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Chromatographic analysis with different detectors in the chemical characterisation and dereplication of African propolis

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ABSTRACT

Propolis or bee glue has very diverse composition and is potentially a source of biologically active compounds. Comprehensive chemical profiling was performed on 22 African propolis samples collected from the sub-Saharan region of Africa by using various hyphenated analytical techniques including Liquid Chromatography (LC)–UltraViolet Detection (UV)–Evaporative Light Scattering Detection (ELSD), LC–High Resolution Mass Spectrometry (HRMS), Gas Chromatography (GC)–MS and LC–Diode Array Detector (DAD)–HRMS/MS. The diversity of the composition of these African propolis samples could be observed by heat mapping the LC–UV and ELSD data. The characteristic chemical components were uncovered by applying Principal Component Analysis (PCA) to the LC–HRMS data and a preliminary dereplication was carried out by searching their accurate masses in the Dictionary of Natural Products (DNP). A further identification was achieved by comparing their GC–MS or LC–DAD–HRMS/MS spectra with previously published data. Generally no clear geographic delineation was observed in the classification of these African propolis samples. Triterpenoids were found as the major chemical components in more than half of the propolis samples analysed in this study and some others were classified as temperate and Eastern Mediterranean type of propolis. Based on the comparative chemical profiling and dereplication studies one uncommon propolis from southern Nigeria stood out from others by presenting prenylated isoflavonoids, which indicated that it was more like Brazilian red propolis, and more significantly a high abundance of stilbenoid compounds which could be novel in propolis.

1. Introduction

Rapid identification of known compounds from chemical profiling of natural resources, referred to as dereplication, is becoming increasingly important for phytochemistry based drug discovery [1–3] via the targeting of novel compounds. With the development of hyphenated analytical techniques the ability to analyse individual components in a complex mixture has been significantly improved [1,3,4]. In the case of LC–MS the enhancement of LC column efficiency has delivered higher peak capacity allowing separation of greater numbers of components and High Resolution MS (HRMS) and Collision Induced Dissociation (CID) techniques offer the measurement of accurate masses of the molecular ions and fragments of the individually eluting molecules providing valuable information for their structure elucidation [5]. Electron Ionisation (EI), as the interface technology of GC–MS, is able to generate reproducible fragmentation patterns for gaseous molecules. Based on this feature an EI–MS standard spectral library including hundreds of thousands of compounds has been built up by National Institute of Standards and Technology (NIST) and is widely used in identification of unknown compounds in many laboratories. LC–Solid Phase Extraction (SPE)–NMR has also been reported as a powerful analytical platform for dereplication [6]. It is able to generate more accurate structural information than MS based hyphenated techniques. However, the sensitivity and the intricate instrumentation configuration limits its wide application for this purpose.

Propolis (bee glue) is a sticky and dark-coloured material harvested by honey bees in order to seal cracks of the hives and more importantly eliminate biological contamination in the colony. It has been reported to have various biological and pharmacological properties, attracting intense interest in its medicinal applications [7–9]. It has the advantage as source of biologically active compounds that it has already been selected by bees for its biological activity and is collected from plants in a non-destructive way. The complex chemical composition of propolis remains as a

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challenge for purification of bioactive components by conventional phytochemistry. In addition, the chemical and bioactive characteristics of propolis are highly dependent on its geographic origin [9–12]. Up to now the propolis from Europe, Asia, North and South America has been well studied presenting a comprehensive information resource for bioactive natural products found in propolis. For instance, polyphenols including flavonoids, phenolic acids and their esters are general major bioactive chemical components in the poplar propolis from the temperate zones of both Northern and Southern hemispheres [9,11] and prenylated benzophenones and diterpenes are characteristic chemical components of the propolis from tropical zones (Northeast Brazil and Cuba) [13] and Eastern Mediterranean regions (Greece, Crete and Turkey) [14], respectively.

Recently chemical profiling and dereplication using LC–MS or GC–MS has been employed for chemical evaluation of commercial propolis products or wild propolis [13–22]. Common chemical components in propolis crude extracts were quickly recognised by comparing DAD, CID–MS^n or EI–MS spectra of the chromatographic peaks with authentic standard compounds' or previously published data leading to uncommon ones being uncovered and then putatively identified by interpreting the DAD and MS data. By applying this strategy a large number of samples can be quickly characterised in chemical composition and unlike in conventional phytochemistry only small amount of propolis is consumed for analysis. By dereplication of the chemical components and comparison with known types of propolis the analysed samples can be categorised or even defined as new types if novel compounds are discovered [13]. From our literature review there is only limited research data on the African propolis but the uniqueness of its chemical composition has been reported [11,23,24]. The aim of this study was to investigate the basic chemical composition of the African propolis collected from 9 countries in the wide region of sub-Saharan Africa. Using various hyphenated analytical techniques we intended to characterise these propolis samples by a comprehensive chemical profiling of their ethanolic extracts and hoped to uncover some novel compounds by dereplication studies for targeted isolation in the future.

