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Full Length Research Paper

Antitumor, cytotoxic and antioxidant potential of *Aster thomsonii* extracts

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The present investigation deals with biological evaluation of *Aster thomsonii*. For this purpose, different biological assays of crude methanolic extract (CME) and its fractions that is n-Hexane fraction (NHF) and aqueous fraction (AQF) were carried out. The results of AQF showed maximum brine shrimp cytotoxic activity with ED_{50} values of 154.69 µg/ml, while the NHF showed significant potato disc antitumor activity with IC_{50} values of 9.55 µg/ml. Evaluation of both NHF and AQF fractions for sulforhodamine B assay on human cell line HT144 showed IC_{50} values of 1.10 and 2.82 mg/ml, respectively. While the IC_{50} of CME and AQF fractions against human cell line H157 were 0.056 and 0.005 mg/ml, respectively. Antioxidant analysis of AQF determined the IC_{50} values of 31.98 µg/ml. DNA protection assay results of all plant extracts were also appreciating for further investigations but the extracts and their fractions did not show antibacterial activity.

Key words: *Aster thomsonii*, brine shrimp toxicity assay, antitumor assay, sulforhodamine-B assay, antioxidant assay, DNA protection assay.

INTRODUCTION

Natural products and their derivatives contribute more than half of all clinically administered drugs (Koehn and Carter, 2005). They possess a significant position in drug discovery for treatment of cancer and other infectious diseases (Fabricant and Farnsworth, 2001). Approximately 40% of newly approved drugs from 1983 to 1994 were of natural origin (Cragg et al., 1997). Plant species, *Aster thomsonii*, belongs to the family Asteraceae with 250 species, mostly belongs to North America. The Asteraceae is cosmopolitan in distribution and cultivated as ornamental (Cronquist, 1955).

Aster genus has been used traditionally for a long time for curing of diseases. It has expectorant, diuretic, antitumor, antibacterial, antiviral and antiulcer activities (Morita et al., 1996; Shao et al., 1997; Shirota et al., 1997; Wang and Yu, 1998). The infusion of the aerial parts of Aster species is considered to have an antidiarrhoeic effect because it increases the intestinal absorption of water and reduces gastrointestinal propulsion (Almeida et al., 1995). Complementary studies showed that the infusion of stalks and roots altered ion transport in the colon. In addition, aqueous and ethanolic extracts of stalks, leaves, and roots of *Aster* plant have low toxicity. Its mouth infusions induced only minor changes in some serum biochemistry (Meneghetti, 1997).

Previously, there was no significant work done on *Aster* species to evaluate their medicinal importance. Medicinal importance of different parts of *Aster* species needed to be determined. There is no single, all embracing bioassay to elucidate the biological activities of this species. The evaluation process generally involves use of many bioassay methods in order to arrive at an appropriate conclusion (Linton, 1983). Biological evaluation of this species carried out by brine shrimp cytotoxicity assay, potato disc antitumor assay, sulforhodamine B assay, antioxidant assay, DNA repair assay and antibacterial assay.

MATERIALS AND METHODS

Plant material

Plant sample was collected from Shugran (Hazara), Pakistan. Plant species was identified as *A. thomsonii* in Herbarium of Islamabad,

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Figure 1. *A. thomsonii* from natural habitat.

Quaid-i-Azam University, Islamabad, Pakistan, by following the description in Flora of Pakistan (Ghafoor, 2002) and comparing with already identified herbarium sheets of same species preserved in the herbarium. The species was given the herbarium number HMP349 and deposited in the herbarium collection (Figure 1).

Maceration and fractionation

Maceration of 500 g poorly homogenized plant sample (stem, leaves and flowers) in 3 L methanol was carried out for 4 weeks at 25°C. Then, methanol was squeezed out from the plant sample and filtered. The filtrate was concentrated in rotary vacuum evaporator (Rotavapor R-200, Buchi) and finally concentrated in vacuum oven at 45°C (Vacucell, Einrichtungen GmbH). This concentrated filtrate was named as crude methanolic extract (CME) of *A. thomsonii*. Then, n-Hexane fraction (NHF) and aqueous fraction (AQF) of CME were isolated by using solvent-solvent extraction.

Biological activities

Following biological activities were performed on the extract and its fractions.

