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Full Length Research Paper

Antimicrobial and phytotoxic screening of various fractions of *Sonchus asper*

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Sonchus asper **is used in the treatment of many diseases ethnopharmacologically in Pakistan. In the present manuscript we demonstrate the antimicrobial and phytotoxic effects of various fractions of *S. asper.* Six (6) different bacteria (*Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, *klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) are used in the screening of extract as well as 4 strains of fungi are characterized. Raddish root inhibition assay was used for phytotoxic screening. Methanolic fraction of *S. asper* showed best activities in all of assays, in inhibition of microbes and phytotoxic activities. The potency of these activities is due to the presence of bioactive flavonoids, saponins and phenolic compounds.**

Key words: *Sonchus asper*, antimicrobial activity, phytotoxicity, *Escherichia coli*.

INTRODUCTION

For thousand years, mankind has learnt about the benefits of plant use to alleviate or cure diseases. Plants possess a number of bioactive compounds which are used as medicine or in preparation of new drugs. Nowadays, 30% of worldwide drugs are based on natural products isolated from medicinal plants (Grabley and Thiericke, 1999). However, for some decades, there has been increasing interest in plant uses and the detection of their constituents with antimicrobial properties. Many efforts have been made to extract new antimicrobial bioactive compounds from various kinds of sources. One of such resources is folk medicinal plants and extracted fractions of medicinal plants. Systematic screening of them may result in the discovery of novel effective compounds. According to WHO reports, most infectious disease has been controlled but still more than 40% deaths have been occurred due to infectious microbes in developing countries. In addition to diseases, preservation of food is becoming a more complex problem, due to new products introduction in market which require more protection against pathogenic microbes (Marino et al., 2001).

There is therefore a great interest for new methods of making food safe which have a natural or green image. One such possibility is the use of essential oils as anti-bacterial additives. Some of the antimicrobial compounds produced by plants are affective against plant and human pathogenic microorganisms (Mitscher et al., 1987). Anti-microbial agents, including food preservatives, have been used to inhibit food borne bacteria and increase the processed food shelf life. Many naturally occurring extracts from herbs, medicinal plants, and as well as from various spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage and pathogens (Bagamboula et al., 2003).

Recently scientist have focused to increase the crop production to meet the needed of world population, but unfortunately, crop yield losses every year due to insects and plant diseases caused by various pathogens, slow biodegradation of herbicides. To control these short-comings researchers have focused on allelochemicals and bio-herbicides, produced by plants themselves.

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Abbreviations: PMF, Proton motive force; **DMSO,** dimethyl sulfoxide; **MIC,** minimum inhibition concentration; **SAME,** *Sonchus asper* methanolic extract; **SAHE,** *Sonchus asper* n-hexane extract; **SAEE,** *Sonchus asper* ethyl acetate extract; **SACE,** *Sonchus asper* chloroform extract; **SABE,** *Sonchus asper* butanolic extract; **SAWE,** *Sonchus asper* water extract.

Sonchus asper (L.) Hill used in various human disorder including wounds and burns (Rehman, 2006; Qureshi et al., 2009; Hussain et al., 2008), cough, bronchitis and asthma (Ahmad et al., 2006; Koche et al., 2008), gastro-intestinal infection, inflammation, diabetis and cardiac dysfunction (Sabeen and Ahmad, 2009), kidney and liver disorders (Zabihullah et al., 2006; Rivera, and Oben, 1993), reproductive disorder like impotence (erectile dysfunction) in humans (Kareru et al., 2007), jaundice (Jan et al., 2009) and cancer (Sammon, 1997; Thomson and Shaw, 2002). *S. asper* contains flavonoids (Giner et al., 1993; Manez et al., 1994), glycosides (Shimizu et al., 1989; Helal et al., 2000), ascorbic acid and carotenoids, possess antioxidant, anticancer; anti-inflammatory properties (Guil-Guerrero et al., 1998). The present investigation was aimed to conform the traditional use of *S. asper* fractions as bacteriocidal, fungicidal and phytotoxic activities as not reported earlier.

