Industrial Crops and Products 86 (2016) 301–310

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Plastotoma rotundifolium aerial tissue extract has antibacterial activities

Jérémie Ngezahayo[a,b,*], Laurent Pottier[a], Sofia Oliveira Ribeiro[a], Cédric Delporte[c], Véronique Fontaine[d], Léonard Hari[b], Caroline Stévigny[a], Pierre Duez[a,e]

[a] *Laboratoire de Pharmacognosie, Bromatologie et Nutrition humaine, Faculté de Pharmacie, Université Libre de Bruxelles, CP 205/09, Boulevard du Triomphe, 1050 Bruxelles, Belgium*
[b] *Centre de Recherche Universitaire en Pharmacopée et Médecine Traditionnelle (CRUPHAMET), Faculté des Sciences, Université du Burundi, BP. 2700 Bujumbura, Burundi*
[c] *Laboratoire de Chimie Pharmaceutique et Plateforme Analytique, Faculté de Pharmacie, Université Libre de Bruxelles, CP 205/05, Boulevard du Triomphe, B-1050 Bruxelles, Belgium*
[d] *Laboratoire de Microbiologie Pharmaceutique et Hygiène, Faculté de Pharmacie, Université Libre de Bruxelles, CP 205/02, Boulevard du Triomphe, 1050 Bruxelles, Belgium*
[e] *Service de Chimie Thérapeutique et de Pharmacognosie, Université de Mons (UMONS), 20 Place du Parc, 7000 Mons, Belgium*

ARTICLE INFO

Article history:
Received 26 December 2015
Received in revised form 1 April 2016
Accepted 1 April 2016
Available online 8 April 2016

Keywords:
Platostoma rotundifolium
Lamiaceae
Antibacterial activity
Bioautography
MRSA
Pentacyclic triterpenoids

ABSTRACT

Despite efforts in recent years, infectious diseases remain a worldwide major public health problem. Several infectious agents have become resistant to conventional antibiotics and there is thus urgent need to discover new antimicrobial drugs to overcome resistances. *Platostoma rotundifolium* (Briq.) A. J. Paton, an African plant, is mainly used to treat microbial infections in traditional Burundian medicine. From the ethyl acetate extract of the aerial parts, five pentacyclic triterpenoid acids were isolated and characterized. Based on spectral analysis, these compounds were elucidated to be 2α, 3α, 19β-trihydroxyurs-12-en-28-oic acid (that was named jeremic acid) (**1**), 3β-hydroxyurs-12-en-28-oic acid (ursolic acid) (**2**), 2α, 3β-dihydroxyurs-12-en-28-oic acid (corosolic acid) (**3**), 2α, 3β, 19α-trihydroxyurs-2-en-28-oic acid (tormentic acid) (**4**) and 19-hydroxy-2-hydroxymethyl norursa-2, 12-dien-28-oic acid (hyptadienic acid) (**5**). Ursolic (MIC = 17.5–68 μM) and corosolic (MIC = 17–68 μM) acids showed significant antibacterial activities against both the Gram-positive *Staphylococcus aureus* (methicillin-susceptible and -resistant strains) and the Gram-negative *Escherichia coli*, which may substantiate the use of *P. rotundifolium* in traditional Burundian medicine. Such hydroxylated pentacyclic triterpenoid acids could point to new antimicrobial strategies that may help overcoming the antimicrobial resistances actually observed throughout the world.

1. Introduction

Infectious diseases remain to this day one of the major public health problems that concerns the whole world. Over 10 years ago, the World Health Organization (WHO) estimated these diseases were the leading cause of morbidity and mortality worldwide, accounting for about half of deaths in tropical countries (WHO, 2003). Even if some improvements are noticeable, the situation remains very alarming. Indeed, according to the latest report by the WHO on global health statistics (WHO, 2014), infectious diseases cause life expectancy to decrease by 70% in the WHO African Region and by 8% in high-income countries. Moreover, apart from the fact that significant progress has been made against child deaths after the first month of birth (measles, −80%; HIV/AIDS, −51 %; diarrhea, −50%; pneumonia, −40%, and malaria, −37%), half of the top 20 causes of premature death worldwide are associated with infectious diseases, complicated by other factors, maternal, neonatal or nutritional (WHO, 2014).

