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ORIGINAL ARTICLE

Phytochemical and pharmacological studies on *Scorzonera alexandrina* Boiss

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Abstract The present study was designed to investigate secondary metabolite contents of *Scorzonera alexandrina* growing in the North–Western coast of Egypt. In addition hepatoprotective activity, reduction in blood sugar and ulcerative colitis were also evaluated . Chromatographic methods and spectroscopic analysis were used for isolation and identification of the compounds. In the hepatoprotective activity; twenty-four adult male Wistar albino rats were divided into four groups of six animals each. Rats of the 1st (normal control) and 2nd (CCl_4-intoxicated control) groups received the vehicle in a dose of 5 mL/kg. The 3rd and 4th groups were treated with the ethanol extract of *S. alexandrina* in doses of 200 and 400 mg/kg, respectively. In the ulcerative colitis study, the same extract was administered orally (200 and 400 mg/kg) to rats once a day for 5 consecutive days and the last dose was administered 2 h before induction of colitis by intra-rectal infusion of acetic acid. The obtained results revealed that *S. alexandrina* contains scopoletin, xanthotoxin, apigenin, luteolin, quercetin-7-*O*-rhaminoside and luteolin-7-*O*-glucoside, also it showed a significant decrease in blood glucose level, liver functions (ALT, AST, TP), also a significant anti-ulcer effect.

1. Introduction

The genus *Scorzonera* encompasses about 160 species and being Ancient Mediterranean in origin, it is widely spread in arid regions of Eurasia and Africa, in Egypt, the genus *Scorzonera* is represented by five species, namely; *Scorzonera pseudolanata* Grossheim, *Scorzonera mollis* Bieb, *Scorzonera schweinfurthii* Boiss, *Scorzonera drarii* Tackh and *Scorzonera alexanderina* Boiss. The first four species are very rare in the Egyptian deserts while the last species is very common and endemic to the North–Western Coastal strip of Egypt. *S. alexanderina* is a small perennial herbaceous plant with tuberous edible root. The flowers are pale mauve, deep purple at the center emitting a nice scent of vanilla. It appears only on rainy seasons (Täckholm, 1974).

Previous chemical investigations of this genus yielded dihydro-isocoumarins, flavonoids, lignans, phenolic acids, sesquiterpene, sesquiterpene lactone, triterpenes and a new class of bibenzyl derivatives (Zidorn et al., 2003; San et al., 2007). In addition to utilizing the fresh leaves as an ingredient in green

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salad, its sub aerial parts are considered to be a potent tonic and continue to be used in traditional medicine as analgesic, antirheumatic, anthelmintic, curing fever, carbuncle, mastitis and treatment of fertility, also it is used as a diabetic diet for containing inulin and laevulin (Baytop, 1984).

A new friedoolean-type triterpene, 3β-acetoxyglutin-5(10)-en-6-oxo, together with seventeen known compounds were isolated from the roots of *Scorzonera austriaca* (Wu et al., 2011). Extracts prepared from the aerial parts of *Scorzonera cana* var. *jacquiniana*, *Scorzonera cana cinerea, Scorzonera cana eriophora, Scorzonera cana incisa* and *Scorzonera cana parviflora* showed a significant inhibitory effect on carrageenan and PGE2-induced hind paw edema model as well as on p-benzoquinone-induced abdominal constriction test (Akkol et al., 2012).

No phytochemical and biological studies have been reported for *S. alexandrina*. Therefore, the present study was designed to investigate the phytochemical and biological activities of this plant.

2. Material and methods

2.1. Plant material

S. alexandrina was collected in the wild form from the North–Western coast of Egypt during spring (2010) and a voucher specimen has been deposited in the herbarium of the Desert Research Center, Cairo-Egypt. The collected plant was dried under shade and then grinded to fine powder.