Brine shrimp cytotoxicity assay

Brine shrimp cytotoxicity assay was performed by using the methodology of Ahmad et al. (2008). Brine shrimp (*Artemia salina*) larvae, used as test organisms, were hatched at 37°C in artificial seawater. Different concentration that is 1000, 100, 10, 1 and 0 µg/ml (control), of CME, NHF and AQF were prepared in methanol and used against brine shrimp larvae. The survival rate of these larvae was observed against all concentrations of different fractions. For this purpose, 0.5 ml sample of each and every fraction was taken in 20 ml vial, solvent from each vials was evaporated, followed by the addition of 2 ml of artificial seawater. Ten shrimps transferred into each vial, final volume was adjusted to 5 ml by artificial seawater and kept under florescence light at 25°C for 24 h. Test was performed in triplicate. After this, survivors were counted, percentage death was calculated (by Abbott's formula), ED_{50} values were determined (by Finney computer program) (Finney, 1971).

Antitumor assay

Antitumor assay was performed by following the standard procedure of Rehman et al. (2001). A 48 h old culture of *Agrobacterium tumefaciens* strain At 10 was used to test the plant extracts. An inoculum of 1.5 ml containing six concentrations of plant samples (10,000, 1000, 100, 10, 1 and 0 µg/ml (control)), bacterial cultures and distilled water was prepared. Red skinned potatoes were surface sterilized by using 0.1% $HgCl_2$ solution followed by washing with distilled thrice. A borer of 8 mm diameter was used to bore out potato cylinders and cut into 2 mm discs. Autoclaved agar solution (1.5%) was poured in petriplates and solidified. Ten discs were placed on agar surface of each petriplate and 50 µl of inoculum was poured on the top of each disc. The plates were sealed with parafilm to avoid contamination and moisture loss. The plates were incubated at 28°C in dark. After 21 days of incubation, potato discs were stained with Lugol's solution (10% KI, 5% I_2), and tumors were counted under dissecting microscope. More than 20% tumor inhibition was considered significant (Ferrigini et al., 1982). Tumor inhibition was calculated by using following formula:

% age of tumor inhibition = (1–Number of tumors in the sample / number of tumors in control) x 100

Sulforhodamine B (SRB) assay

The human cancer cell lines H157 (lung carcinoma) and HT144 (malignant melanoma) were cultured in RPMI1640 (Gibco BRL, Life Technologies, Inc) supplemented with 10% heat inactivated fetal bovine serum in a humidified incubator at 37°C with 5% CO_2. The cells grew as monolayer in tissue culture flasks and sub-cultured approximately once every four days by 98% trypsin EDTA buffer of pH 7.2. Vincristine and methotrexate were used as standard drugs. Growth inhibition of H157 and HT144 cell lines was determined by using the modified SRB assay of Skehan et al. (1990). Briefly, cells were seeded at a density of 1 x 10^6 cells/well in 96-well microplates. After 24 h, the cells were exposed to drugs for continuous 3 days. The culture medium was removed and trichloroacetic acid (50%, 100ul) was added for fixation. Then the plates were air-dried

Table 1. Percentage tumor inhibition and IC_{50} value of potato disc assay for *A. thomsonii*.

Name	%Tumor inhibition					IC_{50} (µg/ml)
	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml	10000 µg/ml	
CME	51.47	61.76	75	80.88	86.76	<1
NHF	33.82	48.52	64.70	70.58	73.52	9.5
AQF	52.94	69.11	75	82.35	89.70	<1

and 0.4% SRB (sigma) in 1% acetic acid was added for 30min. Un-bound dye was washed out with 1% acetic acid. After air-drying, SRB dye within cells were dissolved with 100 µl solution of tris-base 10 mM (pH 10.5). The optical density of the extracted SRB dye was measured with a microplates reader (Platos R 496) at 490 nm. The 50% inhibitory concentration (IC_{50}) of the test drugs was calculated using graphical method.

