statutory requirements of the AERB, GOI and the biological waste was disposed according to the IAEC, NIPER and AERB, GOI protocols. Skin penetration and permeation studies were performed according to protocols which were previously reported (Panchagnula et al. 2004). Ex vivo skin permeation studies were performed ($n = 4$) with unjacketed vertical type Franz diffusion cells with a diffusion surface area of 0.785 cm^2 and 5.2 ml of receptor cell volume, stirred at 800 rpm using a dry block heating and stirring module. Receptor compartments were filled with fluids containing binary combinations of EtOH and PBS (1 : 1, 1 : 2, and 1 : 3). In order to attain ~32 °C at skin surface, receptor phase was maintained at 37 ± 0.5 °C (Qvist et al. 2000). Frozen skin pieces were thawed to room temperature and mounted over diffusion cells with dermal-side in contact with receptor phase, equilibrated for 3 h, and then air bubbles were removed. Subsequently, donor compartments were filled with 300 l of ^3H_2O, and covered with laboratory film to prevent evaporation of donor phase. Samples (200 µl) were withdrawn until 72 h, at specified intervals from receptor compartment, followed by replacement with fresh receptor solution. At the end of the study drug was quantified in receptor samples by radiochemical method.

Transdermal epidermal weight loss (TEWL) was determined after equilibrating skin for 10 h. TEWL was measured up to 84 h with various receptor phases containing increasing concentration of EtOH in PBS, by placing the collared probe on the receptor compartment. Always the standard deviation of measurement was 2% of mean values collected over the previous 20 s. All measurements were performed in a single ventilated room having controlled temperature between 28 and 30 °C, and relative humidity of 38–40%. Vehicle influence on skin barrier was studied after pre-treatment with neat vehicle for 4 h. For this purpose, skin was first equilibrated with optimized receptor phase containing EtOH : PBS 1 : 3 for 12 h. After that, vehicles (500 µl) were applied for 4 h and then wiped carefully with tissue paper. Subsequently, ^3H_2O was applied (300 µl) and its permeation was studied for 48 h using the procedure mentioned above. Flux values (µg cm^{-2} h^{-1}) were calculated from slopes of steady state portion of the Cartesian plots constructed between cumulative amounts of water permeated (µg cm^{-2}) as ordinate and time (h) as abscissa. Lag times (h) were calculated by extrapolation of the steady state portion to abscissa. Flux and lag time were compared between various treatments using unpaired t-test at $P = 0.05$ (Sigmastat 2.03, SPSS Inc., USA).

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Inhibitory effects on the digestive enzyme α-amylase of three *Salsola* species (Chenopodiaceae) *in vitro*

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Hypoglycaemic effects of *Salsola kali*, *S. soda*, and *S. oppositifolia* (Chenopodiaceae) aerial parts were examined using *in vitro* assay based on the inhibition of α-amylase. The *S. kali* ethyl acetate fraction was the most active with a IC_{50} value 0.022 mg/ml. Through bioassay-guided fractionation processes two flavonol glycosides, isorhamnetin-3-*O*-glucoside and isorhamnetin-3-*O*-rutinoside, were isolated by silica gel column chromatography and characterized by spectroscopic methods. Isorhamnetin-3-*O*-rutinoside showed an interesting activity (IC_{50} 0.129 mM).

The plant kingdom is a wide field to search for natural effective oral hypoglycaemic agents that have slight or no side effects. More than 1200 plant species have been recorded to be used empirically for their alleged hypoglycaemic activity (Marles and Farnsworth 1995).