MATERIALS AND METHODS

Plant collections

S. asper at maturity was collected from District Bannu (Pakistan) during the September 2007, identified and a specimen was submitted at herbarium of Pakistan, Quaid-i-Azam University Islamabad, Pakistan. All parts of the plant (leaves, stem, flowers, seeds and roots) were shade dried for two weeks, chopped, and grinded mechanically of mesh size 1 mm.

Preparation of plant extract

5 kg powder of *S. asper* was extracted in methanol to get crude methanolic extract (SAME). This crude extract was further fractionated with solvents of increasing polarity; starting from n-hexane (SAHE), ethyl acetate (SAEE), chloroform (SACE), butanolic (SABE) and water (SAWE). The reflux time for each solvent was 5 h. The fractions were cooled at room temperature, filtered and evaporated under reduced pressure in rotary evaporator. All the fractions were stored at 4°C for further *in vivo* investigations.

Phytotoxicity bioassay

This test was performed according to the modified protocol of McLaughlin (1998). Various fractions of *S. asper* were incorporated at different concentrations that is, 100, 1000 µg/mL in respective solvents in replicates. Radish seed was washed with dH_2O and then with 1 % mercuric chloride. Filter paper was put in each autoclaved petri plates. 5 ml of each fraction was poured in each plate and the respective solvent was evaporated. 10 seeds were placed in each plate and incubated in growth room for five days. After 3 days root and shoot inhibition was noted and again after 5 days. Fresh weight and dry weight was also recorded.

Antibacterial activities

The minimum inhibition concentration (MIC) of antibacterial activity was determined by agar well diffusion method. A loop full of a 104-106 suspension of 24 h old broth of each bacterium was streaked on the surface of Mueller- Hinton agar (BBl-USA) plates. Wells were dug in the agar with the help of sterile borer. Dilutions of the stock solution containing were prepared in DMSO and 100 µl of each dilution was added in the respective wells. The plates were then incubated at 37°C for 24 h and zone of inhibitions were measured in millimeters (mm) and compared with the control (Atta-ur-Rahman, 1991). Antibacterial activity of fractions of *S. asper* was studied against *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, *klebsiella pneumoniae*, *Staphylococcus aureus and Pseudomonas aeruginosa* using replicates. Roxithromycin and Cefixime-USP were used as standard drugs.

Antifungal assay

Antifungal activity was determined according the modified protocol of (Atta-ur-Rahman, 1991). Sabouraud dextrose agar (MERCK) was used to grow fungus for inoculums preparation. The samples for antifungal assay were prepared from initial stock of 12 mg/ml to get final concentration of 200 µg/ml. Solutions of terbinafine 12 mg/ml in DMSO were prepared for positive control. Pure DMSO was used as negative control. Test tubes were marked to 10 cm mark, put 4 ml media and autoclaved. Tubes were allowed to cool and were loaded with 67 µl (200µg/ml) from the stock solution. Tubes were then allowed to solidify in slanting position at room temperature and inoculated with 4 mm diameter piece of inoculums, taken from a seven days old culture of 4 fungal strains. Positive and negative control test tubes with DMSO and terbinafine were also inoculated. The test tubes were incubated at 28°C for 7 days. Cultures were examined twice weekly during the incubation and their % inhibition was calculated as: Percentage inhibition of fungal growth = [(100 - linear growth in test in mm) / (linear growth in control in mm)] × 100.

RESULTS

Phytotoxicity assessment

Phytotoxic (allelopathic) effects of *S. asper* were evaluated against reddish growth under control environmental condition in growth room. Various parameters were studied of allelopathy in this study as shown in Table 1. Root and shoot growth was markedly inhibited by SAME at both concentration when studied after three and 5th day of the experiment. Total fresh weight and dry weight of all groups were studied and found that SAME was most effective as compare to control and other fraction of *S. asper*, while growth of root and shoot was inhibited by SAME and SABE both at 3rd and 5th day of treatment both at 100 and 1000 ppm respectively.

Antibacterial assay (MIC)

The minimum inhibitory concentration of various fractions of *S. asper* was evaluated for antibacterial activity (Table 2). Gram negative bacteria such as *S. aureus* was inhibited by MIC value of SABE (5 µg/ml), SAME (2.5 µg/ml), SAWE (5 µg/ml), respectively. MIC for *E. coli* included SAME (5 µg/ml). However, MIC for *K. pneumoniae* growth was 1µg/ml SAHE and SAME and for SAEE it was (5 µg/ml). In case of gram positive bacteria SAME

Table 1. Phytotoxicity assay of various fractions of *Sonchus asper*.

