Saudi Pharmaceutical Journal xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Phytochemical contents and biological evaluation of *Ruta chalepennsis* L. growing in Saudi Arabia

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ARTICLE INFO

Article history:
Received 9 December 2017
Accepted 5 February 2018
Available online xxxx

Keywords:
Phytochemical analysis
Medicinal plants
Ruta chalepensis
Antimicrobial
Antioxidant
Anticoagulant

ABSTRACT

Phytochemical screening of *Ruta chalepensis* L. exhibited the presence of different chemical groups. The dried aerial parts of the plant was total extracted by ethanol and successively using chloroform, ethyl acetate and Butanol, out of the successive extracts four compounds namely, scopletin, kaempferol, quercetin, quercetin 3-*O*-α-L-rhamno glucopyranosyl (Rutin) were isolated and biological evaluations. Total ethanol and successive extracts; chloroform, ethyl acetate and Butanol were produced excellent antimicrobial activities against gram negative bacteria, gram positive bacteria and fungi. Ethyl acetate extract was the best for inhibition of the microorganism's growth. All extracts (total ethanol, and successive extracts) showed DPPH radical scavenging activity in a concentration–dependent manner. The best antioxidant activity was obtained by ethyl acetate & *n*-butanol extract (94.28%, IC_{50} = 56.6 µg/ml). Also All extracts (total ethanol, and successive extracts) showed anticoagulant activity at higher concentration with prolonged clotting time 6:30 and 4:30 s at 10 mg/ml concentrations, respectively.

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1. Introduction

Infectious diseases caused by bacteria, fungi, viruses are a critical challenge to health and they are believed to be one of the main causes of increasing the rates of morbidity and mortality worldwide (Drusano, 2004). Numerous infections and disorders caused by bacterial and fungal pathogens including *Salmonella*, *Staphylococcus*, *Bacillus*, *Klebsiella*, *Proteous*, *Pseudomonas Aspergillus*, *Candida*, *Cryptococcus* and *Trichophyton* (Bibi et al., 2011). For several decades, natural remedies and medicinal plants were the main, and in fact the only, resource for the physicians. Until the present, most of the people, especially in developing countries, depend on plants for medicines (Amabye and Shalkh, 2015).

The significance of plants to homeopathy and modern medicine is correlated to their chemical constituents such as such as terpenoids, phenolics, alkaloids, flavonoids, amino acids, saponins, glycosides, diterpenes, triterpenes and their compatibility with the human body. It is expected that more than 30% of the worldwide sales of drugs is based mainly on plant products (Patwardhan et al., 2004, De Fatima et al., 2002). Plants of the family Rutaceae are a source of huge variety of natural products with antibacterial, antifungal, antioxidant, spasmolytic, antihelmintic, emmenagogue, antitumoral, analgesic, anti-inflammatory, and antidepressant activities (Raghav et al., 2006, Di Stasi et al., 2002, Zeichen de et al., 2000, Atta and Alkofahi, 1998).

Ruta chalepensis (Rue) is an aromatic evergreen shrub which is originally from the Mediterranean region and is at present distributed worldwide (Akkaria et al., 2015). In many countries, it is cultivated for its pharmacological and biological activity and it is widely used for treatment of gastric, diuretic, inflammation, headache and rheumatism disorders. Analysis of the chemical constituents of *R. chalepensis* extracts revealed that the aerial parts contain alkaloids, phenols, flavonoids, amino acids, saponins and furocoumarins (Kacem et al., 2015). The present study was conducted for determination of the phytochemical composition and the antimicrobial, anticoagulant, and antioxidant activities of different of *Ruta chalepensis*.

2. Material and methods

2.1. Phytochemical contents

2.1.1. Plant material

Ruta chalepennsis L. was collected in March 2016 from Jizan province, KSA. The plant was identified by Dr. Ahmed Al-Farhan;

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Peer review under responsibility of King Saud University.

https://doi.org/10.1016/j.jsps.2018.02.008
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Please cite this article in press as: Alotaibi, S.M., et al. Phytochemical contents and biological evaluation of *Ruta chalepennsis* L. growing in Saudi Arabia. Saudi Pharmaceutical Journal (2018), https://doi.org/10.1016/j.jsps.2018.02.008

Professor of Plant Taxonomy, College of Science, King Saud University. For phytochemical analysis and biological activities, the aerial parts of the plant were air-dried, grounded to powder, packed and stored in a clean, tightly, and closed container.

2.1.2. Phytochemical screening

The plant powder of *Ruta chalepennsis* L. was subjected to preliminary phytochemical screening for determination of its contents of biologically active phytochemical groups according to the method described by Ayoola et al. (2008).

2.1.3. Plant extraction

The powdered plant materials (2 kg) were percolated in 3 L of ethanol (95%) for 3 days. The obtained solvent was filtered by cotton piece and the marks lift was re-extracted for 4 times by the same way (Awaad et al., 2016). The total alcohol extracts were concentrated using rotatory evaporator at low temperature. The obtained alcohol free gummy residue (157 g) was dissolved in boiled-water and filtered using piece of cotton, the non-filtered part (non-polar materials chlorophyll and fatty matters) was removed.

The filtered (aqueous) was re-extracted successively till exhaustion using, ether, chloroform, ethyl acetate and *n*-butanol (water-saturated) respectively. Each extract was passed over an anhydrous sodium sulphate then concentrated using reduced pressure, at low temperature, and residues; 8.3, 10.6 and 30.5 g, were obtained from chloroform, ethyl acetate and *n*-butanol, respectively.