Antioxidant activity

The free radical scavenging activity was measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay. DPPH radical assay was performed according to the procedure described by Obeid et al. (2005). DPPH solution was prepared by dissolving 3.92 mg in 100 ml of 82% methanol. A volume of 2800 µl of DPPH solution was added to glass vials followed by the addition of 200 µl of CME, leading to the final concentration of 100, 50, 25, 10, 5 and 0 µg/ml (negative control). Mixtures were shaken well and incubated in dark at 25°C for 1 h. Absorbance was measured at 517 nm by using spectrophotometer (DAD 8453, Agilent). Ascorbic acid (AsA) was used as positive control. Each test was performed in triplicates and percentage inhibition was measured according to formula and IC_{50} values were calculated by graphical method. Same procedure was then repeated with both the fractions that is NHF and AQF.

(%) Scavenging effect = [(Ac-As)/Ac] x100

Where; "Ac" is Absorbance of negative control and "As" is Absorbance of test sample.

DNA protection assay

DNA protection assay was performed according to the protocol of Tian and Hua (2005). The reaction was carried out in an eppendorf tube at the total volume of 15 µl containing 0.5 µg pBR322 DNA in 3 µl of 50 mM phosphate buffer (pH 7.4), 3 µl of 2 mM $FeSO_4$ and 5 µl of tested samples (CME) at concentrations 1000, 100 and 10 µg/ml. Then, 4 µl of 30% H_2O_2 were added and the mixture was incubated at 37°C for 1 h. The mixture was subjected to 1% agarose gel electrophoresis. DNA bands (open circular, supercoiled and linear) were stained with ethidium bromide and were analyzed qualitatively by scanning with Doc-IT computer program (VWR). An antioxidant effect on DNA was based on the increase or loss of supercoiled monomer, compared with the control value. To avoid the effects of photoexcitation of the samples, experiment was done in the dark and untreated supercoiled DNA and supercoiled DNA treated with 2 mM $FeSO_4$+30% H_2O_2 were used as control along with the tested sample. The same experimental procedure was repeated with both the fractions that is, NHF and AQF.

Antibacterial assay

For antibacterial activity of CME, AQF and NHF, the agar well diffusion assay was carry out by following the methodologies of Rehman et al. (2009). Three bacterial strains, one Gram positive (*Staphylococcus aureus*, ATCC 6538), two Gram negative (*Salmonella setubal*, ATCC 19196; *Pseudomonas picketii*, ATCC 49129) were used. The organisms were maintained on nutrient agar medium at 4°C.

RESULTS

Crude methanolic extract of *A. thomsonii* was prepared and partitioned into two fractions that is n-Hexane (NHF) and aqueous fraction (AQF). The plant crude extract and their partitions were evaluated for their biological activities (brine shrimp cytotoxicity, antitumor, SRB, antioxidant and DNA protection activities). Results of the brine shrimp cytotoxicity assay showed that CME and NHF of *A. thomsonii* had ED_{50} (Effective dose at 50% conc.) values >1000 µg/ml while its AQF fraction showed effective ED_{50} value of 154.69.

Anticancer assay was done by two methods (a) potato disc antitumor assay and (b) SRB assay. Both CME and its fractions showed significant results as shown in Table 1. Highest tumor inhibition was exhibited by CME at concentration 10,000, 1000 and 100 µg/ml while there was less inhibition as the concentration decreased gradually. The CME and AQF showed IC_{50} values <1 µg/ml, at all concentration tested, which indicate that crude extract and AQF are most effective of all. On the basis of these results from potato disc assay, SRB assay was also performed to check the effectiveness of crude extract and its fractions as anticancer drug.

In SRB assay, before SRB staining, cytotoxic effect of CME and its fractions on human cancer cell line H157 is shown in Figure 2. CME shows nearly 100% cell death when incubated in RPMI1640 feeding media after 24 h. These results were visualized under inverted microscope before SRB staining. Percentage growth inhibition (%GI) was calculated by comparing that inhibition with the standard drugs used. Standard drug vincristine showed 100% inhibition at all concentrations to both cell lines as compared to methotrexate. All the extracts showed cytotoxic effect with the most effective extract AQF having IC_{50} value 0.005 mg/ml on H157 cell line. CME on H157 cell line shows highest cytotoxicity and had IC_{50} value of 0.056 mg/ml as compared to its NHF (0.141 mg/ml). Malignant melanoma cytotoxic effect of *A. thomsonii* showed that NHF was the most effective in this

