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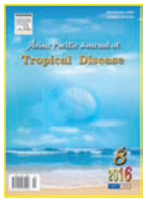

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Phenolic profiling of *Rorippa palustris* (L.) Besser (Brassicaceae) by LC-ESI-MS: Chemosystematic significance and cytotoxic activity

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ABSTRACT

Objective: To investigate the phenolic profile of *Rorippa palustris* (*R. palustris*) compared to Egyptian *Rorippa* species to evaluate their chemosystematic significance as well as screen cytotoxicity of *R. palustris* extract.

Methods: The chemical components of defatted hydroalcoholic *R. palustris* extract were analyzed using liquid chromatography-electrospray ionization mass spectrometry technique. The cytotoxic activity was evaluated by using MTT assay against four carcinoma cell lines.

Results: Sixteen compounds corresponding to flavonoids (kaempferol and quercetin derivatives), phenolic acids (gallic, coumaric and ferulic acids derivatives) and an anthocyanin (delphinine derivative) were identified or tentatively characterized, of which ten compounds were detected for the first time from *R. palustris*. Moreover, *R. palustris* extract showed a moderate activity against MCF7 and A549 cell lines at 100 mg/mL with cell viability of 47.3% and 65.4%, respectively.

Conclusions: The phenolic profile and the morphological characters of *R. paluatris* seem to be in relation with those of *Rorippa indica*, which can be considered as an indicative parameter for its medicinal importance.

1. Introduction

The genus *Rorippa* Scop. is one of the largest genera in the tribe Arabideae of family Brassicaceae, comprising approximately 80 species distributed worldwide except Antarctica[1,2]. It includes an important Chinese traditional herb *Rorippa indica* (L.) Hiern (*R. indica*), which is used as animal forage and treatment for fever, cough, rheumatism and inflammation[3]. In Egypt, the genus *Rorippa* is represented by three wild species: *R. indica*, *Rorippa palustris* (L.) Besser (*R. palustris*) and *Rorippa integrifolia* Boulos[4]. *R. palustris*, a species closely morphologically related to *R. indica*, is the widely distributed species of the genus, commonly named as bog yellowcress, yellow watercress and marsh yellowcress[5].

The chemical constituents of *Rorippa* species were found to contain phenolic acids, flavonoids, glucosinolates and isothiocyanate[6-10]. Some *Rorippa* species possess a highly antioxidant activities[11] and cytotoxicity against human MDA-MB-231 breast cancer cells[12]. Also, they have the ability to reduce lymphocyte DNA damage which could be related to a reduced risk of cancer[13]. In the present study, *R. palustris* was selected for further phytochemical investigation using liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) to evaluate its chemosystematic importance in relation to related taxa of Egypt.

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Additionally, the cytotoxic activity of its extract against breast (MCF7), hepatocellular (HEPG 2), lung (A549) and colon (HCT116) carcinoma cell lines was investigated.

2. Materials and methods

2.1. Plant material

R. palustris was collected and identified by Dr. Mona M. Marzouk from Bin El-Bahrin Island, near Pharos garden, Giza, in March 2009. A voucher specimen (No. M42) was deposited in the herbarium of the National Research Center (CAIRC).

2.2. Extraction

The air-dried powdered aerial parts of *R. palustris* were extracted three times with 70% MeOH. The solvent was evaporated under reduced pressure at 50 ˚C. The dried hydroalcoholic extract was defatted with petroleum ether[14].

2.3. Acid hydrolysis and paper chromatography

A total of 100 mg of *R. palustris* extract (RPE) was hydrolyzed with 10 mL hydrochloric acid (2 mol/L) at 100 ˚C for 2 h. The acidic solution was fractioned with 10 mL ethyl acetate after cooling. Ethyl acetate layer was dried with anhydrous Na_2SO_4 then evaporated. The hydrolyzed extract was subjected to one dimension paper chromatography (PC) Whatman No. 1 (Whatman Ltd., Maidstone, Kent, England) using solvent systems; 50% AcOH (H_2O: AcOH, 1:1) and BAW (*n*-BuOH–AcOH–H_2O 4:1:5, upper layer) to detect the aglycones. Also the aqueous layer was carefully neutralized, then subjected to PC investigation using BBPW (benzene: *n*-BuOH: pyridine: H_2O; 1:5:3:3, upper layer) to detect the sugars[14]. Flavonoid aglycones (Fluka AG, Bucns SG, Switzerland) and sugar samples (E. Merck, Darmstadt, Germany) were used as authentic references.

