Antioxidant, anticancer and antibacterial potential of Zakhm-e-hayat rhizomes crude extract and fractions

Madiha Ahmed[1], Abdul Rehman Phul[1], Ihsan-ul-Haq[1], Gulnaz Bibi[2], Kehkashan Mazhar[3], Tofeeq Ur-Rehman[1], Muhammad Zia[4]* and Bushra Mirza[2]

[1]Department of Pharmacy, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan
[2]Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan
[3]Institute of Biomedical and Genetic Engineering, KRL Hospital, Islamabad, Pakistan
[4]Department of Biotechnology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan

Abstract: *Bergenia ciliata* (locally known as Zakhm-e-hayat; wound healer) is commonly employed for wound healing, curing diarrhea and vomiting, fever, cough and pulmonary affections. Local community uses this plant as tea decoction with table salt. *B. ciliata* crude extract and its fractions were subjected to antibacterial, antioxidant effects as well as determination of total flavonoids and phenolics, DNA damage and anticancerous activities following standard protocols. Increased percentage inhibition of free radical in DPPH assay as well as elevated phenolic and flavonoid contents revealed antioxidant potential of this potent herb. Ethyl acetate and aqueous extracts showed IC_{50} of 0.7 and 0.3 mg/ml respectively, against H157 cell line. Antibacterial analysis showed MIC 0.4-10mg/ml for crude extract and fractions. The results obtained conclude that extracts of *B. ciliata* contain remedial latent and can be used as possible source for drug development by pharmaceutical industries.

Keywords: Antibacterial; anticancer; antioxidant; *Bergenia ciliata;* DNA protection assay.

INTRODUCTION

Plants are imperative source of safe and effective medication throughout the world. Therefore, even today in developing countries almost 80% of the human population depends on plant resources for healthcare. More than 50,000 out of 4,22,000 flowering plants have been reported to be used for medicinal purposes (Govaerts, 2001). Screening, isolation and characterization programs for new active components are gaining attention because of horicon in diseases.

Zakhm-e-hayat (zakham; lesion/wound, hayat; life/heal; *Bergenia ciliata*) is included in most popular plants used in Northern areas of Pakistan, against a number of remedies including vomiting, fever, cough and pulmonary affections and for healing of wounds, diarrhea (Bhakuni *et al*., 1947). The native people use its leaves and rhizome in fresh or dried form. *B. ciliata* belongs to family Saxifragaceae. This family is composed of 30 genera and 580 species, mostly of profound medicinal importance. The genus *Bergenia* consists of six species distributed throughout the cold and temperate regions of the Himalayas, between 7000-10,000 ft and Central and East Asia. Few species are also found common in and around the Murree areas in Pakistan. There are two species of this genus commonly found in Pakistan, namely *B. ciliata* and *B. strachey* (Islam *et al*., 2002). It is a perennial herb of creeping rhizomes; with 10 inches leaf diameter; grows closely to rocks. Several researchers have documented its traditional benefits. In the North West and trans-Himalayan region as well as in Jammu and Kashmir, table salt containing decoction of roots of *B. ciliata* is used for the treatment of asthma. The hot aqueous extract of whole dried plant of *B. ciliata* is traditionally taken by oral route for renal or urinary calculi. Roots of this plant are also employed as a tonic and anti-scorbutic (Islam *et al*., 2002). Present investigation envisaged that this peculiar specie is not only potent antibacterial compound containing but also bears anticancer, DNA protecting and antioxidant properties.

MATERIALS AND METHODS

The rhizomes of the *Bergenia ciliata* were collected from Hidden valley, Nathia Gali, Khyber Pakhtunkhwa Pakistan in July 2008 and were identified by taxonomist, Dr. Rizwana Aleem Qureshi, Department of Plant Sciences, Quaid-i-Azam University, Islamabad by comparing with already identified herbarium sheets of same plant species preserved in the herbarium. A voucher specimen was also deposited in the Herbarium of Pakistan, Quaid-i-Azam University, Islamabad.

