Industrial Crops and Products 96 (2017) 165–172

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Utilization of the ethyl acetate fraction of *Zanthoxylum rhetsa* bark extract as an active ingredient in natural sunscreen formulations

Ramesh kumar Santhanam[a], Muhammad Tayyab Akhtar[a], Syahida Ahmad[b], Faridah Abas[a], Intan Safinar Ismail[a], Yaya Rukayadi[a], Khozirah Shaari[a,*]

[a] *Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[b] *Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

ARTICLE INFO

Article history:
Received 27 July 2016
Received in revised form
26 November 2016
Accepted 28 November 2016
Available online 7 December 2016

Keywords:
Z. rhetsa
Sunscreen
Formulations
UVA/UVB
SPF
LC–MS/MS

ABSTRACT

Sunburn, premature skin aging, skin cancers and suppression of the immune system are linked to exposure of the skin to UV light. In recent years, plant extracts are becoming a popular active ingredient in natural sunscreen formulations. In the present study, the ethyl acetate fraction of *Zanthoxylum rhetsa* bark (commonly called as Indian prickly Ash) was used as an active ingredient in two sunscreen cream formulations (F1 and F2). Primarily, the constituents present in the active fraction were identified using LC–MS/MS analysis. Coumaric acid, benzoic acid, *p*-hydroxybenzoic acid and its isomers, hesperitin, trihydroxyoctadecenoic acid and columbamine were identified in the ethyl acetate fraction of *Z. rhetsa* bark extract. The UV protection properties of the formulated creams were evaluated by assessment of parameters such as their SPF values (F1: 3.60 ± 0.28, F2: 6.90 ± 0.57), UVA effectiveness (moderate for both test formulations) and critical wavelengths (F1: 365.4, F2: 360.3). Moreover, the physicochemical and microbial count of the formulated creams was also assessed based on various parameters such as colour, pH, centrifugation, viscosity and microbial load over a storage period of 28 days. Both formulations showed pseudo plastic behaviour and were stable at all conditions except for samples kept at 40 °C. Altogether, these results suggested that the ethyl acetate fraction of Z. *rhetsa* bark has great potential to reduce exposure to harmful UVA/UVB radiations and may be utilized as an active ingredient in natural sunscreen formulation.

1. Introduction

Sunscreen products such as suntan lotions, gels and sprays, absorb or reflect harmful ultraviolet (UV) radiation, providing some protection against sunburns and skin cancer (American Cancer Society, 2015; Centers for disease control and prevention, 2014; Skin Cancer Foundation, 2016). Common ingredients for these products include inorganic and organic substances which are mainly derived synthetically. Long-term uses of such ingredients have been reported to cause adverse effects (Mancebo et al., 2014; Sambandan and Ratner, 2011). Currently, regulatory bodies and consumers strictly prefer the use of safe and effective ingredients in the sunscreen formulation to prevent UV-induced damages (Mercola, 2014). UV-induced damage in human skin is a sequential process. It is initiated when the skin is repeatedly exposed to UV radiations which stimulate the production of free radicals, alters the signalling pathways (NF-κB, AP-1, TGF), induce inflammatory cytokines and matrix metalloproteinases (MMPs). These damage the extracellular proteins (*e.g.* collagen, elastin) and finally lead to events such as photo aging and skin cancer. The UV radiation responsible for these alterations in the skin are predominantly received from the sun (Diffey, 2002). They are of three types *i.e.* UVA (320–400 nm), UVB (290–320) and UVC (200–280 nm). The UVA and UVB radiations are responsible for the serious effects to the skin. Many studies have reported the deleterious effects of UVA and UVB in the skin. UVB radiation immediately targets the epidermis of the skin and causes sun burn, suntan and reddening, while, UVA radiation penetrates deep into the skin and slowly affects the skin by causing wrinkle formation, premature aging and skin cancer (Skin Cancer Foundation, 2016). Most of the commercially available sunscreen products only safeguard against UVB rays. Very few products target both UVA and UVB. Therefore, Food and Drug Administration (FDA) has announced that the product

* Corresponding author.
E-mail addresses: rameshcra@gmail.com (R.k. Santhanam), tayyabakhtar@hotmail.com (M.T. Akhtar), syahida@upm.edu.my (S. Ahmad), faridah_abas@upm.edu.my (F. Abas), safinar@upm.edu.my (I.S. Ismail), yr32@yahoo.com (Y. Rukayadi), khozirah@yahoo.com.my, khozirah@upm.edu.my (K. Shaari).

http://dx.doi.org/10.1016/j.indcrop.2016.11.058
0926-6690/

which blocks both the radiations is considered as best sunscreen and should be labelled as broad spectrum sunscreen (Skin Cancer Foundation, 2015). Currently, broad spectrum protection is feasible only by incorporating titanium oxide and zinc oxide with other UVB absorbers such as *para*-aminobenzoic acid, cinnamates and salicylates (American Melanoma Foundation, 2016). Due to certain limitations and risks related to the use of iron oxides and UVB absorbers (Environmental Working Group, 2015), researchers are bioprospecting from natural biosources and traditional medicine to discover and develop new and safer broad spectrum sunscreens.

