Antiglycation, Cytotoxicity and Phytotoxicity of Crude Extract of *Sonchus eruca*

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Abstract Three fractions methanol (SEM)**,** ethylacetate (SEE) and butanol (SEB) of *Sonchus eruca* were subjected to test against antiglycation, cytoxicity and phytotoxicity. In case of antiglycationbioassay, methanol fraction showed highest inhibitory activity 65.60 % fallowed by ethyl acetate 45.20 % and butanol 20.40 %. All three fractions did not show any significant cytotoxic activity. The ethyl acetate fraction exhibited significant phytotoxic activity.

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1. Introduction

Genus *Sonchus* belong to the family Asteraceae. It is the largest family of angiosperms comprises of over 1535 genera and 23000 species, distributed in three subfamilies and 17 tribes. The number of genera is rather increasing as more than 10 genera are described each year and several are resurrected from or reduced to synonymy (Bremer, K, 1994).

The genus Sonchus L. (Asteraceae) has a world wide distribution except for Central and South America (Heywood VH 1978). It is commonly considered to be related to Aetheorhiza Cass., Reichardia Roth and Launaea Cass (Stebbine, G. L., R., 1953 and Sell PD 1975). but it is a more isolated and distinct genus (Jeffrey, C 1966). Sonchus is represented with seven taxa in Turkey (Davis, PH 1988). some of which are used as a food in Turkey (Ali K, et al 2006). The genus *Sonchus*, representing 8 species in China, has long been used as folk medicine for the treatment of fever, stasis, and inflammation, as well as for detoxication and mobilization of blood circulation (Jiangsu New Medical College, 1976). Genus *sonchus* showed a marked reduction in its relative growth rate at elevated levels of ozone (A. A. EI-Khatib 2003). Flowers are perfect and generally self-incompatible. Perennial sowthistle can reproduce by seed and vegetatively is pollinated by insects including honeybees and other bees, hover flies, and blister beetles (Stevens OA 1924). Pollen grains of Asteraceae may be resolved into two major groups i.e., lophate and non-lophate grains (Qureshi SJ, et al 2002). Within the genus Sonchus,probably rising from Launaea (Saad, SI 1961). The most primitive taxa, included in the subg.Origosonchus Boulos, show pollen characters very similar to those of Launaea Saad, SI 1961 and Pons A, 1972).

2. Materials and Method

2.1 Plant materials

The plants *Sonchus eruca* was collected at Parachinar Kurram agency, N.W.F.P Pakistan, in July, 2008 and was identified by Plant taxonomist. The voucher specimen has been deposited in the Herbarium of our Botany department Kohat University of Science and Technology Kohat (KUST).The whole plants were air-dried for 10 days and milled into powder with electrical grinder and finally stored in airtight bottles before analysis.

The objective of the present study was to screen the active fractions of *S.eruca* and assay for antiglycation, cytotoxicity (Brine Shrimp Bioassay), and phytotoxicity (*Lemna* Bioassay), properties.

2.2 Extraction

The whole plant, including roots (3 kg) of *Sonchus eruca* was crushed and extracted x 3 with methanol (20 L each) at room temperature. The resulting methanol extract (155 g) was suspended in water and successively portioned to provide n-hexane (19 g), chloroform (57 g), and ethyl acetate (40 g) fractions.

2.3 Methodology Antiglycation

BSA (Bovine Serum Albumin) was used as 10 mg/ml, dissolved in 67 mM phosphate buffer (pH 7.4). Glucose as 50 mg/ml, dissolved in 67 mM phosphate buffer (pH 7.4). Sodium azide (3mM) was

added in phosphate buffer to inhibit bacterial growth. Each fraction (1 mg/1000 µl concentration) was used to calculate antiglycation activity along with standard inhibitor. The dissolved sample (60 ml in each well of 96-well plate) was incubated for a week at 37 °C. After a week, the samples were taken out and cooled at room temperature. Then 6 µl of 100% TCA (trichloroacetic acid) was added to each of the well. The samples were centrifuged at 14,000 rpm for 4 min. Pellets were obtained and supernatants were removed from each well. Then 60 µl of PBS (Phosphate Buffer Saline; pH 10), was added to dissolve the pellets. The fluorescence intensity was measured at 370 nm excitation and 440 nm emission, using Spectrofluorimeter (11,12,13) Rutin was used as the standard inhibitor (14) Percentage inhibition was alculated by the equation % inhibition = 100 – (OD (sample)/OD (blank) x 100

2.4 Brine shrimp lethality bioassay

Brine shrimp (*Artemia salina* larvae) eggs were hatched in a shallow rectangular plastic dish, filled with artificial seawater, which was prepared by mixing a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, OH, USA) with double distilled water (Meyer et al., 1982).An unequal partition was made in the plastic dish with the help of a perforated device. 50 mg of eggs were sprinkled into larger compartment, which was placed under the dark condition while the smaller compartment was opened to ordinary light. After two days naupils were collected. A sample of the test fraction was prepared by dissolving 20 mg of each fraction in 2 ml of methanol. From this stock solution, 1000, 100 and 10 µg/mL was transferred to 12 vials; three for each dilution, and three vials were kept as control having 2 ml of methanol only. The solvent was allowed to evaporate overnight. When shrimp larvae were ready, 1 ml of sea water was added to each vial along with 10 shrimps and the volume was adjusted with sea water to 5 ml per vial. After 24 h, the number of surviving shrimps was counted. Data were analyzed by a Finney computer program to determine the LD50 (Finney, D.J. 1971 and Meyer, B.N, 1982) Each experiment was replicated thrice.