The resistance of microorganisms to existing conventional antimicrobial drugs represents an equally important issue impeding treatments. The antibiotic era (1940–1970) commonly known as "the golden age" (the period during which most of today's antibiotics were discovered) was promising but, unfortunately, resistances have rapidly occurred, favored by major selective

* Corresponding author at: Laboratoire de Pharmacognosie, Bromatologie et Nutrition humaine, Faculté de Pharmacie, Université Libre de Bruxelles (ULB), Campus de la Plaine—CP205/9, Boulevard du Triomphe, B-1050 Brussels, Belgium.
E-mail addresses: jngezaha@ulb.ac.be, jrmienge2000@yahoo.fr (J. Ngezahayo).

http://dx.doi.org/10.1016/j.indcrop.2016.04.004
0926-6690/

pressures mainly due to clinical abuse in humans and overuse in animal feed and aquaculture (Looke et al., 2013; O'Neill, 2014; Wang and Schaffner, 2011). A return to the pre-antibiotic period appears as a dreadful possibility, with a major risk of untreatable diseases increase; O'Neill (2014) estimates that over 10 million people would die every year within the next 35 years because of these antimicrobial resistance problems.

Medicinal plants have long been used against a number of diseases (Petrovska, 2012), notably infections (Cowan, 1999; Iwu et al., 1999; Ngezahayo et al., 2015; Rios and Recio, 2005). Indeed, part of their secondary metabolites present antimicrobial activities, either direct (bactericide or bacteriostatic) (Kim and Ausubel, 2006) or indirect (reversion of resistances, modulation of quorum sensing) (Okusa et al., 2007; Rasamiravaka et al., 2015b). These could certainly help to address the current problems of antimicrobial resistance (Hatano et al., 2005; Okusa et al., 2009; Rasamiravaka et al., 2015a).

In fact, the interest for herbal medicines remains important: (i) in its strategy for 2014–2023, the WHO encourages the development and modernization of traditional medicine as an integral part of emerging healthcare systems (WHO, 2013); (ii) Newman and Cragg (2012), confirming previous studies (Rout et al., 2009; Wachtel-Galor and Benzie, 2011), reported that about 50% of the modern medicines developed over 1981–2010 were natural substances or were inspired by natural substances.

In spite of its negation during the decades of colonization, Traditional African Medicine (TAM) has resisted and remains extremely vivid in Africa (Kahumba et al., 2015), notably in Burundi. Indeed, TAM represents the primary health care needs for 80% of both rural and urban population, be it in daily or emergency cares (Kasilo et al., 2010). The main motivations for its use are the cultural attachment, the confidence in its efficacy but also the scarcity and cost of quality modern drugs (Elujoba et al., 2005). TAM is mainly based on plants, but also on some animal and mineral products.

In an ethnobotanical survey conducted recently on medicinal plants used against infections by traditional healers and herbalists of the city of Bujumbura, capital of Burundi (Ngezahayo et al., 2015), *Platostoma rotundifolium* (Briq.) A. J. Paton (perennial herb with a small bush, of the Lamiaceae family and up to 1.5 m high) was the most used species, cited by 75% of interviewees. It is primarily used against microbial infections, including skin disorders. In addition, a preliminary phytochemical study carried on the aerial parts of this species showed antibacterial activity against Gram-positive and −negative bacteria, antibiotic-sensitive and resistant; the most active fractions were the aerial parts dichloromethane and ethyl acetate extracts (Ngezahayo et al., 2014).

In the present work, the *P. rotundifolium* aerial parts ethyl acetate extract was subjected to bio-guided fractionation, applying TLC-bioautography (Okusa et al., 2010), to isolate and characterize different active molecules that may justify its use in traditional Burundian medicine and possibly provide a clue to new antimicrobial strategies.

2. Experimental

2.1. General experimental procedures

Column and flash chromatography were performed with silica gel 60 (40–63 μm, Merck, Germany) and pre-packed silica Redi*Sep*® R_f Colomn (Teledyne Isco, 4 g Flash Column, CV 4.8 ml, 18 ml/min, max. pressure 1200 psi (13.8 bar), 20–400 mg sample, USA), respectively. Thin Layer Chromatography (TLC) was carried out on precoated silica gel 60 F_{254} (Merck) plates eluted with dichloromethane-ethyl acetate (80:20), dichloromethane-methanol (96-4) or chloroform-methanol (93:7). The TLC plates were sprayed with 1% ethanolic vanillin, followed by 10% ethanolic surfuric acid and heated at 110 °C for 10 min. Preparative TLC (TLC silicagel 60 F_{254}, 20 × 20, Merck, Germany) were used during purification processes. TLC-bioautography was performed on 10 × 5 cm precoated silica gel 60 F_{254} glasses plates (Merck, Darmstadt, Germany). Melting points were measured with a Stuart equipment. IR spectra were recorded with an IRAffinity-1 FTIR spectrophotometer (Shimadzu). Optical rotation values were measured on a Perkin-Elmer 241 polarimeter. High-resolution ESI–MS were determined using an Agilent 6520 Accurate-Mass Q-TOF LC–MS (Palo Alto, CA, USA). NMR experiments (^{1}H, ^{13}C (BBD and Dept 135), COSY, HSQC, HMBC and NOESY) were performed on Bruker Avance 300, 400 or 600 MHz.