Extraction and fractionation

Fresh rhizomes of the *B. ciliata* were thoroughly washed under running tap water to remove soil contaminants. The rhizomes were thin sliced and dried under shade. The dried plant material was pulverized in grinder. The rhizome crude extract was prepared by soaking the powder (5 kg) in methanol (12 L) for 72 hrs with occasional shaking. The mixture was filtered through

*Corresponding author: e-mail: ziachaudhary@gmail.com

Whattman filer paper No. 1 and the residue was again dipped in methanol for additional 72 hrs. The mixture was filtered thereafter, and the filtrates were combined. The filtrate was then concentrated under reduced pressure at 45ºC in rotary evaporator (Rotavapor R-200 Buchi, Switzerland). The methanol was completely evaporated by heating the concentrate in vacuum oven (Vacucell, Einrichtungen GmbH) at 45ºC, resulting in the formation of crude methanol extract.

The crude extract was subjected to fractionation following the protocol described by Haq *et al*. (2013). In brief; 300 g crude extract was suspended in 250 ml of distilled water and subjected to solvent-solvent extraction to yield fractions (Bibi *et al*., 2011). All the fractions were concentrated and stored at -20^{o}C till further analysis.

Determination of antioxidant activity, Flavonoids and phenolic contents

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) assay modified by Sowndhararajan *et al*. (2010). DPPH solution was prepared by dissolving 3.2 mg in 100 ml of 82% methanol. DPPH solution (2.8 ml) was added into glass vial, followed by the addition of 200 µl of sample solution (crude extracts and fractions) leading to the final concentration of 100, 50, 25, 10, 5 and 1 µg/ml. Mixture was shaken well and kept in dark at controlled room temperature (25-28ºC) for one hour. Absorbance was measured at 517 nm by using spectrophotometer (DAD 8453, Agilent). Mixture of 2800 µl of 82% methanol and 200 µl of methanol was used as blank, while 200 µl of methanol and 2800 µl of DPPH solution was taken as control. The test was performed in triplicate.

Percentage inhibition was measured according to following formula:

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

Where "Ac" means Absorbance of control and "As" means Absorbance of test sample. IC_{50} value was calculated by graphical method.

For total flavonoid determination, ammonium chloride colorimetric method was used (Chang *et al*., 2002). The crude extract and fractions (0.5 ml of 1:10 g/ml in methanol) were separately mixed in 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The resulting mixture was kept at room temperature for 30 min. After that absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA).

The total phenolic contents were determined according to the method of Sowndhararajan *et al.* (2010) using Folin-Ciocalteu reagent. The crude extract and fraction solutions were prepared at a concentration of 1 mg/ml. 100 µl of each solution was transferred into a test tube and 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionized water) was mixed thoroughly. The resulting mixture was kept at room temperature for 5 min, following addition of 0.75 ml of 6% (w/v) sodium carbonate solution. The mixture was kept at room temperature for 90 min and absorbance was measured at 725 nm using a UV-Vis spectrophotometer. Gallic acid was used as positive control. The total phenolic content was expressed as gallic acid equivalent in percentage (w/w).

DNA protection assay

To study the effects of samples on DNA protection, the assay was performed according to Hussain *et al.* (2007). The reaction mixture was composed of 3 µl (0.5 µg/3 µl in 50 mM phosphate buffer having pH 7.4) of pBR322 plasmid DNA, 3 µl of 2 mM $FeSO_4$, 4 µl of 30% H_2O_2 and 5 µl of crude extract and fractions (with final concentrations of 1000, 100 and 10 µg/ml). The mixture was incubated at 37ºC for one hour. Four controls, untreated DNA, DNA treated with 2 mM $FeSO_4$, DNA treated with 30% H_2O_2, DNA treated with 2 mM $FeSO_4$ and 30% H_2O_2 (Final volume made up to 15 µl with 50 mM phosphate buffer having pH 7.4) were run on 1% agarose gel electrophoresis simultaneously with the reaction mixtures. The gel was stained with ethidium bromide and was observed for qualitative analysis by scanning with Doc-IT VWR. Evaluations of antioxidant or pro oxidant effects on DNA were based on the increase or loss of supercoiled monomer percentage, compared with the control value. To avoid the effects of photo excitation of samples, experiments were performed in the dark.