One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise. A number of naturally occurring reversible glycosidase inhibitors are known such as nojirimycin, castanospermine, swainsonine, and acarbose, and these have been subjected to intensive study including the synthesis and testing of a number of analogues. *Salsola* species are well known in traditional medicine (Woldu and Abegaz 1990). In Russia *S. collina* was one component of the biologically active food additive "Heparon" recommended as a hepatoprotector when the hepatic cells are exposed to alcohol, medications and various toxins. It has also an anti-inflammatory and mild cholagogue effect. Aqueous extracts of *S. tuberculatiformis* have been used by bushmen women as an oral contraceptive (Swart et al. 2003). *S. soda* has been used as a food plant; the main edible parts are the buds. Chemical constituents of members of the genus have been studied to some extent. *S. kali* are reported to contain tetrahydroisoquinoline alkaloids such as salsoline and salsolidine, fatty acids, and flavonoids (List and Hörhammer 1969–1979; Tomàs

et al. 1985). The phytochemical screening and biological activity testing of *S. oppositifolia* have to the authors' present knowledge not been carried out before. Just one study evaluated the content of protein, lipid and neutral sugars in nine species of Egyptian *Salsola*, including *S. oppositifolia* (Turki 2000). The aim of the present study was to evaluate the hypoglycaemic activity of *Salsola kali, S. soda*, and *S. oppositifolia* by the *in vitro* assay for the inhibition of the digestive enzyme α-amylase.

In order to evaluate the ability of α-amylase inhibition of *Salsola* species, methanolic extracts were prepared and tested *in vitro* (Table 1). The methanolic extract of all *Salsola* species possessed a significant inhibition activity against α-amylase with a IC_{50} value ranging from 0.655 and 0.217 mg/ml.

The methanolic extract was subjected to a bioassay-guided fractionation process. As the first fractionation step, the methanolic extract was consecutively partitioned with *n*-hexane, dichloromethane, ethyl acetate, and diethyl ether. The methanolic extract and *n*-hexane, dichloromethane, ethyl acetate and diethyl ester fractions content data are shown in Table 2. The activity of each fraction was studied using α-amylase inhibition assay at concentration ranging from 1 mg/ml to 0.0156 mg/ml. The *n*-hexane extracts of *S. kali*, *S. oppositifolia*, and *S. soda* showed an IC_{50} ranging from 0.316 to 0.756 mg/ml. Comparing the dichloromethane extracts activity of three *Salsola* species, *S. kali* showed an interesting IC_{50} value of 0.048 mg/ml; this value is quite the same as that of the positive control. Just *S. oppositifolia* and *S. kali* diethyl ether extracts inhibited α-amylase with IC_{50} values of 0.399 and 0.591 mg/ml, respectively.

Table 1: α-Amylase inhibitory activity (IC_{50}) of *S. oppositifolia*, *S. kali* and *S. soda* extracts and isolated compounds

Extracts and isolated compounds	IC_{50} (mg/ml)
S. oppositifolia extracts	
Methanol	$0.655 \pm 1.5^{**}$
n-Hexane	$0.482 \pm 1.8^{**}$
Dichloromethane	$0.587 \pm 1.4^{**}$
Ethyl acetate	$0.193 \pm 1.2^{**}$
Diethyl ether	$0.399 \pm 1.2^{**}$
S. kali extracts	
Methanol	$0.217 \pm 1.8^{**}$
n-Hexane	$0.316 \pm 1.4^{**}$
Dichloromethane	$0.048 \pm 0.2^{\wedge}$
Ethyl acetate	$0.022 \pm 0.2^{**}$
Diethyl ether	$0.591 \pm 1.7^{**}$
S. soda extracts	
Methanol	$0.623 \pm 1.6^{**}$
n-Hexane	$0.756 \pm 1.2^{**}$
Dichloromethane	$0.177 \pm 0.2^{**}$
Ethyl acetate	$0.028 \pm 0.5^{**}$
Diethyl ether	–
Isolated compounds	IC_{50} (mM)
Isorhamnetin-3-*O*-glc	$0.619 \pm 0.9^{**}$
Isorhamnetin-3-*O*-rut	$0.129 \pm 0.7^{**}$

Acarbose was used as positive control (IC_{50} 0.025 ± 0.002 mM). The α-amylase inhibitory activity (%) was defined as the percent decrease in the maltose production rate over the control. Data are given as the mean of at least three independent experiments ± S.D. Differences within and between groups were evaluated by one-way analysis of variance (ANOVA) test completed by a with a multicomparison Dunnett's test
** $p < 0.01$; ^ $p > 0.05$ compared with the control experiment

