Journal of Applied Research on Medicinal and Aromatic Plants 2 (2015) 77–86

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

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

Phytochemical and *in vitro* biological evaluation of *Artemisia scoparia* Waldst. & Kit for enhanced extraction of commercially significant bioactive compounds

Komal Khan[a], Humaira Fatima[a], Malik Mumtaz Taqi[a], Muhammad Zia[b], Tofeeq ur-Rehman[a], Bushra Mirza[c], Ihsan-ul- Haq[a,*]

[a] *Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan*
[b] *Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan*
[c] *Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan*

ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form 16 April 2015
Accepted 21 April 2015
Available online 23 May 2015

Keywords:
Artemisia scoparia
HPLC–DAD
Artemisinin
Cytotoxicity
Protein kinase

ABSTRACT

The validation of ethno botanical data from the under explored folk plant remedies represent an inexhaustible reservoir of novel compounds for drug discovery. The present work was aimed to evaluate the phytochemical analysis and various biological activities (antioxidant, antimicrobial, antileishmanial, cytotoxic, anticancer, and protein kinase inhibition) of *Artemisia scoparia* whole plant extracts prepared by employing an eclectic range of solvents polarities. Among 14 extraction solvents employed, maximum percent extract recovery (10%) was obtained when distilled water was employed as extraction solvent. The highest amount of gallic acid equivalent total phenolic (26.7 μg GAE/mg extract) and quercetin equivalent flavonoid contents (18.7 μg QE/mg extract) were quantified in ethanolic extract. Reverse phase HPLC–DAD based quantitative evaluation showed significant amount of rutin, quercetin, caffeic acid, kaempferol, apigenin and artemisinin ranging from 0.06 to 6.55 μg/mg extract. Ethanolic extract also exhibited highest DPPH scavenging (45.82%) reduction potential and total antioxidant capacity (110 and 60 μg ascorbic acid equivalent/mg extract, respectively). The remarkable toxicity profile against brine shrimps and leishmania (reported for the first time) was manifested by chloroform extract with LC_{50} 272 μg/ml and 141 μg/ml, respectively. Only methanolic extract exhibited a conspicuous *in vitro* anticancerous activity against THP1 human leukemia cell line with IC_{50} 6.93 μg/ml. The ethanolic extract also exhibited significant inhibitory activity against *Streptomyces* 85E with 15 mm bald zone which provides a useful evidence of its protein kinase inhibition potential. This study provides strong evidence that multiple solvent system is very crucial to explore the comprehensive biological potential of medicinal plants and extraction of commercially important compounds.

1. Introduction

Artemisia scoparia Waldst. & Kit (red stem Wormwood: jhahoo) is a bitter, aromatic and branched annual herb widespread in Southwest Asia (Kaur et al., 2010) abundantly found in India up to 2100 m altitude and grow in summer season in semi desert areas, stony grounds and

* Corresponding author. Tel.: +92 5190644143.
E-mail addresses: Sumbal-khan1993@hotmail.com (K. Khan), ihaq@qau.edu.pk (I.-u. Haq).

http://dx.doi.org/10.1016/j.jarmap.2015.04.002
2214-7861/

low hills. The plant has been used as herbal medicine throughout the world possessing antioxidant, antibacterial, antipyretic, purgative and vasodilator activities and also proved efficacious in the treatment of jaundice and hepatitis (Singh et al., 2009). Some researchers have documented phytotoxic potential of *A. scoparia* against weeds *via* ROS generation thus inhibiting emergence and growth of weeds by inducing oxidative stress (Kaur et al., 2012). A hydromethanolic extract of the plant possesses protective effects on acetaminophen and CCL4 induced liver toxicity (Gillani and Janbaz, 1993) as well as anti-inflammatory and antifebrile activities by attenuating acetic acid induced writhing and yeast induced pyrexia respectively (Habib and Waheed, 2013). The smoke of the plant has been considered remarkably effective for burns (Ahmad and Javed, 2007). *A. scoparia* is familiar to its volatile constituent that is "Phytotoxin" (Singh et al., 2009). Extensive phytochemical investigation of *A. scoparia* have resulted in the isolation of scoparone, an anti-atherogenic (Hoult and Payá, 1996), scopariachromane, an inhibitor of nitric oxide production and triglyceride accumulation in pre adipocytes (Yahagi et al., 2014) and scoporal (Ali and Jahangir, 2008).