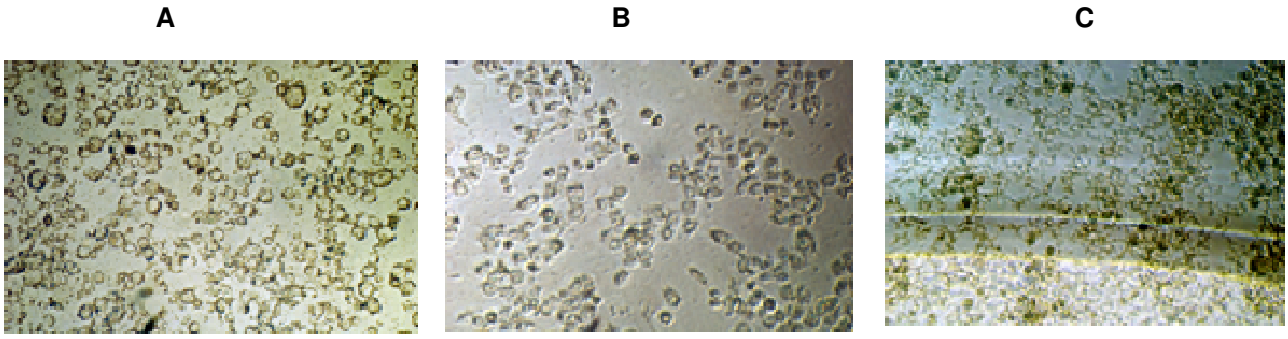

Figure 2. Cytotoxic effect of crude extract and its fractions on human cancer cell lines H157. A = CME at 5 mg/ml, B = NHF at 5 mg/ml and C = AQF at 5 mg/ml.

Table 2. IC_{50} of *A. thomsonii* on H157 and HT144 cancer cell lines.

Name	IC_{50} (mg/ml)	
	H157	HT144
CME	0.056	10.69
NHF	0.141	1.10
AQF	0.005	2.82

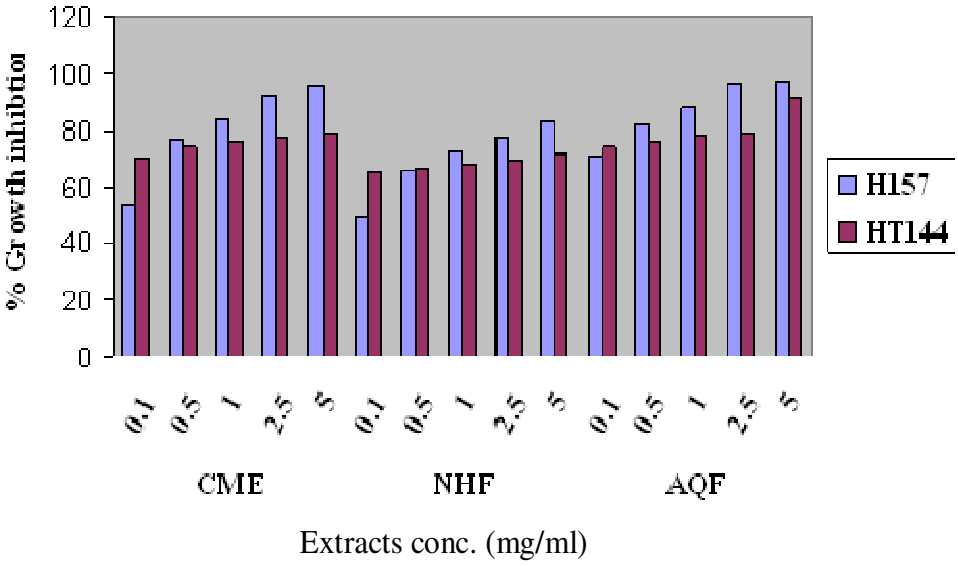

Figure 3. % GI of crude extract and fractions of *A. thomsonii* on human cancer cell lines H157 and HT144.

cell line with IC_{50} value of 1.10 mg/ml and its AQF fraction also shows significant activity (2.82 mg/ml) while CME was least effective as compared to its fractions as shown in Table 2.

