Carbonic Anhydrase and Urease Inhibitory Effects of *Sonchus Asper*

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Abstract: In present research, an attempt was made to explore the pharmacological potential of *Sonchus asper*. The crude extract of *Sonchus asper* and its resultant fractions were tested for the carbonic annhydrase and urease inhibitory actions. The chloroform fraction of Sonchus asper exhibited maximum carbonic anhydrase II inhibition of 58.0%, followed by the plant crude extract, n-hexane fraction and aqueous fraction with 42.6, 30.2 and 21.3% inhibition respectively. In urease inhibitory bioassays, n-hexane fraction possesses highest urease inhibition capacity of 24.1%, followed by chloroform fraction (19.1%), crude extract (5.4%) and aqueous fraction (3.4%). These results indicate that *Sonchus asper* exhibits carbonic anhydrase and urease inhibitory activities. The higher carbonic anhydrase and urease inhibitory profile observed respectively for chloroform and n-hexane fractions of *Sonchus asper* propose concentration of respective active compounds, which warrants further detail studies for their isolation and molecular identification. [Ihsan Ullah Khan, Naser M. AbdEIslam, Farman Ullah Khan, Arif-Ullah Khan, Muhib Ullah, Syed Badshah, , Hidayatullah Khan, Riaz Ullah. **Carbonic Anhydrase and Urease Inhibitory Effects of *Sonchus Asper.*** *Life Sci J* 2013;10(1):2705-2707] (ISSN:1097-8135). http://www.lifesciencesite.com. 320

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1. Introduction

The genus *Sonchus* belongs to largest family of angiosperms namely, Asteraceae, which consist of almost 1535 genera and 23000 species (Bremer, 1994). In China, 8 species of the genus *Sonchus* has long been used as traditional remedy for the treatment of fever and inflammation as well as for purification and mobilization of blood circulation (Jiangsu, 1976). Studies on enzyme inhibition remain an important area of pharmaceutical research, since these studies have led to the discoveries of drugs useful in a variety of pathologies. The enzyme inhibitors can interact with enzymes and block their activity towards natural substrates. Carbonic anhydrase inhibitors, a group of medicines (acetazolamide) are well known for their diuretic effect (Ives, 2009). The urease inhibitors have recently attracted much attention as potential new anti-ulcer drugs. Ironically, urease was the first enzyme crystallized, but its mechanism of action is still largely misunderstood (Amtul et al., 2002). Unfortunately, few natural products have been discovered so far as carbonic anhydrase and urease inhibitors (Hussain et al., 2011), hence there is need to explore more inhibitors of aforementioned enzymes from natural resources. In this investigation, we reported that *Sonchus asper* crude extract and its resultant fractions exhibits carbonic anhydrase and urease inhibitory properties.

2. Material and Methods

Plant materials and extraction: The plant *Sonchus asper* was collected at Parachinar Kurram agency, KPK, Pakistan in June 2009 and was identified by a taxonomist Zafar Iqbal, lecturer in Botany Department, Kohat University of Science and Technology, Kohat, Pakistan. The voucher specimen# 105DBK has been deposited in the herbarium of Botany Department, Kohat University of Science and Technology, Kohat, Pakistan. The whole plant was air-dried in shade for 20 days, chopped and crushed into fine powder with electrical grinder. The powdered plant (4.0 kg) was initially extracted with methanol (10 days x 3) at room temperature with occasional shaking. It was filtered through a muslin cloth and then through a Whatman qualitative grade 1 filter paper (Williamson et al., 1998). This procedure was repeated thrice and the combined filtrate was evaporated on rotary evaporator to a concentrated greenish syrupy mass, the crude extract of *Sonchus asper,* yielding approximately 3.25%. Activity-guided fractionation

was carried out by using solvents of increasing polarity to obtain organic and aqueous fractions. The *Sonchus asper* crude methanolic extract was partitioned into n-hexane, chloroform and aqueous fractions.

Chemicals: The chemicals used include, dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), ethanol, lithium chloride ($LiCl_2$), phenol, sodium hydroxide, sodium hypochlorite (NaOCl), sodium nitroprusside and ureases were purchased from Sigma St. Loius, MO, USA. The solvents: methanol, n-hexane and chloroform were obtained from Merck, Darmstadt, Germany. All chemicals used were of the analytical grade available.