Anticancer activities by sulforhodamine B (SRB) Assay

The human cancer cell lines H157 (lung carcinoma) and HT144 (malignant melanoma) were cultured in RPMI 1640 (Gibco BRL, Life Technologies, Inc) supplemented with 10% heat inactivated fetal bovine serum in a humidified incubator at 37ºC with 5% CO_2. The cells were grown as monolayers in tissue culture flasks and were sub cultured approximately once every four days by 98% trypsin EDTA buffer (pH 7.2). Growth inhibition of H157 and HT144 cell lines was determined by using the modified SRB assay as described by Ambrose *et al* (2012). Briefly, cells were seeded at a density of 5×10^3 cells/well in 96-well micro plates. After 24 hrs, serial dilutions of test agents (crude extract and fractions) and anticancer drug (Methotrexate) solutions were added in quadruplicate for each concentration. The cells were exposed to drugs for continuous 3 days. The culture medium was removed; fixed by adding 100 µl trichloroacetic acid (50%) and air-dried. Thereafter 0.4% SRB (Sigma) in 1% acetic acid was added and after 30 min unbound dye was washed with 1% acetic acid. The samples were re-air dried and SRB dye within cells was dissolved in 100 µl of 10 mM tris-base (pH 10.5). The OD was measured with a microplate reader (Platos R 496)

at 490 nm. The 50% inhibitory concentration (IC_{50}) of the test drugs was calculated using a Probit analysis program. Chemo-sensitivity of cells H157 and HT144 transfected with control vector was determined by SRB assay described above.

Determination of antibacterial activities

Antibacterial assay was performed by agar well diffusion method (Bibi *et al*., 2011). Six bacterial strains, four gram negative *Bordetella bronchiseptica* (ATCC 4617), *Escherichia coli* (ATCC 15224), *Salmonella setubal* (ATCC 19196), *Salmonella typhimurium* (ATCC 14028) and two gram positive, *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 6538) were used in this study. Bacterial strains from 24 hrs old culture in nutrient broth (MERK) were mixed with sterile physiological saline (0.9% NaCl) to match Mac Farland turbidity standard of $0.5x10^6$ colony forming unit (CFU) per ml. One ml of this standardized suspension of bacteria was used to seed 100 ml of the nutrient agar (MERK). Petri plates (14 cm) were prepared by pouring 75 ml of seeded nutrient agar and were solidified. Ten wells each having diameter of 8.0 mm were made in each petri plate with the cork borer. The wells were sealed with nutrient agar and marked followed by the addition of 100 µl of crude methanol extract and fractions at final concentrations of 5 mg/ml, 2.5 mg/ml, 2 mg/ml, 1.5 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml into their respective wells. As all the concentrations were prepared in dimethyl sulfoxide (DMSO), therefore pure DMSO was used as negative control, while solution of antibiotic Roxithromycin (1 mg/ml in DMSO) was used as positive control. All the plates were incubated at 37°C in incubator (YAMATO IC83) for 24 hrs. The susceptibility of each microorganism to test samples was determined by measuring the size of inhibitory zones around each well. The values below 8 mm were considered as inactive against bacteria. All of the experiment was performed in triplicate. The average of these plates was taken and standard deviation was calculated. The results are reported as minimum inhibitory concentration (MIC) defined as the lowest concentration at which zone of inhibition was observed after incubation at 37°C for 24 hrs.

The percentage growth inhibition was calculated by:
Percentage inhibition = (TS-SC) /PC × 100
Where TS, SC and PC represents test sample, solvent control and positive control, respectively.

Phytochemical analysis

The crude methanol extract and its fractions were screened phytochemically for the presence of tannins, alkaloids, saponins, carbohydrates, flavonoids, steroids, phlobatannins, terpenoids, cardiac glycosides by following the standard methods of analysis (Bibi *et al*., 2012).