Results of % GI of SRB cytotoxic effect of crude extract and its fractions on human cancer cell lines (H157 and HT144) showed significant growth inhibition at all concentrations tested with gradually increase in %GI on increasing concentrations as shown in Figure 3. Maximum % growth inhibition was shown by AQF (97.28 and 91.60%) in H157 and HT144 cell lines respectively. CME had 95.52% growth inhibition in H157 which was greater

Table 3. DPPH scavenging antioxidant activities of *A. thomsonii.*

Name	Percent scavenging effect					IC_{50} (µg/ml)
	100 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	
CME	78.56	59.79	33.30	20.67	7.23	49.24
NHF	17.34	14.61	12.29	11.72	2.76	>100
AQF	80.12	74.69	40.24	20.10	9.46	31.98
AsA	95.04	94.83	90.00	86.45	44.71	5.54

Table 4. DNA protection effect of crude extract and fractions.

Conc. (µg/ml)	Plasmid DNA Treated with $FeSO_4$ and H_2O_2			Plasmid DNA with out $FeSO_4$ and H_2O_2		
	CME	NHF	AQF	CME	NHF	AQF
1000	++	+	+++	-	-	-
100	+++	+++	+++	-	-	-
10	+++	++	++	-	-	-

+++ = Significant Protection; ++ = Moderat protection; + = Weak protection; and - = No effect.

than HT144 (79.39%), where NHF showed less % growth inhibition as compared to CME.

Results of antioxidant assay indicated that crude extract and both the fractions had significant antioxidant activity as shown in Table 3. IC_{50} of CME of *A. thomsonii* had value of 49.24 µg/ml while, its AQF fraction showed much stronger IC_{50} value of 31.98 µg/ml while NHF proved to be the least active fraction.

The results of DNA protection assay showed that when plasmid DNA was treated with $FeSO_4$+H_2O_2, super-coiled form completely disappear indicating complete damage to plasmid DNA as shown in Table 4. However, when the same was repeated in the presence of CME, NHF and AQF no or very less DNA damage had occur, at almost all the concentrations as shown in Figure 4(a). While in third case the plasmid DNA was treated alone with samples without $FeSO_4$+H_2O_2 so is to confirm that if our plant extract may affect super-coiled form of plasmid DNA as shown in Figure 4b. This proves that our plant extract is highly DNA friendly.

According to the results of antibacterial assay, crude extract and its fractions showed no activity against all bacterial strains while standard drugs showed maximum activity.

DISCUSSION

Brine shrimp cytotoxicity assay has been considered as prescreening assay for antimicrobial, antitumor, antimalarial, antifungal, and insecticidal activities. Brine shrimp assay is suggested to be a convenient probe for the pharmacological activities in plant extracts (Mayerhof et al., 1991). In the present study, AQF of *A. thomsonii* showed ED_{50} values of 154.69 µg/ml. This ED_{50} value is much better than that of *Thymus serpyllum* that showed the ED50 value of 466 µg/ml (Rehman et al., 2009). Another scientific research work on 60 medicinal plant species from Brazil reported brine shrimp cytotoxicity and only 10% plants showed ED50 < 1000 µg/ml (Maria et al., 2000). Jacques et al. (2003) screened 226 plant extracts for brine shrimp toxicity and identified several important cytotoxic plant species.

In antitumor assay, although, plant extracts and their fractions did not show 100% inhibition of bacterial growth (Table 2). However, when IC_{50} was calculated, AQF and CME showed IC_{50}<1 µg/ml. These results are much better than the results of the antitumor assay performed on *T. serphyllum* (Rehman et al., 2009). Potential antitumor activity performed to screen the selected plant species, *in vitro*. The advantage of potato disc antitumor assay is cost effective, rapid response, easy to perform and reproducible results (Galasky et al., 1981). The significant results of this plant not only increases its pharmacological importance but also encouraged to test the anticancer activity of these plants on human cell lines by using SRB assay.