Table 2: Yield % in comparison to the weight of the aerial parts of methanolic extracts and fractions of *Salsola* species

Extract and fractions	Yield (%) ± S.D.		
	S. kali	*S. soda*	*S. oppositifolia*
Methanol	10.81 ± 0.033	7.61 ± 0.025	22.17 ± 0.042
n-Hexane	0.70 ± 0.015	0.76 ± 0.026	1.07 ± 0.034
Dichloromethane	0.23 ± 0.007	0.13 ± 0.009	0.49 ± 0.021
Ethyl acetate	0.27 ± 0.005	0.22 ± 0.023	0.61 ± 0.018
Diethyl ether	0.11 ± 0.013	0.03 ± 0.002	0.17 ± 0.012

Results are shown as mean ± S.D., Standard deviation (n = 3)

The ethyl acetate fractions obtained from all species exhibited the highest activity. Among them, the *S. kali* ethyl acetate fraction showed an IC_{50} value of 0.022 mg/ml. For this reason, this fraction was selected for further fractionation processes to isolate the inhibitors. Two compounds were isolated by SiO_2 column chromatography and identified as isorhamnetin-3-*O*-glucoside (**1**) and isorhamnetin-3-*O*-rutinoside (**2**) by comparing their spectral data to those previously reported. Compound **1** inhibited α-amylase with a IC_{50} of 0.619 mM, while compound **2** showed an IC_{50} of 0.129 mM. Structure-relationship studies revealed that the rhamnoglycoside moiety plays an important role in the interaction with the enzyme.

Isorhamnetin-3-*O*-glucoside, isolated from *Salicornia herbacea* (Chenopodiaceae), have been reported to possess inhibitory effects on rat lens aldose reductase (RLAR) and sorbitol accumulation in streptozotocin-induced diabetic rat tissues (Lee et al. 2005). In particular, the flavonol exhibited a potent RLAR inhibition *in vitro*, its IC_{50} being 1.4 μM. A recent study demonstrated the high inhibitory potency against α-amylase of some flavonoids, such as luteolin and luteolin-7-*O*-glucoside (Funke and Melzig 2005). Moreover, due to the presence of aromatic hydroxyl groups, flavonoids have strong antioxidant properties. They are scavengers of reactive oxygen and nitrogen species and, therefore, inhibit peroxidation reactions. It was reported that a flavonoid, (−)-epicatechin, protects normal rat islets from inducing-diabetes alloxan, normalizes blood glucose levels and promotes β-cell regeneration in islets of alloxan-treated rats (Chakravarthy et al. 1982).

Hyperglycaemia results in the generation of free radicals which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation. Increased formation of glucose-derived and increased glucose flux through the polyol pathway belongs to the biochemical mechanisms of hyperglycaemia-induced tissue damage (Andallu and Varadacharyulu 2003).

In order to establish a correlation with the hypoglycaemic activity of *S. kali* ethyl acetate fraction, isorhamnetin-3-*O*-glucoside and isorhamnetin-3-*O*-rutinoside concentrations were determined as 0.9% w/w and 3.5% w/w, respectively. The significant activity of *S. kali*, in relation to the percentage of two flavonols in the ethyl acetate fraction, suggests the existence of additional hypoglycaemic components in this fraction acting synergistically. Our studies will continue in the characterization of compounds in the fractions that have shown more activity.

In conclusion, the present work indicates for the first time that the methanolic extract, fractions and pure compounds from three *Salsola* species could be useful in lowering the post-prandial enhancement of blood glucose levels by inhibiting α-amylase activity. Particularly important are the results on the inhibition of α-amylase shown by the *S. kali* ethyl acetate fraction. This investigation reveals the potential of *S. kali*, *S. soda* and *S. oppositifolia* for use as a natural oral agent for reducing post-prandial glucose peak or

as a food supplement for people with a risk of onset of diabetes or obesity, since clinical studies demonstrated that administration of *S. kali* extracts is safe and well tolerated (Garde et al. 2005).