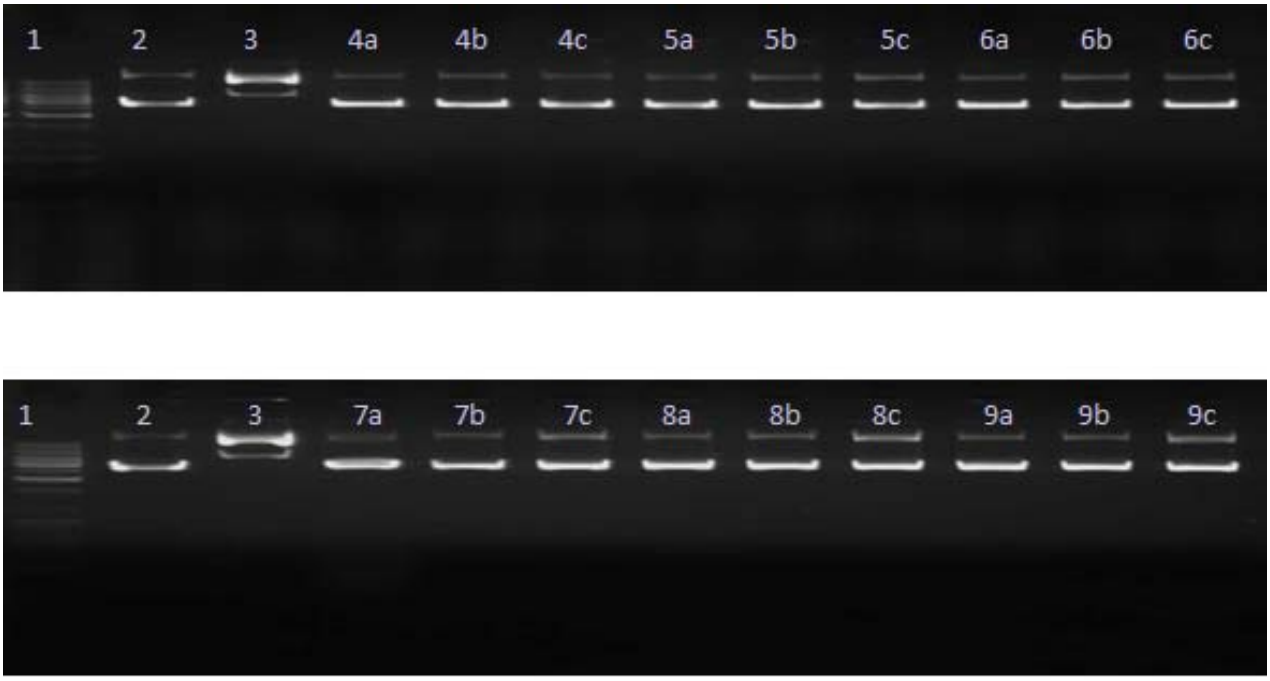

Fig. 1: DNA protection effect of crude methanol extract and different fractions of *Bergenia ciliata* rhizome. The labeling is; 1=1 Kb DNA Ladder, 2=Plasmid DNA (pBR 322), 3=Plasmid DNA treated with $FeSO_4$ and H_2O_2, a=1000 µg/ml, b=100 µg/ml, c=10 µg/ml, 4=Crude extract, 5=n-hexane fraction, 6=n-butanol fraction, 7=ethyl acetate fraction, 8=aqueous fraction (ppt.), 9=aqueous fraction (spt.)

STATISTICAL ANALYSIS

All the tests were performed in triplicate as independent experiments. The data obtained from cytotoxic activity of fractions was further analyzed for analysis of variance (ANOVA) and Least Significant Difference (LSD) test at $p<0.05$ using MSTATC to determine the significance of percentage inhibition values between the extracts.

RESULTS

In the present investigation, crude extract as well as fractions of *B. ciliata* showed effective free radical scavenging activity. Crude extract showed promising antioxidant activity with IC_{50} 80.50 µg/ml while among fractions, n-butanol fraction had maximum antioxidant activity (IC_{50} 142.62 µg/ml).

