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Identification and characterization of potential bioactive compounds from the leaves of *Leucosidea sericea*

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ABSTRACT

Ethnopharmacological relevance: *Leucosidea sericea* is a southern African tree used for treating different diseases including microbial infections and inflammatory-related conditions.
Aim of the study: To isolate and identify the chemicals in *Leucosidea sericea* which possibly account for the diverse therapeutic effects of the species.
Materials and methods: Leaf material was extracted using 20% methanol and subsequently partitioned with different solvents (hexane, dichloromethane, ethyl acetate and butanol). Resultant fractions were subjected to bioactive (antimicrobial)-guided isolation and the structural elucidation was conducted using NMR (1D and 2D) spectroscopic methods. Given the broad uses of *Leucosidea sericea* in traditional medicine, the extract, fractions and isolated compounds were evaluated in five (5) biological assays *in vitro* (antimicrobial, antioxidant, acetylcholinesterase (AChE) and anti-inflammatory inhibition as well as cytotoxicity effect).
Results: As the most active fractions, from ethyl acetate yielded 5,7-dihydroxychromone **(1)**; 1-hydroxy-2-oxopomolic acid **(2)**; 3,5,7,3´,4´-pentahydroxyflavone **(3)** and Tiliroside **(4)**. For the first time, these four (4) compounds were isolated from leaves of *Leucosidea sericea*. These aforementioned compounds demonstrated broad-spectrum antibacterial activity (1.95–125 μg/mL) and noteworthy antifungal (3.9–250 μg/mL) potential. In addition to its noteworthy antimicrobial activity, compound **3** also demonstrated significant antioxidant (EC_{50} = 14 μg/mL in DPPH assay) and anti-inflammatory (inhibited the level of ELAM by approximately 36% and decreased also the viability of endothelial cells) activities *in vitro*. Overall, AChE inhibition activity and cytotoxic response was generally weak for the extracts, fractions and isolated compounds.
Conclusion: The pool of chemicals in *Leucosidea sericea* were enriched with the isolation and identification of four (4) compounds obtained from the leaf extract. Among these compounds, the significant antimicrobial activity of compound **3** provides strong evidence that support the use of *Leucosidea sericea* for microbial-related infections in folk medicine.

1. Introduction

Leucosidea sericea Eckl. & Zeyh. (Rosaceae) is the only species in the genus and is commonly found in southern Africa (Pooley, 1993). It is an important economic tree species and medicinal plant which is well-known for its multiple therapeutic values in traditional medicine (Mafole et al., 2017). In South Africa, different ethnic groups use the plant for different purposes. For instance, the Sotho are known to prescribe '*Mošino*' or '*Cheche*' (local name of *Leucosidea sericea*) for the treatment of infectious diseases including skin infection (herpes), HIV and cough (Moteetee and Van Wyk, 2011; Seleteng Kose et al., 2015). '*umTshitshi*' as the plant is known among the Zulu is a therapy against inflammation of the eyes (Hutchings et al., 1996). Records by Watt and Breyer-Brandwijk (1962) referred to the plant as an astringent medicine

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and its utilization as vermifuge by Basuto tribes. Even though most of the parts of the plant possess healing powers, the leaves remain the most commonly used part and is often prepared as a ground paste or crushed and boiled in water (Hutchings et al., 1996; Mafole et al., 2017; Watt and Breyer-Brandwijk, 1962).

On the basis of the diverse uses of *Leucosidea sericea* in traditional medicine and the need to validate the biological efficacy, researchers have screened the extracts from the plant in more than five (5) biological assays (Mafole et al., 2017). The extracts prepared from different solvents have exhibited noteworthy therapeutic potential in biological assays including antimicrobial, antioxidant, anti-inflammatory, anti-parasitic and acetylcholinesterase inhibitory activities (Aremu et al., 2011; Bosman et al., 2004; Mafole et al., 2017). Some of these aforementioned biological activities have been attributed to the rich pool of essential oils found in the plants (Pitso and Ashafa, 2015). Further attempt to identify the active principle in *Leucosidea sericea* have resulted in the isolation and identification of nine (9) compounds mainly from the leaves (Mafole et al., 2017). In one study, Sharma et al. (2014) reported on four known compounds (phytol acetate, triacontanol, phytol and alpha kosin) and one new compound, (E)-3,7,11,15-tetramethylheptadec-2-ene-1,17-diol, which were isolated for the first time from *Leucosidea sericea*. The authors highlighted the potential of alpha kosin against *Propionibacterium acnes*, which is known as acne inducing bacteria. Two phloroglucinols (aspidinol and desaspidinol) were isolated from the leaves and flowers of the plant (Bosman et al., 2004). Particularly, aspidinol demonstrated a strong antibacterial activity against two (2) Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Nair et al. (2012) also identified two (2) cholestane triterpenoids (*β*-sitosterol and *β*-sitostenone) from the stem, which possibly corroborate the traditional uses related to the use of *Leucosidea sericea* for alleviation of inflammation in folk medicine.

The current study aims to identify and characterize the compound (s) responsible for some of the biological activities exhibited by crude extracts of *Leucosidea sericea* using gravity column chromatography and NMR analysis. The biological activities investigated were antimicrobial, antioxidant, acetylcholinesterase (AChE) inhibitory, anti-inflammatory and cytotoxicity.

