Saudi Pharmaceutical Journal (2014) 22, 460–471

King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

Phytochemical and pharmacological study of *Ficus palmata* growing in Saudi Arabia

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Received 26 October 2013; accepted 14 December 2013
Available online 25 December 2013

KEYWORDS

Ficus palmata;
Isolation;
Hepatoprotective;
Nephroprotective;
Antiulcer;
Anticoagulant;
Antioxidant

Abstract Phytochemical study of the aerial parts of *Ficus palmata* utilizing liquid–liquid fractionation and different chromatographic techniques resulted in the isolation of a new isomer of psoralenoside namely, *trans*-psoralenoside (5) in addition to, one triterpene: germanicol acetate (1), two furanocoumarins: psoralene (2), bergapten (3), one aromatic acid vanillic acid (4) and the flavone glycoside rutin (6). Structures of the isolated compounds were established through physical, 1D- and 2D-NMR and MS data. The total extract and fractions of the plant were examined *in vivo* for its possible effects as hepatoprotective, nephroprotective, antiulcer and anticoagulant activities in comparison with standard drugs. Hepatoprotective activity was assessed via serum biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin. Tissue parameters such as non-protein sulfhydryl groups (NP-SH), malonaldehyde (MDA) and total protein (TP) were also measured. In addition to tissue parameters, nephroprotective effect was evaluated by measuring the serum levels of sodium, potassium, creatinine and urea. Histopathological study for both liver and kidney cells was also conducted. Antiulcer activity was explored by observing stomach

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.jsps.2013.12.010

lesions after treatment with ethanol. Whole blood clotting time (CT) was taken as a measure for the anticoagulant activity of the extract. Antioxidant activity of the total extract and fractions of the plant was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and ascorbic acid as standard.

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1. Introduction

Ficus is the genus of the family *Moraceae* that comprises about 800 species (Harrison, 2005). Most of the members of the family are very high trees, shrubs and rarely herbs often with milky juice (Hutchinson et al., 1958). There are five species of *Ficus* growing in Saudi Arabia; *Ficus vasta*, *Ficus carica*, *Ficus salicifolia*, *Ficus palmata* and *Ficus glumosa* (Migahed, 1996). A number of *Ficus* species are used in folk medicine as anti-tumor, anti-inflammatory and tonic medicament (Lansky et al., 2008; Kitajima et al., 1999) Microbial diseases such as epilepsy and jaundice (Noumi and Fozi, 2003; Betti, 2004), bronchitis, influenza whooping cough, tonsillitis, toothache, bacillary dysentery, enteritis and bruises are also reported to be treated by *Ficus* extracts. Antioxidant activities were also reported for *Ficus* extracts. (Abdel-Hameed, 2009; Çalişkan and Polat, 2011). The chemical review on genus *Ficus*, reveals the presence of sterols and/or terpenes (Kuo and Li, 1997; Kuo and Chaiang, 1999), coumarins (Chunyan et al., 2009), furanocoumarin glycosides (Chang et al., 2005), isoflavones (Li and Kuo, 1997), lignans (Li and Kuo, 2000) and chromone (Basudan et al., 2005).

2. Materials and methods

2.1. Plant materials

The plants of *F. palmate* Forsk. were collected in March, 2008 from the Agabat Tanoma in the kingdom of Saudi Arabia. The plant was identified by Dr. Mohammed Yusuf, Taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# 15362) has been deposited at the herbarium of the department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

2.2. General experimental procedures

Melting points were determined in open capillary tubes using *Thermosystem FP800 Mettler FP80* central processor supplied with *FP81 MBC* cell apparatus, and were uncorrected. Ultraviolet absorption spectra were obtained in methanol and with different shift reagents on a *Unicum Heyios* α UV–Visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a UltraShield Plus 500 MHz (Bruker) (NMR Unite at the College of Pharmacy, Salman Bin Abdulaziz University) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the internal standard TMS or residual solvent peak, the coupling constants (J) are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) were obtained using standard *Bruker* program. MS were obtained using Liquid Chromatography/Mass Spectrometer (*Quattro micro API*) equipped with a Z-spray electrospray ion source (*Micromass*®, *Quattro micro*™, *WATERS*). Silica gel 60/230–400 mesh (EM Science) and RP C-18 silica gel 40–63/230–400 mesh (Fluka) were used for column chromatography, while silica gel 60 F254 (Merck) was used for TLC.

2.3. Extraction, fractionation and purification

Dry leaves of *Ficus palmata* (1900 gm) were extracted with 95% ethanol to exhaustion and the solvent was evaporated under reduced pressure using rotary vacuum evaporator to obtain viscose extract. Equal volume of water was added and the resulted extract successively partitioned with petroleum ether (60–80 °C) (3 × 500), chloroform (3 × 500), ethyl acetate (3 × 400) and butanol (2 × 300). The solvents were evaporated to obtain 36.9, 9.7, 8.0 and 27.5 gm of petroleum ether, chloroform, ethyl acetate and butanol, respectively. The left aqueous layer was dried to give 33.4 gm.

A portion of petroleum ether layer (17.2 gm) was and chromatographed over silica gel column (400 gm, 5 cm i.d.). Elution the column with petroleum ether; petroleum ether: $CHCl_3$ (90:10–0:100) in a gradient system. On the bases of TLC behavior, similar fractions were pooled together affording three main fractions (A–C). Fraction A (2.6 gm) was further purified on silica gel column (50 gm, 2 cm i.d.) eluted with petroleum ether: $CHCl_3$ (90:10) followed by crystallization from MeOH afforded 45 mg of 1. A similar treatment of fraction B (3.2 gm) afforded 140 mg of β-sitosterol. Fraction C (2.7 gm) was rechromatographed over silica gel column eluted with petroleum ether: EtOAc mixtures in a gradient system resulted in the isolation of 75 mg of 2 and 14 mg of 3 after crystallization from MeOH.

Part of the chloroform layer (5 gm) was chromatographed over silica gel column (200 gm, 3 cm i.d.) eluted with petroleum ether: $CHCl_3$ (90:10) and polarity was gradually increased with $CHCl_3$ till 100% then MeOH was used in an increasing ratio. Fractions eluted with petroleum ether: $CHCl_3$ (85:15) (700 mg) afforded 40 mg of 2 on crystallization from MeOH. Fraction D eluted from the column with $CHCl_3$:-MeOH (75:25) was subjected to reversed phase RP-18 PTLC using MeOH:H_2O (70:30) as a developing system to yield 4 (5.5 mg).

The EtOAc layer (8 gm) was chromatographed over silica gel column. The column eluted with $CHCl_3$:MeOH:H_2O (100:0:0–40:60:6) and then with MeOH. Fraction E eluted from the column with $CHCl_3$:MeOH:H_2O (75:25:2.5) afforded 15 mg of 5 after crystallization from MeOH.

A portion of ButOH layer (2.5 gm) was chromatographed on medium pressure RP-18 silica gel column started with 5% ACN in H_2O, increasing polarity with ACN afforded 6.5 mg of compound 6.

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