2.4. LC-ESI-MS analysis of RPE

LC-ESI-MS analysis system consists of high performance liquid chromatography (Waters Alliance 2695) and mass spectrometry (Waters 3100). The mobile phases were prepared daily by filtering through 0.45 μm membrane disc filter and degassed by sonication before use. The mobile phase for gradient elution consists of two solvents: solvent A (0.1% formic acid in H_2O) and solvent B (0.1% formic acid in CH_3CN/MeOH (1:1; v/v). The linear gradient profile was as follows: 95% A (5 min), 95%–90% A (10 min), 90%–50% A (55 min), 50%–95% A (65 min), and 95% A (70 min). The injection volume was 10 μL. The flow rate (0.6 mL/min) was split 1:1 before the MS interface. The negative ion mode parameters were as follows: source temperature 150 ˚C, desolvation temperature 350 ˚C, cone gas flow 50 L/h, cone voltage 50 eV, capillary voltage 3 kV, and desolvation gas flow 600 L/h. Spectra were recorded in the ESI negative mode between 50–1 000 *m/z*. The peaks and spectra were processed using the Maslynx 4.1 software[15]. Known peaks were identified by comparing their retention time and mass spectrum with the flavonoid standards (95% purity; UV, nuclear magnetic resonance) which obtained from this research group (Phytochemical and Plant Systematic Department, NRC)[10,16-21]. Other peaks were tentatively identified by comparing the mass spectrum with literatures.

2.5. Cell culture and sample treatment

The investigated human carcinoma cell lines were breast (MCF7), hepatocellular (HepG2), lung (A549) and colon (HCT116). They were purchased from American Tissue Culture Collection. HepG2, MCF7 and HCT116 cells lines were cultured in RPMI 1640 medium while A549 cell line was cultured in Dulbecco's modified Eagle's medium media. Media are supplemented with 1% antibiotic antimycotic mixture (10 000 IU/mL potassium penicillin, 10 000 μg/mL streptomycin sulphate and 25 μg/mL amphotericin B), 1% L-glutamine and 10% fetal bovine serum. According to the cells growth profile, cells were seeded with a density of 1×10^4 cell per well. This number was sufficient to give a reliable reading with the MTT assay, which corresponded well with the cell number and was the one that gave exponential growth throughout the incubation period with the tested sample[20].

3. Results

3.1. Acid hydrolysis

The PC of the ethyl acetate extract gave two spots which have R_f and color reaction similar to kaempferol and quercetin aglycones. Galactose, glucose, arabinose and rhamnose were detected as sugar moieties in the aqueous extract. Complete hydrolyzing indicated that all glycoside were in *O*-glycoside form.

3.2. Identification of phenolics using LC-ESI-MS analysis

Nineteen peaks were detected in RPE chromatogram (Table 1, Figure 1). The flavonoid contents were identified for peaks 1, 6–10, 13, 15, 17 and 18. The peaks 1, 9, 10, 17 and 18 were predicted to be kaempferol derivatives, while the others were related to quercetin aglycone.

Peaks 1, 9 and 10 presented the same molecular ion peak at *m/z* 593 provided the presence of kaempferol and a disaccharide residue (rhamnose and hexose). The hexose moiety was confirmed to be glucose or galactose by direct comparison with standards in the acidic hydrolyzed extract.

The flavonoid 1 (Rt = 19.90 min) showed fragments of *m/z* 447 (by loss of 146) and *m/z* 285 (loss of 162), suggesting that the rhamnose moiety was in a terminal position in the disaccharide residue, while the hexose one was directly attached to the aglycone (*i.e.* rhamnosyl-hexoside)[22]. It was further identified as kaempferol 3-*O*-β-(2"-*O*-α-rhamnopyranoside)-glucopyranoside by direct comparison with an authentic standard and previously isolated from *R. palustris*[10].

Another kaempferol isomers 9 (Rt = 33.20 min) and 10 (Rt = 34.48