Concentration	Samples	Root growth after 3rd day (cm)	Shoot growth after 3rd day (cm)	Root growth after 5th day (cm)	Shoot growth after 5th day (cm)	Total fresh weight (g)	Total dry weight (g)
Control	dH_2O_2	7.56 ± 0.03	6.98 ± 0.05	9.25 ± 0.08	9.27 ± 0.02	6.76 ± 0.08	2.14 ± 0.002
100 ppm	SAHE	1.20 ± 0.006	1.27 ± 0.003	2.7 ± 0.003	3.42 ± 0.005	1.09 ± 0.0004	0.155 ± 0.0005
1000 ppm	SAHE	1.51 ± 0.003	1.39 ± 0.007	2.9 ± 0.002	3.67 ± 0.005	1.6 ± 0.0003	0.195 ± 0.003
100 ppm	SAEE	0.85 ± 0.002	1.07 ± 0.0008	1.95 ± 0.0006	2.65 ± 0.0007	1.2 ± 0.0008	0.18 ± 0.003
1000 ppm	SAEE	0.99 ± 0.007	1.28 ± 0.0004	2.17 ± 0.002	5.15 ± 0.002	2.1 ± 0.0004	0.22 ± 0.002
100 ppm	SASC	0.66 ± 0.001	0.16 ± 0.0002	1.42 ± 0.004	0.98 ± 0.0008	0.57 ± 0.002	0.305 ± 0.008
1000 PM	SACE	0.86 ± 0.009	1.1 ± 0.0007	2.7 ± 0.003	3.2 ± 0.0006	2.2 ± 0.09	0.355 ± 0.001
100 ppm	SABE	0.093 ± 0.0004	0.11 ± 0.0001	0.13 ± 0.0007	0.06 ± 0.00004	0.55 ± 0.0002	0.09 ± 0.00003
1000 ppm	SABE	0.42 ± 0.005	0.35 ± 0.003	1.2 ± 0.0002	1.2 ± 0.0007	0.78 ± 0.0008	0.17 ± ± 0.0002
100 ppm	SAME	0.004 ± 0.00005	0.003 ± 0.00006	0.675 ± 0.0005	0.87 ± 0.0005	0.04 ± 0.0003	0.001 ± 0.00003
1000 ppm	SAME	0.009 ± 0.00008	0.007 ± 0.00005	1.5 ± 0.0001	2.1 ± 0.0002	0.08 ± 0.00001	0.002 ± 0.00005
100 ppm	SAWE	0.37 ± 0.004	0.526 ± 0.0034	1.02 ± 0.00021	1.23 ± 0.0009	158 ± 0.0006	0.16 ± 0.005
1000 ppm	SAWE	1.3 ± 0.02	1.126 ± 0.02	2.12 ± 0.002	3.2 ± 0.002	2.8 ± 0.005	0.35 ± 0.005

(2.5 µg/ml) showed MIC against *M. luteus*, while *B. subtilis* was inhibited by 1 µg/ml of SAME and SAEE however, SAME and SAEE inhibited the growth of *Enterobacter aerogenes* with MIC (5 µg/ml). All the remaining fractions did not inhibit the growth of the said negative and positive bacteria.

Antifungal assay

In the present study of antifungal assay, all the extracts of *S. asper* were evaluated against fungal strains as shown in Table 3. Growth of *Aspergillus niger* was inhibited more than 70% by SAME (91.1 ± 3.11%). In case of *Fusarium solani* SAEE (88.7 ± 2.12%), SACE (75 ± 3.21%) and SABE (93.75 ± 3.02%) markedly inhibited the growth of the said fungus. However, SAWE (12.5 ± 0.08%) showed inhibition. *Aspergillus flavus* was inhibited by SAME (91.1 ± 3.11) and SAWE showed less than 30% inhibition. SAEE (86.8 ± 2.98%) and SAME (88.15 ± 2.54%) inhibited the growth of *Aspergillus fumigatus*.

