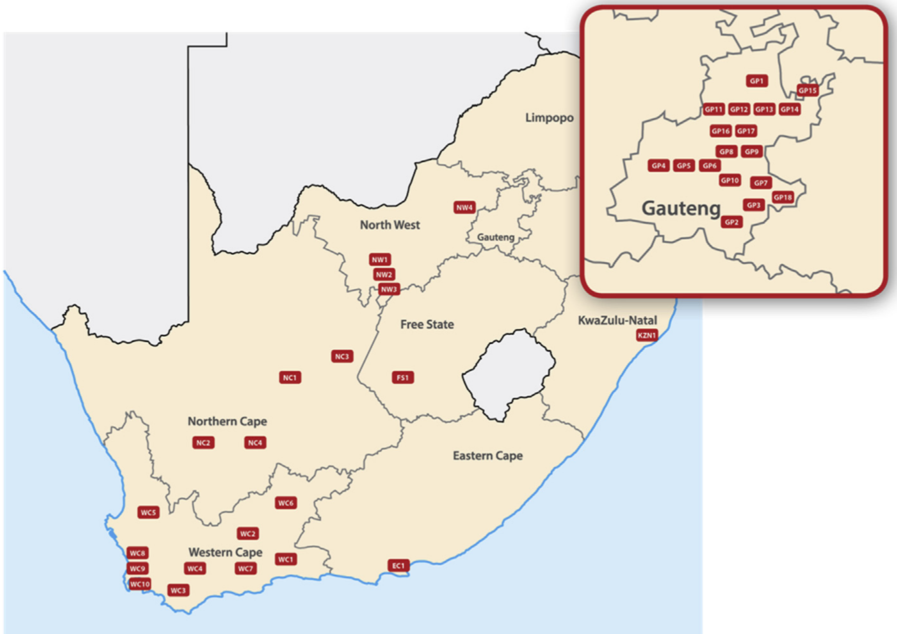

Fig. 1. Localities where propolis samples were sourced in South Africa.