Carbonic anhydrase inhibitory assay: Carbonic anhydrase is a zinc containing metallo-enzyme, which catalyzes hydration of carbon dioxide and dehydration of carbonic acid. In carbonic anhydrase, zinc ion covalently bound to active site. If zinc removes in active site, apo carbonic anhydrase (apoCA) will obtain, which has no activity. The apoCA can show activity when Zn^{2+} is added to the reaction medium, which is proportional to Zn^{2+} added, which is basic principle of the method used (Kobayasshi et al., 1981). 20 µl of enzyme solution, 0.1 mg/2000 µl of deionize water, 2.22 units of enzyme, 20 µl of acetazolamide with 1 mM concentration and 1 mg/200 ml of plant material were incubated for 15 minutes. Then add 20 µl of substrate solution which was 0.6 mM, weigh 2.60 mg/2000 µl of ethanol and read the plate at 25ºC for 30 min at wavelength of 400 nm, through Spectrophotometer (Hussain et al., 2011).

Urease inhibition: For urease enzyme inhibitory activity, reaction mixtures comprising 25 µl of enzyme (Jack bean urease) solution and 55 µl of buffers containing 100 mM urea were incubated with 5 µl of each of the test sample respectively (1 mM) at 30°C for 15 minutes in 96-well plates. Urease inhibitory activity was determined by measuring ammonia production using the indophenol's method as described previously (Weatherburn, 1967). Briefly, 45 µl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a micro plate reader (Molecular Device, USA). All reactions were performed in triplicate in final volume of 200 µl. The results (change in absorbance per min.) were processed by using Soft Max Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M-$K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M LiCl). Percentage inhibition was calculated from the formula. % inhibition = 100-(OD test well/OD control) x 100.

3. RESULTS AND DISCUSSION

The *Sonchus asper* crude extract and subsequent fractions were evaluated for carbonic anhydrase and urease inhibitory activities, to explore its therapeutic potential in causing diuresis and healing of gastrointestinal ulcers. In carbonic anhydrase II inhibitory assay, among the tested plant samples, chloroform fraction was found to be most effective, exhibiting 58.0% inhibition, followed by crude extract (42.6%), n-hexane (30.2%) and aqueous fraction (21.3%) respectively (Table 1). Acetazolamide used positive control caused 85.5% inhibition, as shown in Table 1. The higher carbonic anhydrase inhibitory potential of chloroform fraction reveals that it might contain prospective carbonic anhydrase inhibitory compounds, which subject it to be of further interest for future detail studies. In case of urease inhibitory profile of *Sonchus asper,* n-hexane fraction showed highest efficiency, causing 24.1% inhibition, followed by chloroform fraction (19.1%), the plant crude extract (5.4%) and aqueous fraction (3.4%), as shown in (Table 2). Thiourea, a standard urease inhibitor (Abdulaziz et al., 2002) exhibited 21% inhibition (Table 2). The observations that urease have urea as a substrate, due to which urease specifically can only bind to a few inhibitors with a similar binding manner as urea. If a substrate attach with an atoms which has lower electro negativity and donates its electrons more effectively to the phenyl ring and thus, this probably positively affects the binding of the molecules to the active site of the enzyme (Ghous et al., 2010). Our results show that n-hexane fraction caused good inhibition as compared to rest of the plant material and even potent than reference agent, thiourea, which reveals that hexane fraction might have compound(s) with less electronegative substitution and may serve as the lead candidates for urease inhibition.

4. CONCLUSION

In conclusion, the results reveal that *Sonchus asper* crude extract and its polar and non-polar fractions exhibit various medicinal activities, such as carbonic anhydrase and urease inhibition, with different efficacies. Among the analyzed *Sonchus asper* samples, chloroform fraction showed highest potential against carbonic anhydrase II enzyme, while hexane fraction displayed significant urease inhibition, subjecting targeted fractions for isolation and characterization of active compounds, accounting for the observed effects. Thus, the current study evidenced the therapeutic usefulness of the plant, *Sonchus asper* in variety of health disorders.

Table- 1 Carbonic anhydrase II inhibitory effect of *Sonchus asper* crude extract and its various fractions

Sample	Concentration	% Inhibition
Crude extract	1mg/200µl	42.6
n-hexane fraction	1mg/200µl	30.2
Chloroform fraction	1mg/200µl	58.0
Aqueous fraction	1mg/200µl	21.3
Acetazolamide	1mM	85.5

Table- 2 Urease inhibitory effect of *Sonchus asper* crude extract and its various fractions

Sample	Concentration	% Inhibition
Crude extract	200µg/25µL	5.4
n-hexane fraction	200µg/25µL	24.1
Chloroform fraction	200µg/25µL	19.1
Aqueous fraction	200µg/25µL	3.4
Thiourea	-	21.0

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