This assay is simple, sensitive and reproducible, gives better linearity, a good signal-to-noise ratio with stable end-result (Fricker and Buckley, 1996; Keepers et al., 1991). This assay is based on aminoxanthine dye, sulphorhodamine B (SRB). Human cell lines contain the basic amino acids that uptakes of the negatively charged pink dye SRB. When growth of the cells increased more dye absorbed in the cell, and when the cells lysed, dye released and showed greater absorbance (Skehan et al., 1990). In present study, the results of SRB assay of CME, AQF and NHF against H157 and HT144 human

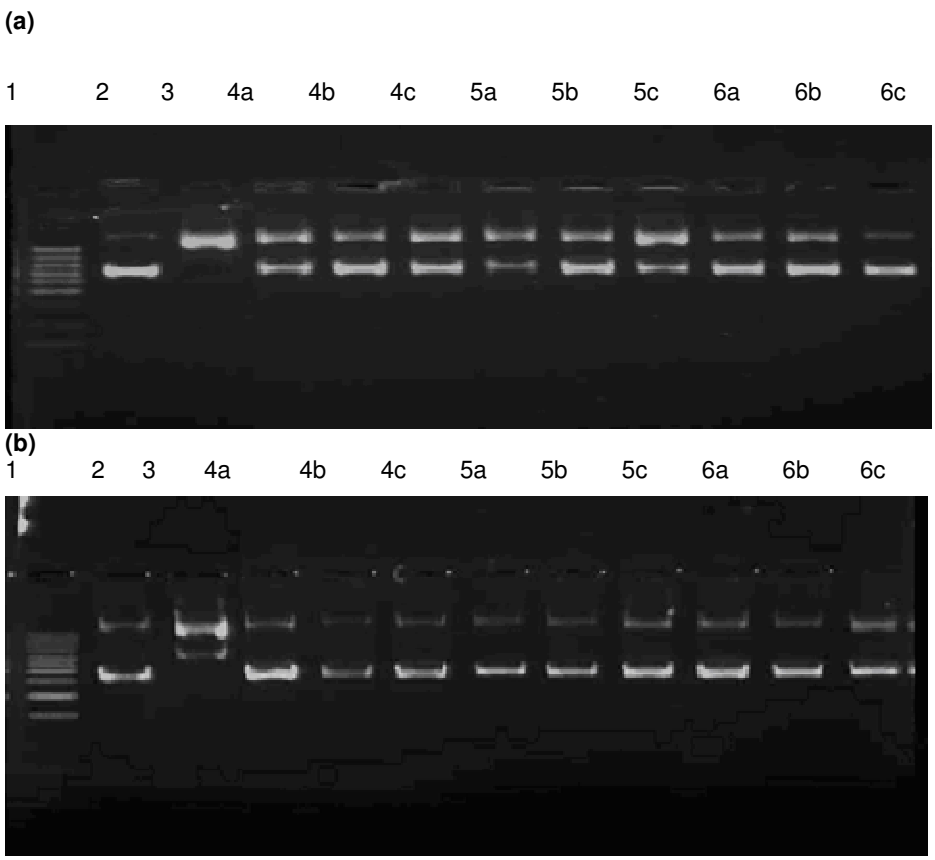

Figure 4. (a) DNA Protection effect of crude extract and fractions treated with $FeSO_4+H_2O_2$ (1 = 1kb DNA Ladder, 2 = Plasmid DNA (pBR322), 3 = Plasmid DNA Treated with $FeSO_4+H_2O_2$, a = 1000µg/ml, b = 100 µg/ml, c = 10 µg/ml 4, 5, 6 = *A. thomsonii* (CME, NHF, AQF). All the extracts were added in addition to $FeSO_4+H_2O_2$. (b). DNA Protection effect of crude extract and fractions treated without $FeSO_4+H_2O_2$.1 = 1kb DNA Ladder, 2 = Plasmid DNA (pBR322), 3 = Plasmid DNA Treated with $FeSO_4+H_2O_2$, a = 1000 µg/ml, b = 100 µg/ml, c = 10 µg/ml, 4, 5, 6 = *A. thomsonii* (CME, NHF, AQF).

cell lines showed that %GI of both cell lines increased as concentration of samples increased. In case of H157 the maximum %GI observed was 97.28 and 82.90% for AQF and NHF respectively at 5 mg/ml as shown in Figure 3. These results are parallel to the findings of Chung et al. (1995), while in case of HT144 the highest activity observed was 91.60% for AQF. Mostly, cytotoxic screening of plant extracts done on the basis of the traditional uses of the plants, without thinking of the fact that naturally may be inactive, but metabolites may be active e.g. senna contain glycosides which hydrolyzed in intestinal and released the active aglycones which increase in peristalsis (Houghton et al., 2007).