Various plant extracts and marine organisms have been screened for their sunscreen properties (Balakrishnan and Narayanaswamy, 2011; Mishra et al., 2011). Several studies revealed the photoprotective properties of different parts of plant species such as the leaves of *Camellia sinensis* (Kaur and Saraf, 2011) and *Lippia sericea* (Polonini et al., 2014), the fruits of *Cucumis sativa* (Maheshwar et al., 2010), the flowers of *Crinum asiaticum* (Kale et al., 2012), the roots of *Scutellaria radix* (Seok et al., 2015) and the seeds of *Zanthoxylum rhetsa* (Kale et al., 2011). Their properties were demonstrated either in the form of crude extracts or sunscreen formulations. Previous study reported that the ethyl acetate fraction obtained from the bark of *Z. rhetsa* possessed good sunscreen properties by preventing the UV radiation and scavenging the free radicals (Santhanam et al., 2013). *Zanthoxylum rhetsa* (Rutaceae), commonly known as Indian Prickly Ash has long been used as a traditional medicine and its therapeutic value has been demonstrated by several studies (Ahsan et al., 2014; Ahsan et al., 2000; Shankaracharya et al., 1994; Tantapakul et al., 2012). The present work is carried out to identify the chemical constituents present in the bioactive ethyl acetate fraction of the extract. The study was also undertaken to further investigate the photo protective properties of a sunscreen formulation containing the bioactive fraction through a battery of well-established tests which included measurements of the sun protection factor (SPF), UVA/UVB ratio, critical wavelength, physicochemical parameters and microbiological stability.

2. Materials and methods

2.1. Reagents and instrumentation

All the solvents used for extraction were of analytical grade and obtained from R&M chemicals (Edmonton, AB, Canada). LC–MS/MS analyses were carried out on AB Sciex 3200 QTrap LC–MS/MS with Perkin Elmer FX15 UHPLC system (Shelton, CT, USA). UV protection properties were carried out using UV-2000S Ultraviolet Transmittance Analyzer from Labsphere, Inc., (North Sutton, NH, USA). Physica MCR 300 rheometer from Anton Paar GmbH (Graz, Austria) was used for viscosity measurement. Cream base was purchased from Kimia Farma, Jakarta, Indonesia. Other chemicals used to prepare the formulation were purchased from Sigma (St. Louis, MO, USA). Media for microbiological assay was purchased from Merck Millipore, Darmstadt, Germany.

2.2. Sample collection

The bark material of *Z. rhetsa* was collected from Pankgor Island, Malaysia. Two batches of specimens were collected. First batch was collected in the month of January 2012 (Santhanam et al., 2013) and the second batch was collected in the month of March 2014. In this study, the second batch samples were used. A voucher specimen (SK2226/13) was deposited at the Herbarium of the Institute of Biosains, Universiti Putra Malaysia.

Table 1
Components and their percentage concentration in test formulation F1.

Sr.No	Ingredients	Components (% w/w)
1	Cetostearyl alcohol	5
2	Stearic acid	4
3	Petroleum Jelly	1
4	Glycerin	5
5	Potassium hydroxide	1
6	Water	85
7	Methyl paraben sodium	0.20
8	Propyl paraben sodium	0.05
9	Ethyl acetate fraction of *Zanthoxylum rhetsa*	10

2.3. Preparation of extract

The extract was prepared according to Santhanam et al. (2013). Briefly, the bark material was cut into small pieces, dried and ground into fine powder by using a Wiley mill. The sample was stored in −20 °C for extraction. Within a week gap the powdered material (2.5 kg) was extracted with double distilled methanol using ultrasound-assisted extraction and dried under reduced pressure at 40 °C to yield 205 g of the crude methanolic extract. The extract was then suspended in water and partitioned using organic solvents of varying polarities to obtain hexane (52 g), chloroform (53.8 g), ethyl acetate (8.4 g) and butanol (15.3 g) soluble fractions. All the solvent fractions were dried, lyophilized and stored at −20 °C. In comparison with other solvent fractions, the ethyl acetate fraction revealed high SPF value, good UVA/UVB absorption spectra, high phenolic and flavonoid contents in our previous study (Santhanam et al., 2013). Similar SPF value and UVA/UVB absorption spectra were observed in the current batch ethyl acetate fraction, which strongly supports the reproducibility of the work. Hence, the bioactive ethyl acetate fraction was used for further analysis.

2.4. LC–MS/MS analysis

The chemical constituents present in the ethyl acetate fraction were analysed using an AB Sciex 3200 QTrap LC–MS/MS equipped with a Perkin Elmer FX15 UHPLC system. The LC separation was carried out on a Phenomenex Aqua C18 column (50 mm × 2.0 mm × 5 μM). The mobile phase consisted of water (A) and acetonitrile (B), acidified with 5 mM ammonium formate and 0.1% formic acid. The gradient programme started from solvent ratio 10: 90 (A: B, v/v) for 8 min followed by 90:10 (A: B, v/v) for 2 min and further equilibrated with initial conditions 10: 90 (A: B, v/v) for 5 min. The flow rate was set at 500 μL/min and the injection volume was 20 μL. The mass spectrum of the samples was attained in negative mode using turbo ion spray by setting the following conditions voltage IS: −4500 V, source temperature: 500 °C, desolvation gas: 40 psi, source gas: 40 psi, scan range: 100–1200 m/z for full scan and 50–1200 m/z for MS/MS scan, declustering potential: 40 V, entrance potential: 10 V, collision energy: spread of 35 eV ± 15 eV.

2.5. Preparation of sunscreen formulations

Two types of formulations (F1 and F2) and their corresponding controls (C1 and C2) were prepared. The ingredients used for each formulation are listed in Tables 1 and 2, respectively.

For formulation F1, the aqueous phase was first prepared by dissolving potassium hydroxide (1% w/w) in deionised water (85% w/w), followed by the addition of glycerin (5% w/w), sodium methyl paraben (0.2% w/w) and ethyl acetate fraction of *Z. rhetsa* (10% w/w). The resulting mixture was heated up to 80 °C with continuous