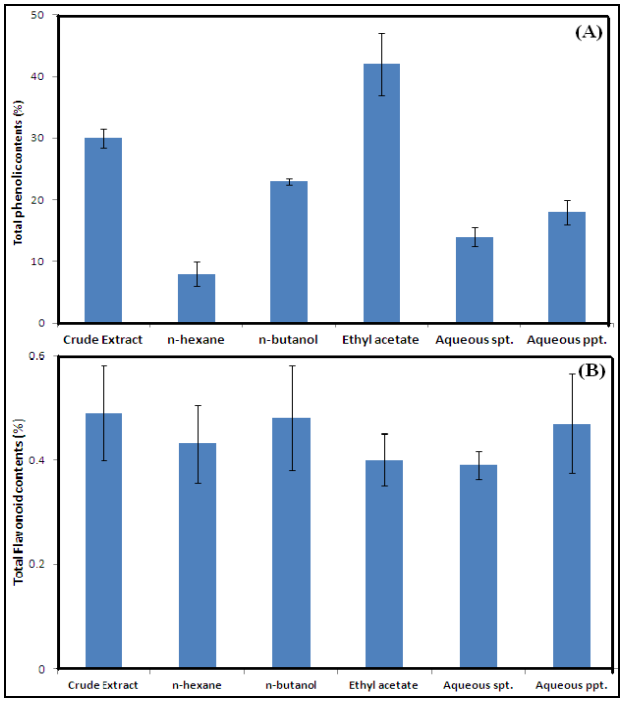

Fig. 2: Total phenolic (A) and total Flavonoid (B) contents of *Bergenia ciliata* crude extract and its fractions.

Other fractions; ethyl acetate fraction, aqueous fraction (ppt.) and aqueous fraction (spt.) had IC_{50} values of 186.71, 198.41 and 247.04 µg/ml, respectively (table 1). The crude extract and fraction scavenged DPPH free radical however it is clear that these extracts did not damage DNA (fig. 1).

The total phenolic content of the plant extract and fractions is shown in fig. 2(A). Among crude extract and fractions, ethyl acetate fraction contained highest phenolic content (42.13%), followed by n-butanol fraction (23.32%), crude extract (30.08%), aqueous fraction (ppt.) (18.57%), aqueous fraction (spt.) (14.05%) and n-hexane

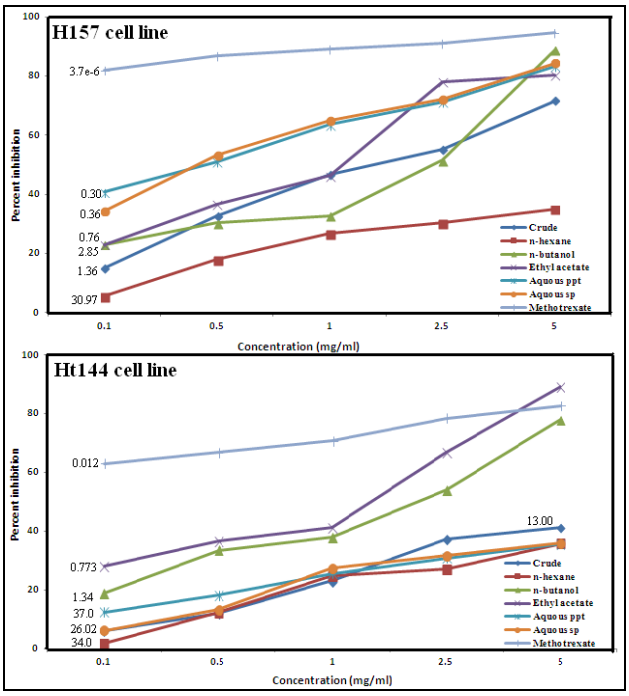

Fig. 3: Inhibition of H157 and Ht144 cell lines by crude extract and various solvent fractions of *Bergenia ciliata*. The values shown with graph lines shows IC_{50} (mg/ml) values of respective extract.