2. Experimental

2.1. Chemicals and solvents

HPLC grade acetonitrile (ACN) and ethyl acetate was purchased from Fisher Scientific, UK. HPLC grade water was produced by a Direct-Q 3 Ultrapure Water System from Millipore, UK. AnalaR grade formic acid (98%) and ethanol were obtained from BDH-Merck, UK. N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was purchased from Sigma-Aldrich, UK.

2.2. Propolis sample collection and preparation

The propolis samples were gradually collected by BeeVital from sub-Saharan African countries in the past 10 years (Supporting information 1) and stored at room temperature with dark and dry conditions before the extraction. Approximately 50 mg of propolis was cut off from the core of each sample and extracted with ethanol by ultra-sonication with heat at 40 °C for 2 hours. The filtered solution was dried by nitrogen flow and the amount of residue was measured by subtracting the weight of empty vial from the total weight. Finally each residue was reconstituted with ethanol at a concentration of 5 mg/ml as the stock solution for the following analysis.

2.3. LC–UV–ELSD

1 ml of each stock solution was dried by nitrogen flow and reconstituted as 5 mg/ml with the mobile phase at the ratio of the initial composition of the LC gradient programme. The LC–UV–ELSD analysis was performed on an Agilent 1100 system (Agilent Technologies, Germany) consisting of a quaternary pump, an autosampler, a degasser and a UV single channel (290 nm) detector coupled with an Evaporative Light Scattering Detector (ELSD) (model: SEDEX75, SEDERE France) at 30 °C. An ACE C18 column (150 × 3 mm, 3 μm) (HiChrom, Reading UK) was employed for separation with 0.1% v/v formic acid in water as mobile phases A and 0.1% v/v formic acid in ACN as B at the flow rate of 300 μl/min. The injection volume was 10 μl. The gradient elution was programmed as follows: 0–15 min linear gradient from 30% to 50% of B, 15–25 min at 50% of B, 25–40 min linear gradient from 50% to 80% of B, 40–50 min at 80% of B, 50–51 min increasing to 100% of B, 51–59 min at 100% of B with the flow rate increasing to 500 μl/min for cleaning the column and 60–70 min back to 30% of B for re-equilibration for next run.

2.4. LC–HRMS and DAD–$HRMS^n$

The same samples and the chromatographic conditions described in Section 2.3 were used on an Accela 600 HPLC system combined with an Exactive (Orbitrap) mass spectrometer from Thermo Fisher Scientific (Bremen, Germany). The MS detection range was from 100 to 1500 m/z and the scanning was performed under ESI polarity switching mode. All detailed MS settings were used the same as described in our previous study [25]. The data dependent MS^n fragmentation was carried out by using Collision Induced Dissociation (CID) at 35 V on a LTQ-Orbitrap mass spectrometer combined with a Surveyor HPLC system from Thermo Fisher Scientific (Bremen, Germany) including on-line DAD (200–600 nm) and UV at 290 nm analysis. Again the chromatographic conditions described in Section 2.3 were used. Each whole MS scan consisted of three segments: MS full scan from 100 to 1500 m/z; MS/MS on the most intense m/z signal in the full scan; MS/MS on the most intense fragment generated in the MS^2. By switching on the dynamic exclusion function each m/z signal would be selected for MS/MS fragmentation only three times within 1 minute and then the selection would move to the next most intense m/z signal and so on.

2.5. GC–Ms

1 ml of stock solution was dried by nitrogen flow and reconstituted with 1 ml of ethyl acetate. For derivatisation evaluation the sample was prepared as described in Ref. [14] with MSTFA instead of bis(trimethylsilyl)-trifluoroacetamide (BSTFA). 1 μl of each prepared sample was injected in splitless mode at 280 °C into the GC–MS (Focus GC-DSQ2) system from Thermo Fisher Scientific (Bremen, Germany) equipped with a 30 m long, 0.25 mm i.d., and 0.25 μm film thickness InertCap 1 MS capillary column from GL Sciences (Japan). The temperature gradient was programmed as follows: initially holding at 100 °C for 2 min, linearly increasing to 280 °C at the rate of 5 °C/min, holding at 280 °C for 15 mins and linearly increasing to 320 °C at the rate of 10 °C/min and holding for 10 mins. The temperature was 250 °C in the ionisation source and the ionisation voltage was 70 eV for EI–MS in positive mode.

2.6. Software and data processing

MZMine 2.10 [26] was used for LC–HRMS data processing. The procedure and the settings were the same as described in our previous study [27]. The generated peak lists from both ESI