2.2. Extraction and isolation

About 500 g of the air dried powder of *S. alexandrina* (aerial and sub aerial parts) were extracted by aqueous ethanol. The combined ethanolic extracts were concentrated under reduced pressure at a temperature not exceeding 35 °C. The ethanolic extract of *S. alexandrina* (25 g) was separated into fractions on a silica gel (500 g) column and eluted with Chloroform (fractions 1–12), after that Chloroform–methanol (fractions 13–45). Fractions 1–12 (1.8 g) were reapplied to a silica gel column eluted with chloroform, from which compounds 1 and 2 were isolated. Fractions 13–45 (6.5 g) were reapplied to a second silica gel column and eluted with chloroform:methanol from this column, compounds 3, 4, 5 and 6 were isolated.

2.3. Pharmacological studies

2.3.1. Experimental animals

Male Wistar albino rats (220–240 g) and albino mice of both sexes (25–28 g) were maintained in the Laboratory Animal Unit of the College of Pharmacy, Salman Bin Abdulaziz University. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. Animals were allowed to adapt to the laboratory environment for one week before experimentation. The care and handling of the animals were in accordance with the internationally accepted standard guidelines. All animal procedures were approved by an institutional review board of Pharmacy College, Salman Bin Abdulaziz University, KSA.

2.3.2. Acute toxicity experiment

Albino mice were divided into control and test groups (6 animals each). Control group received the vehicle (3% Tween 80) while the test groups got graded doses (1000–4000 mg/kg) of *S. alexandrina* ethanol extract orally and were observed for mortality till 48 h and the LD_{50} was calculated (Ghosh, 1994).

2.3.3. Effect of prolonged administration

Eighteen male Wistar albino rats were randomly divided into 3 groups of 6 animals. The 1st group was kept as control (5 mL/kg of 3% Tween 80), while 2nd and 3rd groups were administered the ethanol extract of *S. alexandrina* in doses of 200 and 400 mg/kg, respectively. All medications were administered orally with the aid of an orogastric cannula for 35 consecutive days. Rats were maintained under identical conditions with food and water *ad libitum* for the entire period with close observation. At the end of the experimental period, blood samples (2 mL) were drawn by puncturing retro-orbital venous sinus of each rat (under ether anesthesia) and centrifuged at 10,000 rpm for 5 min. Sera were separated to be used for the biochemical estimations.

2.3.4. Experimental induction of hepatic damage

CCl_4 was dissolved in corn oil in the ratio 1:1 v/v. Liver damage was induced in rats following subcutaneous (SC) injection of CCl_4 in the lower abdomen at a dose of 3 mL/kg (Theophile et al., 2006).

2.3.5. Hepatoprotective activity

Twenty-four adult male Wistar albino rats were randomly divided into four groups of six animals each. Rats of the 1st (normal control) and 2nd (CCl_4-intoxicated control) groups received the vehicle in a dose of 5 mL/kg. The 3rd and 4th groups were treated with the ethanol extract of *S. alexandrina* in doses of 200 and 400 mg/kg, respectively. All medications were administered orally by gastric intubation for 7 consecutive days. Two h after the last dose, normal control rats were given a single dose of corn oil (3 mL/kg, SC), while animals of the 2nd–4th groups received a single dose of CCl_4 (3 mL/kg, SC).

2.3.6. Measurement of liver and kidney function markers

Liver functions were evaluated by measuring the serum activity of alanine transaminase (ALT) and aspartate transaminase (AST), following the method of Reitman and Frankel (1957). The serum concentrations of total bilirubin (TB) (Walter and Gerarade, 1970), total protein (TP) (Henary et al., 1974) and albumin (Alb) (Doumas et al., 1971) were estimated. Serum levels of urea (Wills and Savory, 1981) and creatinine (Kroll et al., 1987) were determined colorimetrically as measures of kidney functions.

2.3.7. Measurement of blood glucose level

Determination of the blood glucose level was done by the glucose-oxidase principle (Rheney and Kirk, 2000), results were reported as mg/dl.

2.3.8. Effect on ulcerative colitis

The total ethanolic extract of *S. alexandrina* and the standard; dexamethasone (DEX) was suspended separately in 3% v/v Tween 80 (vehicle).

Thirty male Wistar albino rats were divided into 5 equal groups. Groups 1 and 2 (normal and colitis control groups,