DISCUSSION

The results of our present screening assays justify the use of the investigated plants in the Pakistani ethnomedicine. The findings of our project shows that gram negative bacteria such as *S. aureus* was inhibited (MIC) by SABE (5 µg/ml), SAME (2.5 µg/ml), SAWE (5 µg/ml), while MIC of *E. coli* includes SAME (5 µg/ml). However, *K. pneumoniae* growth was inhibited (MIC) by SAHE, SAME (1 µg/ml) and SAEE (5 µg/ml). In case of gram positive bacteria, SAME (2.5 µg/ml) showed MIC against *Micrococcus luteus*, while *B. subtilis* was inhibited by SAME and SAEE (1 µg/ml), however LPBE, LPME, SAME and SAEE inhibited the growth of *E. aerogenes* (5 µg/ml). This indicates that the plant possesses antibacterial, antifungal and antitumor potential. SAME of *S. asper* showed activity against *E. coli. S. aureus* was inhibited by SABE, SAME and SAWE. *K. pneumoniae* was inhibited by SAHE, SAME and SAWE. *B. subtilis* was inhibited SAME and SAWE, while *M. luteus* and *E. aerogenes* was controlled by SAME and SABE. Narod et al. (2004) reported that anti-bacterial activity of hexane, methanol and water extract of leaf and stem of *Toddalia asiatica* were active against gram-negative and gram positive bacteria.

Duraipandiyan and Ignacimuthu (2009) reported similar results during screening of antibacterial activities of various fractions of traditional medicinal plant, *T. asiatica* (L.) Lam. Algiannis et al. (2001) proposed a classification based on MIC values of antifungal activities of extracts, where extracts with MIC up to 500 µg/ml are considered as strong inhibitors, 600 - 1500 µg/ml as moderate

Table 2. MIC of various fractions of various fractions of *Sonchus asper* against bacterial strains.

Group	*S. aureus*		*E.coli*		*K. pneumoniae*		*M. luteus*		*E. aerogenes*		*B. subtilis*	
	Conc.	Zone	Conc.	Zone	Conc.	Zone	Conc.	Zone	Conc.	Zone	Conc.	Zone
Cefix	1	26	1	28	1	16	1	17	1	10	1	24
Rox	1	22	1	18	1	20	1	25	1	24	1	32
SAWE	-	-	-	-	-	-	-	-	-	-	-	-
SABE	5	12	-	-	-	-	-	-	-	-	-	-
SAME	2.5	12	5	14	1	12	2.5	10	5	14	1	12
SACE	-	-	-	-	-	-	-	-	-	-	-	-
SAEE	5	10	-	-	-	-	-	-	5	12	1	10
SAHE	-	-	-	-	1	10	-	-	-	-	-	-

- = No effect; Conc.= concentration of extract, antibiotic (mg /ml); and Zone = diameter of zone (mm).

Table 3. Antifungal activity of various fractions of *Sonchus asper*.

Group	% Inhibition of various fungal species			
	Aspergillus niger	*Fusarium solani*	*Aspergillus flavus*	*Aspergillus fumigatus*
Control	88.8 ± 2.33	85 ± 3.45	89.2 ± 3.22	90.5 ± 3.09
SAWE	61.1 ± 2.07	12.5 ± 0.08	5.06 ± 0.003	27.7 ± 0.67
SABE	61.1 ± 1.98	93.75 ± 3.02	5 ± 0.004	36.84 ± 1.09
SAME	91.1 ± 3.11	31.25 ± 1.21	6 ± 0.001	88.15 ± 2.54
SACE	33.3 ± 1.32	75 ± 3.21	64.5 ± 1.56	60.52 ± 3.0
SAEE	55.5 ± 1.27	88.7 ± 2.12	55.6 ± 2.11	86.8 ± 2.98
SAHE	66.6 ± 3.76	47.5 ± 1.02	59.4 ± 2.07	53.9 ± 1.9