Experimental

^{1}H NMR, ^{13}C NMR, and 2D spectra were recorded on a Bruker 300 MHz spectrophotometer in deuterio-methanol. Chemical shifts are given in δ units. Purifications of bioactive constituents from *S. kali* were carried out by column chromatography (CC) using silica gel (Merck Silica Gel 0.040–0.063 mm diameter) as absorbent. Detection of spots was accomplished by visualizing the spots under UV light at 254 and 365 nm and by spraying with 50% v/v H_2SO_4 followed by heating for 5 min at 120 °C. Chemicals and reagents used for the study of hypoglycaemic activity were purchased from Sigma-Aldrich Co. (Milan, Italy); other chemicals, solvents and reagents used in chromatography were purchased from Merck, Italy.

The aerial parts of *S. kali*, *S. soda* and *S. oppositifolia* used in this study were collected in Calabria (2003) and authenticated by Dr. N. G. Passalacqua and Dr. L. Peruzzi of the Natural History Museum of Calabria and Botanical Garden of Calabria University, Italy. Voucher specimens were deposited in the Botany Department Herbarium at the University of Calabria (CLU), Italy.

Air-dried samples of aerial parts (200 g) were powdered and exhaustively extracted with methanol for 48 h (3 × 5 l) through maceration at room temperature. Combined methanol solutions of each species were concentrated under reduced pressure and dried, then suspended in distilled water and in sequence partitioned with *n*-hexane, dichloromethane, and ethyl acetate. The aqueous residue was alkalized with NH_4OH (up to pH 8) and extracted by diethyl ether no more alkaloids could be detected in aqueous phase (TLC, silica gel, eluent CH_2Cl_2–MeOH–NH_4OH 85 : 14 : 1, detection by Dragendorff's reagent). The organic portions were dried over anhydrous sodium sulphate and the solvent evaporated to dryness to obtain the four fractions (Table 2).

As shown in Table 1, the ethyl acetate fraction of all *Salsola* species exerted more hypoglycaemic activity than other fractions. Due to its activity, with the purpose to identify the active compounds, the ethyl acetate fraction of *S. kali* (540 mg) was subjected to column chromatography on silica gel 60 (20–45 μm; Merck, Germany) with gradient systems of chloroform-methanol (starting with $CHCl_3$–MeOH 95 : 5, ending with $CHCl_3$–MeOH 20 : 80) to afford isorhamnetin-3-*O*-glucoside (**1**, 5 mg) and isorhamnetin-3-*O*-rutinoside (**2**, 19 mg). The flavonol structures were elucidated using ^{1}H and ^{13}C NMR as well as 2D-NMR techniques and were found to be identical with those previously described (Slimestad et al. 1995; Sharaf et al. 1997).

α-Amylase inhibition determined according to Conforti et al. (2005). Briefly, *Salsola* species extracts and fractions were dissolved in buffer to give final concentrations ranging from 1 mg/ml to 0.0156 mg/ml, while pure compounds were dissolved in buffer to give final concentrations between 0.50 mg/ml and 0.062 mg/ml. To both control and plant samples starch solution (0.5% w/v) was added and left to react with α-amylase (EC 3.2.1.1) solution in alkaline condition at 25 °C. The generation of maltose was quantified every 3 min by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction (corresponding to colour change from orange-yellow to red) is detectable at 540 nm. In the presence of α-amylase inhibitors maltose should be reduced and the absorbance value would be decreased. Data are given as the mean of at least three independent experiments ± S.D. Differences within and between groups were evaluated by using one-way analysis of variance (ANOVA) test completed by a multicomparison Dunnett's test. Differences were considered significant at $p < 0.05$. The inhibitory concentration 50% (IC_{50}) was calculated from dose-response curve obtained by plotting the percentage of inhibition versus the concentrations by the Graph Pad Prism 4.0 software.

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