2.2. Plant material

Fresh aerial parts of *P. rotundifolium* (Briq.) A. J. Paton were collected in July 2012 from Nyabiraba area (1730 m, S 03.45325°, E 029.47607°) in Bujumbura Rural Province (Burundi). The plant was identified by the specialists of the Herbarium of the National Botanical Garden of Meise (Belgium) where a voucher specimen has been deposited under the number BR0000013315900.

2.3. Extraction and isolation

Powdered aerial parts (1.7 kg) were percolated successively with 8 l of each of five solvents: *n*-hexane (yield, 16.4 g), dichloromethane (yield, 49.9 g), ethyl acetate (yield, 18.4 g), methanol (yield, 52.8 g) and water (yield, 125.6 g). The most active fraction, the ethyl acetate extract (18.4 g), was subjected to fractionations by column chromatography (360 g silica; 80 × 6 cm i.d), eluting with 10.5 l of dichloromethane-ethyl acetate mixtures with increasing polarities (10 to 100% EtOAc). The fractions were pooled according to their TLC profile to give eight fractions (FI-FVIII) with respective yields 0.92, 0.11, 0.10, 1.47, 1.20, 0.95, 3.34 and 4.47 g.

Portions of 3 active fractions (420 mg of FIV, 890 mg of FV and 849 mg of FVIII) were subjected to flash chromatography, eluting with dichloromethane-ethyl acetate gradients (0 to 100% EtOAc). The obtained active subfractions were purified by preparative TLC using chloroform-methanol (93:7) and dichloromethane-methanol (96:4) as mobile phases, yielding compounds (**1**) (23 mg), (**2**) (42 mg), (**3**) (10 mg), (**4**) (30 mg) and (**5**) (8 mg).

Compound **1** (Jeremic acid): white powder; R_f (eluent: dichloromethane/methanol, 96:4) = 0.12; mp 245 °C; $[\alpha]_D^{20}$ + 20.5° (*c* = 0.07, methanol); IR (cm^{-1}) = 3566, 2936, 2874, 2682, 1455, 1288, 1222, 1160, 1038, 938; ^{1}H- and ^{13}C— NMR data, see Tables 1 and 2; ESI-HRMS (positive mode): *m/z* 489.3576 $[M+H]^+$ (theoretical *m/z*, 489.3575); 506.3836 $[M+NH_4]^+$ (theoretical *m/z*, 506.3840); 511.3395 $[M+Na]^+$ (theoretical *m/z*, 511.3394); 527.3174 $[M+K]^+$ (theoretical *m/z*, 527.3133) (See supplementary data); ESI-HRMS/MS (positive mode): *m/z* (rel. int.) 489.3552 $[M+H]^+$ (75), 471.3454 $[(M+H)-H_2O]^+$ (96), 453.3348 $[(M+H)-2H_2O]^+$ (65), 425.3405 (56), 407.3285 $[C_{29}H_{43}O]^+$ (57), 219.1714 $[C_{15}H_{23}O]^+$ (18), 207.172 [retro-Diels-Alder (RDA) ion, $[C_{14}H_{23}O]^+$] (17), 205.1575 $[C_{14}H_{21}O]^+$ (83), 203.1788 $[C_{15}H_{23}]^+$) (7), 201.163$[C_{15}H_{21}]^+$ (100), 189.1636 (RDA ion − H_2O, $[C_{14}H_{21}]^+$) (17), 187.1473 $[C_{14}H_{19}]^+$ (41), 177.1627 $[C_{13}H_{21}]^+$ (14), 159.1159 $[C_{12}H_{15}]^+$ (13), 147.1159 $[C_{11}H_{15}]^+$ (28), 145.0986 $[C_{11}H_{13}]^+$ (10), 133.0996 $[C_{10}H_{13}]^+$ (13), 131.0857 $[C_{10}H_{11}]^+$ (13), 121.101 $[C_9H_{13}]^+$ (13), 119.0862 $[C_9H_{11}]^+$ (21), 107.086 $[C_8H_{11}]^+$ (11).

2.4. Bacterial strains

Five bacterial strains (four Gram-positive and one Gram-negative) were used in this work: *Staphylococcus aureus* (C 98506, C 100459, ATCC 33591 and ATCC 6538) and *Escherichia coli* ATCC