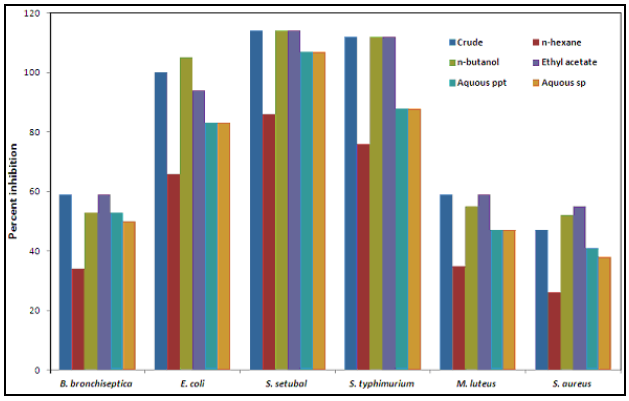

Fig. 4: Percent inhibition of bacterial cultures by crude extract of *Bergenia ciliata* and its various solvent fractions.

fraction (8.71%). Determination of total flavonoid contents in crude extract and fractions revealed that crude extract presented highest content (0.493%) followed by n-butanol fraction (0.49%) >aqueous fraction (ppt.) (0.47%) >n-hexane fraction (0.42%) >ethyl acetate fraction (0.40%) >aqueous fraction (spt.) (0.39%) (fig. 2(B)).

Crude extract and fractions also showed increase in activity against both cell lines (H157 Lung Carcinoma and HT144 Malignant Melanoma) at increasing concentrations. It is worth mentioning that ethyl acetate fraction exhibited more percent inhibition of Ht144 cells as compared with methotrexate (positive control) at equal

concentration and approximately same activity was shown by n-butanol fraction. These fractions along with aqueous fraction (spt.) also showed pronounced activity against H157 cell line. The aqueous (ppt.) of *B. ciliata* crude extract (fig. 3) showed highest cytotoxicity on H157 cell line (IC_{50} value 0.30 mg/ml) followed by aqueous spt. and ethyl acetate fraction (0.36 mg/ml and 0.76 mg/ml, respectively). Against Malignant Melanoma (HT144) cells; the cytotoxic effect of ethyl acetate fraction was highest (fig. 3) with IC_{50} value of 0.773 followed by n-butanol fraction having IC_{50} of 1.34 mg/ml. The crude extract of *B. ciliata* shows nearly same results as that of H157 cell line and its IC_{50} value was 13.0 mg/ml.

The crude extract and all fractions except n-hexane fraction presented significant activities against all bacterial strains tested. The crude extract demonstrated 16-20 mm zone of inhibition against Gram positive and Gram negative bacterial strains while amongst fractions, ethyl acetate fraction showed more prominent zone of inhibition followed by n-butanol fraction (table 2). It is worth mentioning that crude extract, n-butanol and ethyl acetate fraction showed more than 100% inhibition as compared with roxithromycin (positive control) against *S. setubal* and *S. typhimurium*. All the fractions showed above 60% inhibition of *E. coli* while percent inhibition was 30-58% against *B. bronchiseptica*, *M. luteus* and *S. aureus* (fig. 4). Ethyl acetate fraction presented 0.4 mg/ml MIC against *B. bronchiseptica* and *M. luteus*. Table 3 shows that crude extract and other fractions also showed significant MIC (0.6-5 mg/ml) against all bacterial strains tested.

Phytochemical analysis reveals presence of different classes of phytochemicals in crude methanol extract and these distributed among the fractions depending upon polarity. Table 4 shows that tannins, phlobatannins and cardiac glycosides were present to higher extant

Table 1: Percentage scavenging and IC_{50} of antioxidant assay of crude extract and fractions of *Bergenia ciliata* rhizome

Extract	Concentrations (µg/ml)					IC_{50}
	1000	500	250	125	62.5	
Crude extract	84	83	81	56	40	80.50
n-hexane	82	40	28	22	20	440
n-butanol	82	80	62	45	34	142.62
Ethyl acetate	96	92	88	80	41	186.71
Aqueous ppt.	84	80	52	36	30	198.41
Aqueous spt.	84	82	51	32	28	247.04