The literature is scarce on the complete spectrum of pharmacological potential of *A. scoparia* plant extracted in variety of polar to non-polar solvents. Most of the bioactive components of the plant adhere to inorganic compounds and proteins. The most commonly employed solvents methanol, ethanol and water do not extract all the bioactive components. The present study was designed to compare the extraction potential of different solvents with reference to the polarity of bioactive compounds present in *A. scoparia* whole plant. We explored the pharmacological activities of standardized extracts by utilizing a battery of assays along with identification of some valued phytochemicals that would be fundamentally useful on large scale basis *via* commercial collaboration. The *in vitro* pharmacological screening of plants has an advantage of low cost, reliability and fast turnover which made the plant to be screened at large scale.

2. Material and methods

2.1. Collection and identification

Plant material (a mixture of 20–30 plants) was collected from Quaid-i-Azam University Islamabad and authenticated by Prof. Dr. Rizwana Aleem Qureshi (Taxonomist), Department of Plant sciences, Faculty of biological sciences, Quaid-i-Azam University, Islamabad. The plant was deposited as an authentic specimen in the Herbarium of medicinal plants, Quaid-i-Azam University, Islamabad, Pakistan with a herbarium number PHM 485.

2.2. Preparation of crude extract

The plant was shade-dried at room temperature for 3 weeks. The dried material was comminuted by using a commercial miller to coarse powder with final dry weight of 700 g. The extracts were prepared by macerating 40 g of the powder separately in 14 different solvents of ranging polarity from highly non-polar to highly polar solvents including n-hexane (NH), chloroform(CH), acetone (AC), acetone + ethyl acetate (AC–EA), ethyl acetate (EA), chloroform + ethanol (CH–E), chloroform + methanol (CH–M), ethanol + ethyl acetate (E–EA), methanol + ethyl acetate (M–EA), ethanol (E), distilled water + acetone (Dw–AC), distilled water + methanol (Dw–M) and distilled water (Dw). Plant powder was soaked in separate Erlenmeyer flask for 3 days and occasionally mixed five times a day by using ultrasonic bath operated at a frequency of 40 KHz and 25 °C. Afterward the extract was filtered through filtration and residue was again dipped in respective solvent. This process was repeated thrice. Later on all the respective filtrates were combined and concentrated by rotary evaporator (Buchi, Switzerland) to obtain the crude extract (of each solvent) for further analysis.

2.3. Determination of extract yield

The percentage yield of the all extracts was determined gravimetrically using the dry weight of concentrate (a) and the plant dry weight (b) as follows: % yield = a/b

2.4. Phytochemical analysis

2.4.1. Total phenolic content estimation

The phenolic contents in the samples (4 mg/ml DMSO) were determined as described previously (Haq et al., 2012). Folin–Ciocalteu was employed as a reagent while gallic acid as a positive standard. Subsequent to 30 min incubation (incubator IC83 Yomato, Japan) of reaction mixtures containing 20 μl samples and 90 μl Folin–Ciocalteu reagent, 90 μl of sodium carbonate was added. Absorbance of the reaction mixture was measured at 630 nm using microplate reader (Biotech USA, microplate reader Elx 800) employing reagent as a blank. The same procedure was followed for the standard and the experiment was performed in triplicate. A calibration curve ($y = 0.0136x + 0.0845$, $R^2 = 0.9861$) was obtained in parallel under the same operating conditions using gallic acid (6.25–50 μg/ml) as a positive control and the resultant phenolics (TPC) were expressed as μg GAE (Gallic acid equivalent) per mg of extract.

2.4.2. Total flavonoid content determination

An aluminum chloride based colorimetric method (Haq et al., 2012) was used for total flavonoid content (TFC) estimation in the given samples. The determination was based on the formation of aluminum–chloride complex. Quercetin was used as positive control for plotting standard calibration curve. Briefly, after incubating a mixture containing 20 μl of each sample (4 mg/ml DMSO), 10 μl potassium acetate, 10 μl of 10% aluminum chloride and 160 μl of distilled water, absorbance was measured at 415 nm by using microplate reader. The calibration curve was drawn by using quercetin at final concentrations of 0, 2.5, 5, 10, 20 and 40 μg/ml. The resultant TFC was expressed as μg QE per mg of sample after triplicate analysis.

2.4.3. Quantitative evaluation by HPLC–DAD

HPLC–DAD analysis of *A. scoparia* extracts for polyphenols was carried out by following the previously described protocol (Jafri et al., 2014). Samples were dissolved in