2.1.4. Isolation and purifications

The obtained successive extracts were chromatographically investigated on pre-coated silica gel GF plates using the following three solvent systems; (a) (Benzene- ethyl acetate 86:14 v/v), (b) (Chloroform- methanol 96:4 v/v), and (c) (Ethyl acetate-Methanol- water 30: 5: 4 v/v/v). Visualization of the spots was carried out under UV- light before and after spraying of TLC with $AlCl_3$ and $SbCl_3$.

Ether & Chloroform extract were collected together (8.3) and symbolized as **D-1**. Also ethyl acetate and *n*-butanol (41.19 g) are collected together and symbolized as **D-2** (Based on similarity of spots (colour, number and R_f).

The fractions D-1 and D-2 were subjected to further chromatographic investigation to isolate and identify their active compound (s) as following:

For isolation of compound(s) from D-1, five grams were applied onto the top of a glass column (120 × 2 cm) packed with 150 g silica gel G, eluted using system chloroform-methanol (95: 5), and 100 fractions (50 ml each) were obtained. All fractions were concentrated under reduced pressure, chromatographically screened on TLC, and reduced (according of number, colour and R_f of spots) to three sub-fractions; D-1-A (2.1 g), D-1-B (1.7 g), and D-1-C (0.5 g). The sub-fraction D-1-C (showed many spots with very pale colour) was ignored.

Sub-Fraction D-1-A (2.1 g) was applied onto top of a glass column (100 × 1.5 cm) packed with 60 g silica gel, eluted with chloroform-methanol (98: 2, v/v), ninety fractions (40 ml each) were collected, concentrated under reduced pressure. A semi-purified compound was obtained; purified using re-crystallization from methanol and compound **(R1)** was isolated.

Sub-Fraction D-1-B (1.7 g) was applied onto top of a glass column (80 × 1 cm) packed with 50 g silica gel, eluted with chloroform-methanol (97: 3, v/v), 30 fractions (30 ml each) were collected, dried from the solvent, re-crystallized (dissolved in methanol), and compound **(R2)** was isolated.

For isolation of compound(s) from D-2, twenty grams were dissolved in methanol, applied onto the top of a column (150 × 5 cm) packed with 200 g Sephadex LH-20, and eluted with methanol. A hundred fractions (100 ml each) were obtained and according of number, colour and R_f of spots were reduced to two sub-fractions; D-2-G (3.7 g) and D-2-H (2.7 g). For isolation of compound(s) from D-2-G, 3.5 g was applied onto top of a glass column (100 × 1.5 cm) packed with 90 g silica gel G, eluted with chloroform-methanol (92:8, v/v), 50 fractions (60 ml each) were obtained, dried from solvent, and compound **R3** was isolated.

For isolation of compound(s) from D-2-H, 2.5 g was applied onto top of a glass column (100 × 1.5 cm) packed with 90 g silica gel G, eluted with chloroform (polarity was gradually-increased with ethyl acetate and methanol), 40 fractions (60 ml each) were collected and chromatography examined using TLC and ethyl acetate- Methanol- water 30: 5: 4, v/v/v. A semi-purified compound was obtained; purified using recrystallization from methanol, dried from solvent, and compound **R4** was isolated.

2.2. Biological evaluations

2.2.1. Antimicrobial activity

2.2.1.1. Test organisms. Strains of microorganisms; namely, *Escherichia coli* (RCMB 010,052), *Klebsiella pneumonia* (RCMB 003-1), *Proteous vulgaris* (RCMB 004-1), *Pseudomonas aeruginosa* (RCMB 0,100,243-), *Salmonella typhimurium* (RCMB 006-1), *Bacillus substilis* (RCMB 015-1), *Staphylococcus aureus* (RCMB 010,010), *Staphylococcus epidermidis* (RCMB 009-2), *Streptococcus mutans* (RCMB 017-1), *Stroptococcus pyogenes* (RCMB 101,001,742), *Aspergillus fumigatus* (RCMB 002,008), *Aspergillus niger* (RCMB 002,005), *Candida albicans* (RCMB 005,003), *C. tropicalis* (RCMB 005,004), *Cryptococcus neoformans* (RCMB 0,049,001), *Geotricum candidum* (RCMB 05,097), *Penicillium expansum* (RCMB 001,001-2), and *Syncephalastrum racemosum* (RCMB 0,016,001-1) were provided from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt and used as test organisms.

2.2.1.2. Antimicrobial assay. The antimicrobial activity of ethanol and collected successive extracts **D1** and **D2** of *Ruta chalepennsis* was determined using well-diffusion method (Zain et al., 2012). Petri plates containing 20 ml of nutrient or malt extract agar medium were seeded with 1–3 day cultures of microbial inoculums. Wells (6 mm in diameter) were cut off from agar and 50 μl of the plant extracts were separately added, in a concentration of 100 mg/ml, and incubated at 37 °C for 24–48 h and 3–5 days for bacterial and fungal strains, respectively. The antimicrobial activity was determined by measurement of the diameter of the inhibition zone around the well.

2.2.1.3. Determination of minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) was determined by well-diffusion method (Zain et al., 2012). The MIC of *Ruta chalepennsis* extracts was determined using twofold dilutions for concentrations from 0.0 to 10 mg/ml. Wells (6 mm in diameter) were cut off from agar and 100 μl of each concentration of the plant extracts were separately added and incubated at 37 °C for 24–48 h and 3–5 days for bacterial and fungal strains, respectively. The lowest concentration (highest dilution) of the plant extract that produced no visible microbial growth (no turbidity) when compared with the control tubes were considered as MIC.

2.2.2. Antioxidant activity (DPPH (1-diphenyl-2-picrylhydrazyl) radical-scavenging assay)

The antioxidant activity of ethanol and collected successive extracts D1 and D2 of *Ruta chalepennsis* was determined using the DPPH free radical scavenging assay according to the method described by Yen and Duh (1994). Freshly prepared (0.004%w/v) methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

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