2. Materials and methods

2.1. General

2.1.1. Chemicals, reagents and instrumentations for compound isolation and structural elucidation

Curcumin was obtained from Sigma Aldrich (St. Louis, MO, USA) and used as anti-inflammatory natural compound. It is known as specific inhibitor of cyclooxygenase-2 (COX-2), a key inducer of inflammation. Neomycin, Amphotericin B, Galanthamine and Trolox were purchased from Sigma-Aldrich, Germany.

Pre-coated plates (MERCK, silica gel 60 F254 0.2 thickness) were used for thin layer chromatography (TLC) analysis which was conducted at room temperature. The detection of spots was viewed under ultraviolet light (254 and 366 nm) while open column chromatography was carried out using Sephadex LH-20 and silica gel. Nuclear magnetic resonance (NMR) data were obtained using a Bruker AVANCE 500 (^{1}H at 500.0 MHz, ^{13}C at 125.7 MHz) spectrometer. Chemical shifts (in ppm, δ scale) were referenced to the solvent signal (CD_3OD, ^{1}H: 3.31 ppm, ^{13}C: 49.0 ppm). Coupling constants (J) are given in Hz. Complete assignment of protons and carbons was done by analysis of correlated homonuclear 2D-gCOSY and heteronuclear ^{1}H-^{13}C gHSQC and ^{1}H-^{13}C gHMBC spectra. Mass spectrum (TOF MS ES) was recorded using a Thermofinnigan Trace GC coupled to a Polaris Q Mass Spectrometer.

2.1.2. Cell culture for anti-inflammatory and cytotoxicity assays

Cell lines from various histopathological origins were used for cytotoxicity screening. T-lymphoblastic leukemia (CEM); breast carcinoma (MCF7); cervical carcinoma (HeLa) cell lines; and normal human foreskin fibroblasts (BJ) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma, MO, USA) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 1% penicillin-streptomycin. Human umbilical vein endothelial cells (HUVECs) were a kind gift from Prof Jitka Ulrichová (Medical Faculty, Palacky University, Olomouc) and were cultured in Endothelial Cell Proliferation Medium (ECPM, Provitro, Berlin, Germany), supplemented with 10% heat-inactivated fetal bovine serum (HyClone, GE Healthcare, Logan, UT, USA). The cell lines and HUVECs were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humid environment. Cells were sub-cultured twice or three times a week using the standard trypsinization procedure.

2.2. Plant collection, extraction and isolation of compounds

2.2.1. Sample collection

Leaf material of *Leucosidea sericea* was collected from the University of KwaZulu-Natal (UKZN) Botanical Garden, Pietermaritzburg, South Africa. The plant was identified by Mrs Alison Young (Horticulturist, University of KwaZulu-Natal, UKZN) and a voucher specimen was prepared (S Pendota 6) and lodged in the Bews Herbarium (NU). The leaves were dried at 50 °C, ground into a powder using an Ultra-Centrifugal Mill (ZM 200, Retsch®, Germany) and stored at room temperature in airtight containers under dark conditions.

2.2.2. Extraction and solvent partitioning of the crude extract

The powdered plant material (1000 g) was extracted with 8 L of 20% aqueous methanol (MeOH) at room temperature for 24 h and filtered. The filtrate was concentrated *in vacuo* at 40 °C to about a third of its original volume. This afforded crude extract of the plant material.

Concentrated crude extract was in turn sequentially extracted with n-hexane (Hex; 3 × 800 mL), dichloromethane (DCM; 3 × 800 mL), ethyl acetate (EtOAc; 3 × 1 L) and finally n-butanol (500 mL). The solvent fractions were concentrated to dryness *in vacuo* to afford four (4) solvent fractions coded as: Hex, DCM, EtOAc and n-butanol fractions. As a result of the favourable antibacterial potential of EtOAc fraction during the preliminary tests, it was selected for subsequent isolation procedures.

2.2.3. Isolation of compounds from the portioned fractions

The EtOAc fraction (21.2 g) was fractionated on a silica gel column (Length = 59 cm, width = 2.4 cm) using DCM/EtOAc (8:2) followed by an increasing gradient of EtOAc up to 100% and then MeOH up to 60%. Eight fractions (A_1-A_8) were obtained based on TLC analysis. Further column fractionation of fraction A_3 on Sephadex LH-20 using EtOAc/MeOH (8:2) followed by an increasing gradient of MeOH up to 20% resulted in the isolation of compound **1** (35 mg) and compound **2** (72 mg) after analysis on TLC (Fig. 1). Purification of fraction 4 on Sephadex LH-20 (Length = 59 cm, width = 2.4 cm) using EtOAC 100% followed by an increasing gradient of MeOH up to 15% (9:1) yielded compound **3** (120 mg) on analysis of the fractions collected on TLC (Fig. 1). Purification of fraction 6 on Sephadex LH-20 using EtOAc 100% followed by an increasing gradient of MeOH up to 20% (8:2) yielded compound **4** (21 mg) (Fig. 1). All TLC analyses were done using DCM/MeOH.

2.2.4. Structure elucidation of isolated compounds

Structure elucidation of the isolated compounds was carried out using spectroscopic techniques: ^{1}H (500.0 MHz) and ^{13}C NMR (125.7 MHz), and DEPT together with 2D experiments.

5,7-dihydroxychromone (compound 1) ^{13}C NMR (125.7 MHz, CD_3OD): 95.68 (CH-8); 101.02 (CH-6); 105.94 (C-10); 111.42 (CH-3); 157.79 (CH-2); 160.08 (C-9); 168.57 (C-7); 183.10 (C-4).^{1}H NMR