The results of DPPH assay showed that CME, AQF and NHF of this plant species have antioxidant compounds. The AQF fraction has good antioxidant activity with IC_{50} value of 31.9 (Table 3) and this determined activity is similar to the findings of Agnieszka et al. (2008) while working of *Carum carvi*. However, this fraction has very low flavonoid contents (results not shown) but had good antioxidant activity which is opposite to the findings of Zielinska et al. (2001).

The results of H_2O_2 induced plasmid pBR322 DNA damage assay showed that CME of the plant species showed high DNA protection activity which increases with decrease in sample concentration (Table 4). The NHF and AQF showed good DNA protection activity with highest protection observed at 100 µg/ml. AQF showed maximum protection at 1000 µg/ml as well. However, the AQF showed opposite behavior to that of CME as its DNA protection ability decreases with decrease in conc. and vice versa (Figure 4). The expected high DNA protection activity of AQF may be due its high anti-oxidant activity. Till now no DNA protection activity was reported for *A. thomsonii,* however the good antioxidant DPPH free radical scavenging activity was the sign that this plant will also have good DNA protection activity as well. As reported by Attaguile et al. (2000) the broad pharmacological properties of flavonoids are related to their ability to protect against the damaging action of free

radicals. However, this report is contrary to our findings of AQF and NHF of *A. thomsonii* which showed significant DNA protection activity with low flavonoid contents. However, the prevailing opinion is that the antioxidant activity of plants are directly related to DNA protection activity, either by chelating the transition metal (Asker et al., 1996) or by inhibiting the enzymes involved in the initiation reaction of DNA break. *A. thomsonii* plant species has good pharmacological importance should be investigated further for the isolation, purification and characterization of valuable compounds.

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REFERENCES

Asker VSABE, Berg VDDJ, Tromp MNJL (1996). Structural aspects of antioxidant activity of flavonoids. Free Radic. Biol. Med., 20: 331-42.

Agnieszka N, Jan DY, Norbert B (2008). Flavonoid content and antioxidant activity of caraway roots (*Carum carvi* L.). Veg. Crop Res. Bull., 68: 127-133.

Ahmad MS, Hussain M, Hanif M, Ali S, Qayyum M, Mirza B (2008). Di and Tri organotin (IV) Esters of 3,4-Methylenedioxyphenylpropenoic Acid: Synthesis, Spectroscopic Characterization and Biological Screening for Antimicrobial, Cytotoxic and Antitumor Activities. Chem. Bio. Drug Design., 71: 568-576.

Almeida CE, Karnikowski MGO, Foleto R, Baldisserotto B (1995). Analysis of antidiarrhoeic effect of plants used in popular medicine. Rev. Saude. Public, 29: 428-33.

Attaguile G, Russo A, Campisi A, Savoca F, Acquaviva RN, Ragusa N, Vanella A (2000). Antioxidant activity and protective e¡ect on DNA cleavage of extracts from *Cistus incanus* L. and *Cistus monspeliensis* L. Cell Biol. Toxicol. 16: 83-90.

Chung CP, Park JB, Bae KH (1995). Pharmocological effects of methanolic extract from the root of *Scutellaria baicalensis* and its flavonoids on human gingival fibroblast. Planta Med., 61: 150-153.

Cragg GM, Newman DJ, Snader KM (1997). Natural Products in Drug Discovery and Development. J. Nat. Prod., 60: 52-60.

Cronquist A (1955). Vascular plants of the Pacific Northwest: Compositae. Univ. Washington Press, Seattle, WA, pp. 5 - 343.

Fabricant DS, Farnsworth NR (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. Environ. Health Perspect., 109: 69-75.

Ferrigini NR, Putna JE, Anderson B, Jacobsen LB, Nichols DE, Moore DS, McLaughlin JL (1982). Modification and evaluation of the potato disc assay and antitumor screening of Euphorbia seeds. J. Nat. Prod., 45: 679-686.

Finney DJ (1971). Probit Analysis 3rd ed. Cambridge University Press, Cambridge.