Ascorbic acid was used as positive control and its IC_{50} value was 5.54 µg/ml

Table 2: Antibacterial activities of crude extract and fractions of *Bergenia ciliata* rhizome

Extracts/Fractions	Zone of inhibition (mm)					
	B. brochiseptica	*E. coli*	*S. setubal*	*S. typhimirum*	*M. luteus*	*S. aureus*
Crude extract	19.0±0.7	18.0±0.5	16.0±0.6	19.0±0.2	20.0±0.2	16.0±0.2
n-hexane	11.0±0.5	12.0±0	12.0±0.0	13±0	12.0±0	9.0±0.3
n-butanol	16.5±1.0	19.0±0.2	16.0±0.5	19.0±0.2	19.0±0.2	18.0±0.1
Ethyl acetate	19.0±0.5	16.5±0.5	16.0±0.5	19.0±0.5	20.0±0.5	19.0±0.1
Aqueous ppt.	17.0±0	15.5±0.2	15.0±0.3	15.0±0.3	15.5±0.3	14.0±0.3
Aqueous spt.	16.0±0	15.5±0	15.0±0.2	15.0±0.3	16.5±0	13.0±0.1
Roxithromycin	32.5±0.1	18.5±1.5	14.0±0.3	17.0±0.5	34.0±0.5	34±0.2
DMSO	0±0	0±0	0±0	0±0	0±0	0±0

Table 3: The results of MIC for antibacterial activities of crude extract and fractions of *Bergenia ciliata* rhizome

Extracts/ Fractions	Minimum inhibitory concentration (mg/ml)					
	B. bronchiseptica	*E. coli*	*S. setubal*	*S. typhimirum*	*M. luteus*	*S. aureus*
Crude extract	0.8	5.0	10.0	10.0	0.6	2.0
n-hexane	2.5	10.0	10.0	10.0	2.5	10.0
n-butanol	0.8	5.0	10.0	10.0	0.6	1.5
Ethyl acetate	0.4	5.0	5.0	5.0	0.4	0.8
Aqueous ppt.	1.5	10.0	10.0	10.0	1.5	5.0
Aqueous Spt.	1.5	10.0	10.0	10.0	1.0	10.0

Table 4: Phytochemical analysis of crude extract and fractions of *Bergenia ciliata* rhizome

Constituents/test	Extracts/Fractions					
	Crude extract	N-hexane	N-butanol	Ethyl acetate	Aqueous ppt.	Aqueous spt.
Alkaloids						
Dragendoff's	-	N.C.	-	-	-	-
Mayer's	-	N.C.	-	-	-	-
Wagners	-	N.C.	-	-	-	-
Flavonoids						
Harborne	+	N.C.	+	+	+	-
Steriods						
Liebermann-Burchard	-	N.C.	-	-	-	-
Saponins						
Frothing Test	++	N.C.	-	++	+	++
Tannins						
$FeCl_3$ Test	+++	N.C.	-	+++	++	+++
Phlobatannins	-	N.C.	-	-	-	-
Terpenoids	+++	N.C.	+	+++	-	-
Cardiac Glycosides	-	-	-	-	-	-
Keller-Kiliani	+++	N.C	+++	+	++	++

N.C.= Not checked, + = weekly present, ++ = moderately present, +++ = highly present and - = not present

approximately in all extracts followed by saponins and steroids.

DISCUSSION

Natural products have been traditionally employed since prehistoric times for the maintenance of general health conditions and management of various ailments and provide clues to investigate and isolate bioactive components in modern era. The ethnic properties of *Bergenia ciliata* aggravated us to investigate pharmacological activities and it is found that this plant is potential candidate for isolation of antioxidant, anticancer and antibacterial components.