inhibitors and those with MIC values above 1600 as weak inhibitors. In this study, replicate results of antifungal show that all fractions showed some extent of antifungal activity at 200 µg/ml concentration of various fractions, however growth of *A. niger* was markedly inhibited by methanolic and ethyl acetate fraction of both plants, while *F. solani* was inhibited by butanolic, methanolic and water fraction of *S. asper*. Similarly, *A. flavus* showed significant growth inhibition. These results suggest that methanolic extracts of *S. asper* were more efficient to inhibit bacterial growth than fungal growth, probably in relation to their active molecules. Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolic composition (Baydar et al., 2006; Rodriguez et al., 2007), and saponins have antifungal properties (Aboaba and Efuwape, 2001; Mohanta et al., 2007). Kabuki et al. (2000) reported that the antimicrobial spectrums of the crude catechin were more effective against gram-positive bacteria than gram-negative bacteria. This tendency of tannin could be explained by that the structures of cell envelope, including cytoplasmic membrane and cell wall component, are different between gram-positive and gram-negative bacteria. Gram-negative bacteria possess an outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. Without outer membrane, the cell wall of gram-positive bacteria can be permeated more easily and tannins can disturb the cytoplasmic membrane, disrupt the proton motive force (PMF), electron flow, active transport and coagulation of cell contents (Burt, 2004). Therefore, the structural difference of bacteria plays an important role in their susceptibility.

The phytotoxic results of all fractions of both plants shows that they inhibited the growth of reddish root as well as shoot as compared to non treated control plant. Water, methanolic and butanolic fractions showed marked growth inhibition of root and shoot while n-hexane and ethyl acetate fraction of both plants showed moderate effects. Our findings showed similarity with results reported by Javaid, (2009) that water extract of *Withania somnifera* and *Datura alba* possessed some bioactive compounds which significantly inhibited the growth of root and shoot of *Rumex dentatus* L., highly competitive weed in wheat during allelopathic screening. Similar investi-gations was found by Kordali et al. (2008) that essential oil isolated from Turkish *Origanum acutidens* and their phenolic compounds completely inhibited the growth of seedling and roots and possessed antifungal activity when compared to standards compounds.

REFERENCES

Aboaba OO, Efuwape BM (2001). Antibacterial properties of some Nigerian species. Biol. Res. Commun. 13: 183-188.

Ahmad M, Khan MA, Manzoor S, Zafar M, Sultana S (2006). Check list of medicinal flora of Tehsil Isakhel, District Mianwali Pakistan. Ethno. Leaflets 10: 41-48.

Algiannis N, Kalpotzakis E, Mitaku S, Chinou IB (2001). Composition and antimicrobial activity of essential oils of two Origanum species. J. Agric. Food Chem. 40: 4168-4170.

Atta-ur-Rahman (1991). Studies in Natural Product Chemistry, p. 383 (Part B) Elsevier Science Publishers, B.V; Netherlands.

Bagamboula CF, Uyttendaele M, Debevere J (2003). Antimicrobial effect of spices and herbs on *Shigella sonnei* and *Shigella flexneri*. J. Food Prot. 66: 668-673.

Baydar NG, Sagdic O, Ozkan G, Cetin S (2006). Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera* L.) seed extracts. Int. J. Food Sci. Technol. 41:799-804.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods. Int. J. Food Microbiol. 94: 223-253.

Duraipandiyan V, Ignacimuthu S (2009). Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. J. Ethno. 123: 494-498.

Giner RM, Ubeda A, Just MJ, Serrano A, Manez S, Rios JL (1993). A chemotaxonomic survey of *Sonchus* subgenus *Sonchus*. Biochem. Syst. Ecol. 21: 617.

Grabley S, Thiericke R (1999). Bioactive agents from natural sources: trends in discovery and application. Adv. Biochem. Eng. Biotechnol. 64: 101-54.

Guil-Guerrero JS, Gimenez-Gimenez A, Rodrguez-Garc I, Torija-Isasa ME (1998). Nutritional composition of *Sonchus* species (*S. asper* L., *S. oleraceus* L., and *S. tenerrimus* L.). J. Sci. Food Agric. 76: 628-632.

Hussain K, Shahazad A, Zia-ul-Hussnain U (2008). An ethnobotanical survey of important wild medicinal plants of Hattar, District Haripur Pakistan. Ethno. Leaflets, 12: 29-35.

Jan G, Khan MA, Gul F (2009). Ethnomedicinal Plants Used against Jaundice in Dir Kohistan Valleys (NWFP) Pakistan. Ethno. Leaflets, 13: 1029-1041.