Fricker SP, Buckley RG (1996). Comparison of two colorimetric assays as cytotoxicity endpoints for an in vitro screen for antitumour agents. Anticancer Res., 16: 3755-3760.

Galasky AB, Kozimor R, Piotrowski D, Powell R (1981). The crown gall potato disc bioassay as a preliminary screen for compounds with antitumor activity. J. Nat. Cancer, 6: 689-692.

Ghafoor A (2002). *Asteraceae*. In: *Flora of Pakistan*. (Eds.): Ali SI, Qaiser M. Missuri Botanical Press.

Houghton P, Fang R, Techatanawat I, Steventon G, Hylands PJ, Lee CC (2007). The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods, 42: 377-387.

Jacques EL, Pohlit AM, Nunomura SM, Da AC, Mustafa EV, Reis SK, Alecrim AM, Brito BR, De CS, Finney EK, Oliveira ED, Santos KD, Pereira LC, Castro LD, Rosha LF, Andrade MM, Henrique MC, Santos MD, Souza PD, Silva SG (2003). Screening of plants in Amazone state for lethality towards brine shrimp. Acta Amaz. 33: 93-104.

Keepers YP, Pizao PE, Peters GJ, Arke-Otte J, Winograd B, Pinedo HM (1991). Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for in vitro chemosensitivity testing. Eur. J. Cancer, 27: 897-900.

Koehn FE, Carter GT (2005). The evolving role of natural products drug discovery. Nat. Rev., 4: 206-220.

Linton AH (1983). Antibiotics: Assessment of Antimicrobial Activity and Resistance. Russell AD, Quernel LB (Eds), Academic Press London, pp. 19-30.

Maria T, Silva AF, Brandao M, Mesquita TS, Fatima ED, Junior AS, Zani CL (2000). Biological screening of Brazilian medicinal plants. Mem. Inst. Oswaldo. Cruz., 95: 367-373.

Mayerhof ER, Koncz-Kalman RZ, Nawrath C, Bakkeren G, Crameri A, Angelis K, Redei GP, Schell JB, Hohn KJ (1991). T-DNA integration: a mode of illegitimate recombination in plants. Embo. J., 10: 697-704.

Meneghetti BH (1997). Avaliação da atividade antidiarréica e toxicidade ubaguda de Aster squamatus (Spreng.) Hieron. (Asteraceae) Universidade Federal de Santa Maria, Santa Maria, Master Dissertation in Pharmaceutical Science and Technology, p. 54.

Morita H, Nagashima S, Uchiumi Y, Kuroki O, Takeya K, Itokawa H (1996). Cyclic peptides from higher plants. XXVIII. Antitumor activity and hepatic microsomal biotransformation of cyclic pentapeptides, astins, from *Aster tataricus*. Tokyo Chem. Pharmaceut. Bull., 44: 1026-1032.

Obeid HK, Allen MS, Bedgood DR, Prenzler PD, Robards K (2005). Investigation of Australian olive mill waste for recovery of biophenols. J. Agric. Food Chem., 53: 9911-9920.

Rehman A, Mannan A, Inayatullah S, Akhtar Z, Qayyum M, Mirza B (2009). Biological evaluation of Wild Thyme (*Thymus serpyllum*). Pharmaceut. Biol., 47(7): 628-633.

Rehman A, Choudhary MI, Thomsen WJ (2001). Bioassay Techniques for Drug Development. Amsterdam: Harwood Academic Publishers, pp. 9-25.

Shao Y, Ho C, Chin C (1997). Aster lingulatosides C and D cytotoxic triterpenoid saponins from *Aster lingulatus*. Phytochem., 44: 337-341.

Shirota O, Morita H, Takeya K, Itokawa H, Iitaka Y (1997) Cytotoxic triterpene from *Aster tataricus*. Nat. Med., 51: 170-172.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990). New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. J. Natl. Cancer Inst., 82(13): 1107-1112.

Tian B, Hua Y (2005). Concentration dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA. Food Chem., 91: 413-418.

Wang C, Yu D (1998) Triterpenoid saponins from *Aster auriculatus*. Planta. Med. 64: 50 – 53.

Zielinska M, Haegele NF, Firschke C (2001). Fulminant thrombosis of mechanical mitral valve prosthesis. Heart, 86: 16.