Free radicals may attack life-important molecules such as DNA, protein, lipid, membrane lipids and carbohydrates and play vital role in the pathology of numerous diseases (Pranas et al., 2010). Antioxidant compounds scavenge or prevent the formation of free radicals and as a result prevent the deterioration caused by them. Due to DNA damaging effects and enhanced toxicity of synthetic antioxidants, natural antioxidants are gaining popularity (Kumar *et al*., 2013). Oxidative DNA damage from ROS plays a vital function in several biological processes such as mutagenesis, aging, carcinogenesis, radiation effects, and the cancer (Christo *et al*., 2012). Evidences have shown an inverse correlation between the intake of antioxidants and the risk of above said chronic diseases (Maria *et al*., 2009). The $DPPH^{\bullet}$ scavenging capacity assay and DNA protection assay are considered to be reliable and easy methods for antioxidant property evaluation (Widad *et al*. 2013). Maximum activity by aqueous fraction is a clue for presence of compounds polar in nature. Logically, water decoctions have traditionally been used to treat ailments (Bibi *et al*., 2011).

Phenolics and flavonoids are secondary metabolites present in various plant parts. These compounds have properties to neutralize the active moieties during the metabolism such as free radicals and may prevent pathological conditions involving free radicals including cancer, cardiovascular and neurodegenerative anomalies (Maria *et al*., 2009). Both phenolics and flavonoids produce their antioxidant activity by acting as hydrogen or electron donor to free radicals, by reacting with radicals to form less reactive compounds or by chelating transition metals which may act as prooxidants and can lead to degeneration of body systems (García-Cruz *et al*., 2013; Abdelhady and Aly, 2013). It is well recognized that the flavonoids show antioxidant activities and have substantial effects on human nutrition and health care (Kahkonen *et al*., 1999). Naturally occurring phenolics and flavonoids act as important antioxidant compounds, scavenge the free radicals and prevent the damage caused by them. Both polyphenols and flavonoids intake can reduce the chances of coronary heart diseases and tumor formation. Similarly also contain anti-inflammatory activities due to the inhibition of lipoxygenase and cyclooxygenase enzymes (Tian and Hua, 2005). There are several reports on the relationships between phenolic content and antioxidant activity (Kahkonen *et al*., 1999). Such reports indicate possible role of phenolics and

flavonoids for the inhibition of free radicals and provide basis for the content quantification in *B. ciliata*. The difference of total phenolic contents in various fractions utilized in the study may be due to the nature of polarity of different solvents and the deviation of phenolics solubilized and eluted from the solvent.

Carcinogenesis is a multistep process involving initiation, promotion and progression. Initiation mainly involves the activation of the process due to the change in the genetic makeup of cells due to the presence of carcinogens or inactivation of DNA repair mechanism. Promotion involves the over production or division of mutated cells whereas progression means the outnumbering of tumor cells in comparison to normal counterparts (Haq *et al*., 2013). SRB assay is considered most sensitive and less expansive to investigate anticancer potential of plant extracts. Many plants have been reported carrying anticancerous potential against different cell lines (Skehan *et al*., 1990; Nisa *et al*., 2013). Significant anticancerous activity by *B. ciliata* extract and fractions against tested cell lines in the current study might be attributed to the presence of anticancerous compounds, need to be identified and isolated for further investigation.

Stupendous antibacterial activities define subject plant as a good candidate for isolation of active constituents against these bacterial strains. Maximum activity by aqueous fraction might be due to presence of compounds that are water soluble in nature (Rizvi *et al*., 2013) and that are consistently produced in plant and cell culture (Bibi *et al*., 2012).

CONCLUSIONS

Present investigation concludes that *Bergenia ciliata* rhizome crude extract and fractions have DPPH activity, flavonoid and phenolic contents and IC_{50} values fall within acceptable range. Along with this, the DNA protection capability is also in good agreement. The ethyl acetate fraction showed IC_{50} 0.76 and 0.73 mg/ml against H157 and Ht144 cell lines, respectively. The aqueous extract is also potent anticancer against H157 cell line with IC_{50} 0.3-0.36 mg/ml. Crude extract and fractions also showed MIC<1.0 mg/ml against different bacterial strains. All other MIC values are <10 mg/ml describing potential antibacterial contents. *B. ciliata* is a good candidate for isolation of antioxidant, anticancer and antibacterial compounds that can be a break through for pharmaceutical industry.

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