2.5 Phytotoxicity bioassay

This test was performed according to the modified protocol of McLaughlin (McLaughlin, J.L, 1988). The test fractions were incorporated with sterilized E-medium at different concentrations i.e. 10, 100, 1000 µg/mL in methanol. Sterilized conical flasks were inoculated with fractions of desired concentrations prepared from the stock solution and allowed to evaporate overnight. Each flask was inoculated with 20 ml of sterilized E-medium and then ten *Lemna minor* each containing a rosette of three fronds were placed on media. Other flasks were supplemented with methanol serving as negative control and reference inhibitor i.e. Parquet serving as positive control. Treatment was replicated three times and the flasks incubated at 30°C in Fisons Fi-Totron 600 H growth cabinet for seven days, 9000 lux intensity, 56±10 relative humidity and 12 h day length. Growth of *L. minor* in fraction containing flask was determined by counting the number of fronds per dose and growth inhibition was calculated with reference to negative control (McLaughlin, J.L, 1988).

3. Result and Discussion

3.1 Antiglycation bioassay

Two fractions have shown good inhibitory potential *in vitro* at given concentration of 1 mg/1000 µL (Table 1). Methyl fraction (SEM) showed highest inhibitory activity (65.60%) at 1 mg/100µl followed by ethyl acetate (SEE) (45.20%) and butanol fractions (20.40%) at same concentration. We could not conduct IC50 studies due to unavailability of samples. However the current study showed that SEM fraction might contain our potential chemical compounds responsible for higher antiglycation activity. Our assumption is supported by one of the studies in which n-butanolic fraction of *Plantagoasiatica* had shown higher antiglycation activity (75%) which was actually due to the presence of a new compound, plantamajoside (Heywood, V.H).

3.2. Brine-shrimp lethality bioassay

The fractions obtained were determined for cytotoxicity in the brine-shrimp lethality bioassay by using the protocol of Meyer (Meyer, et al 1982) of these fractions were screened at three concentration levels i.e. 1000,100 10 /ml and LD50= values were calculated by using Finny computer program (Finney, D.J. 1971). Standard drug used was etoposide**.** All these three fractions did not show any significant cytotoxic activity

Results

Sample Code No	Conc. (mM)	% Inhibition	IC_{50}^{+} SEM [mM]
SEM	1 mg/100µl	65.60	-
SEE	1 mg/100µl	45.20	-
SEB	1 mg/100µl	20.40	-

Remarks: Fraction SEM and SEE have shown resonably good inhibitory potential against protein glycation in vitro and given concentration

Table 2. Mortality rates (%) of brine shrimps caused by treatments of *S.eruca* fractions.

Dose (µg/mL)	No. of Shrimps	SEM	SEE	SEB	STD.Drug	LD_{50}(µg/mL)
1000	30	20	18	17		
100	30	23	20	20	Etoposide	7.4625
10	30	25	28	27		

No. of Replicates: 03 **Remarks**: No Cytotoxicity of SEM, SEE and SEB

3.3 Phytotoxicity bioassay

The phytotoxicity of all fractions obtained from the crude methanolic extract was carried out against *L. minor*. This assay was performed at three different concentrations i.e.1000,100 and 10 µg/ml. S.eruca ethyl acetate (SEE) and methanol (SEM) fractions have

Table 3: Phytotoxic studies of various fractions of *Sonchus eruca* against *Lemna minor*

Conc. (µg/mL)	No. of Fonds				% Growth regulation			
	SEM	SEE	SEB	Control	SEM	SEE	SEB	Control
1000	18	18	18		88.5	100	40.5	
100	20	20	20		19.8	11.5	32.4	
10	22	22	22	16	-25.8	-11.7	17.3	0.015

Tremendous phytotoxic activity at highest dose while butanol fraction (SEB) showed significant activity at the highest dose (Table 3). In conclusion, *Sonchus eruca* exhibits a variety of biological activities, such as antiglycation, cytotoxicity and phytotoxicity in various organic fractions, with variable effects in different bioassays. The methanol fraction showed highest inhibitory potential in antiglycation bioassay which may be the potential fraction for characterization and isolation of targeted compounds, while ethyl acetate fraction showed significant antiglycation activity.The ethyl acetate fraction exhibited significant phytotoxic activity. Thus, the present study, provide an evidence for the therapeutic usefulness of the plant in a wide range of disorders.

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