Javaid A (2009). Role of Effective Microorganisms in Sustainable Agricultural Productivity. In: Advances in Sustainable Agriculture. Springer Publishers. (in Press).

Kabuki T, Nakajima H, Arai M, Ueda S, Kuwabara Y, Dosako S (2000). Characterization of novel antimicrobial compounds rom mango (*Mangifera indica* L.) kernel seeds. Food Chem. 71: 61-66.

Kareru PG, Kenji GM, Gachanja AN, Keriko JM, Mungai G (2007). Traditional medicine among the Embu and Mbeere peoples of Kenya. Afr. J. Traditional Compl. Altern. Med. 4: 75-86.

Koche DK, Shirsat RP, Imran S, Nafees M, Zingare AK, Donode KA (2008). Ethnomedicinal Survey of Nageria Wild Life Sanctuary, District Gondia (M.S.). India- Part II. Ethno. Leaflets, 12: 532-537.

Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish Origanum acutidens and its three components, carvacrol, thymol and p-cymene. Bioresour. Technol. 99: 8788-8795.

Manez S, Recio MC, Giner RM, Sanz MJ, Terencio MC, Peris JB, Stubing G, Rios JL (1994). A chemotaxonomic review of the subtribe Crepidinae based on its phenolic constituents.. Biochem. Syst. Ecol. 22: p. 297.

Marino M, Bersani C, Comi G (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. Int. J. Food Microbiol. 67: 187-95.

McLaughlin JL, Rogers LL (1998). The use of biological assays to evaluate botanicals. Drug Inform. J. 32: 513-524.

Mitscher LA, Drake S, Gollapudi SR, Okwute SK (1987). A modern look at folkloric use of anti-infective agents. J. Natl. Prod. 50: 1025-1040.

Mohanta TK, Patra JK, Rath SK, Pal DK, Thatoi HN (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* L.f. Sci. Res. Essay, 2: 486-490.

Narod FB, Gurib-Fakim A, Subratty AH (2004). Biological investigations into *Antidesma madagascariense* Lam. (Euphorbiaceae), *Faujasiopsis flexuosa* (Lam.) C. Jeffrey (Asteraceae), *Toddalia asiatica* (L.) Lam. and *Vepris lanceolata* (Lam.) G. Don (Rutaceae). J. Cell Mol. Biol. 3: 15-21.

Qureshi R, Waheed A, Arshad M, Umbreen T (2009). Medico-ethanobotanical inventory of tehsil Chakwal, Pakistan. Pak. J. Bot. 41: 529-538.

Rehman EU (2006). Indigenous knowledge on medicinal plants, village Barali Kass and its allied areas, District Kotli Azad Jammu and Kashmir, Pakistan. Ethno. Leaflets 10: 254-264.

Rivera ND, Oben DCC (1993). Ethnopharmacology of Murcia (SE Spain). Conference internationale Ethnomedecine. Heidelb2e.4rg-2, 7.

Rodriguez N, Amils R, Jimenez-Ballesta R, Rufo L, dela-Fuente V (2007). Heavy metal content in Erica andevalensis: an endemic plant from extreme acidic environment of Tinto River and its soils. Arid Land Res. Manage. 21: 51-65.

Sabeen M, Ahmad AA (2009). Exploring the Folk Medicinal Flora of Abbotabad City, Pakistan. Ethno. Leaflets, 13: 810-833.

Sammon AM (1998). Protease Inhibitors and Carcinoma of the Esophagus. Cancer, 83: 405-408.

Shimizu S, Miyase T, Ueno A, Usmanghani K (1989). Sesquiterpene lactone glycosides and ionone derivative glycosides from *Sonchus asper*. Phytochemistry, 28: p. 3399.

Thomson B, Shaw IA (2002).Comparison of Risk and Protective Factors for Colorectal Cancer in the Diet of New Zealand Maori and non-Maori. Asian Pacific J. Cancer Prevention, 3: 319-324.

Zabihullah Q, Rashid A, Akhter N (2006). Ethanobotnical survey in kot Manzary baba valley malakand agency, Pakistan. Pak. J. Plant Sci. 12: 115-121.