Phytochemical screening, antiglycation and antioxidant activities of whole plant of *Boerhavia repens* L. from Cholistan, Pakistan

Fariha Nazneen[1], Munir A. Sheikh[1], Amir Jameel[1] and Zia-ur-Rahman[2]*
[1]Department of Biochemistry and University of Agriculture, Faisalabad, Pakistan
[2]Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

Abstract: Present study was aimed to explore a traditionally used indigenous medicinal plant *Boerhavia repens* (Nyctaginaceae family) of the Cholistan desert, Pakistan. Crude aqueous and methanolic extracts of the whole plant were investigated *in vitro* for preliminary phytochemical screening, antioxidant and antiglycation activities. Antioxidant activities were determined by total phenolic contents, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and inhibition of lipid peroxidation. For antiglycation activities browning production was noted and thiobarbituric acid (TBA) technique was used to determine glycation level. *Boerhavia repens* expressed considerable amounts of phytochemicals. Extract yield was found to be 4.59%-7.85% g/100g of dry matter with total phenolics ranging from 47.9- 190.77mg/GAE per g for aqueous and methanol extract respectively. Strong inhibitory effect was exhibited by methanolic extract in linoleic acid per oxidation system (86.11%, EC_{50}=0.99mg/mL) and DPPH assay (88.65%, EC_{50}=212.33µg/ml). In term of browning maximum inhibition (81.50%) was exhibited by methanolic extract at 37°C at third week of incubation. Both extracts expressed significant (P>0.05) and comparable inhibition of glycation level. In conclusion, *Boerhavia repens* showed promising antioxidant and antiglycation activities validating its therapeutic potential.

Keywords: *Boerhavia repens*, antioxidant, antiglycation.

INTRODUCTION

Herbal remedies are known to cure different ailments of mankind since the ancient times. Medicinal plants are benefiting through various useful phytoconstituents, such as vitamins, nitrogen compounds, phenolics, terpenoids and secondary metabolites. Many valuable bioactivities are expressed including antioxidant, anti-inflammatory, anti-carcinogenic, antimutagenic, antitumor, antiviral, antibacterial and antifungal activities. Thus present day research of chemists, biochemists and pharmaceutics mainly focuses medicinal plants to explore the scientific reasoning behind the traditional usage (Maridass and Britto, 2008).

Reactive oxygen species (ROS) generation damages biomolecules and triggers mechanisms underlying various chronic and degenerative diseases. Antioxidants play a vital role to combat the harmful effects of free radicals and ROS. Consequently, phytochemicals are the essential antioxidant source and inhibit lipid per oxidation and scavange free radicals effectively exerting remedial action, thus oxidative stress is countered in prophylactic manner (Sen *et al*., 2010; Siham *et al*., 2014).

Non enzymatic binding of glucose to amino groups containing biomolecules like proteins, lipids or nucleic acids give rise to a reversible glycosylation product known as a Schiff base. Further chemical rearrangements cause production of irreversible advanced glycation end products (AGE), which are stable and resistant to proteolysis. It takes a long time to establish the phenomenon of advanced glycation end products and their deposition can have worst consequences (Golon *et al*., 2014). In diabetes hyperglycemia causes Oxidative stress and enhance free radical formation due to auto-oxidation of glucose, further interrupts the electron transport chain increasing the formation of AGE (Elosta *et al*., 2012; Neelofar and Ahmad 2015).

Therefore, diabetes and its complications can be treated by natural extracts or compounds that possess antioxidants as well as antiglycation bio activities and offer great curative potential (Chen *et al*., 2011; Esmaeili 2014 and Khan *et al* 2014).

Boerhavia repens L. of family Nyctaginaceae is a potential ethno pharmacological herb throughout the world (Mishra *et al*., 2014). Several natural products are established by phytochemistry and its various extracts are in use for treating diseases with no or minimum side effects (Najam *et al*., 2008). A lot of work has been done on the *B. diffusa* roots for chemical constituents like flavonoids, alkaloids, lignans (liriodendrons), β-sitosterols, rotenoids (boeravinone A-F), amino acids, tetracosanoic, esacosanoic, ursolic and stearic acid (Rajpot and Mishra, 2011;Riaz *et al*., 2014). Roots of this plant were sub fractionated by different polar solvents like hexane, ethanol, chloroform and ethyl acetate in a study. Triterpenes were present in hexane fraction, glycosides in ethyl acetate, flavonoids in chloroform fraction and tannins were found in ethanolic extract (Darsini *et al*., 2009).

**Corresponding author:* e-mail: drziar@yahoo.com

Traditionally this plant is useful in treating fever, inflammations, constipation, anemia, cardiac ailments, jaundice, ophthalmia, bronchitis and dyspepsia (Nadeem *et al*., 2012; Dey *et al.*, 2013). Current study was carried out to investigate *B. repens* with respect to various phytoconstituents, antioxidant and antiglycation activities to reveal the rationale behind the ethano medicinal usage of this important plant.

MATERIAL AND METHODS

Plant collected from Cholistan desert, Pakistan was authenticated from Dr Mansoor Hameed (Associate Professor) at Department of Botany University of Agriculture Faisalabad, Pakistan (voucher specimen No. 2086) and deposited at departmental Herbarium.

Chemicals and reagents

Acetic acid, ammonium hydroxide, diethyl ether, *n*-butanol, sodium chloride, methanol, ethanol, glacial acetic acid, 1,1 Diphenyl-2-picrylhydrazyl radical (DPPH) (sigma, 90.0%), Folin-ciocalteau reagent etc. were purchased from Merck (Darmstadt Germany) and sigma chemicals co (st, Louis, MO, USA). Analytical grade chemicals and reagents were used in this study.

Pytochemical qualitative analysis

Qualitative test of Boerhavia plant was performed to identify the cardiac glycosides, saponins, tanins and steroids using the method of Harborne (1998). Plants flavonoids were determined as described by Sofowara (1993).

Quantitative analysis

Air dried samples of plants were ground into a fine powder in a grinding mill (Tector-Cemotec 1090 sample mill, Hognas, Sweden) to obtain crude extract.

Alkaloids

Alkaloids were quantitatively determined according to the method of Harborne (1998).

% yield of Alkaloid=(Weight of Alkaloid obtained/Total weight of sample)×100

Flavonoids

Flavonoids were quantitatively determined as described by Edeoga *et al*., 2005.

% yield of Flavonoids=(Weight of flavonoids obtained/Total weight of sample)×100

Saponins

Quantitative determination of saponins was done according to Obadoni and Ochuko (2001). % yield of Saponins= (Weight of Saponins obtained/Total weight of sample)×100

Preparation of plant extract

Pulverized plant sample (40g) was extracted with 400mL of 80% methanol in water (80:20 v/v) using an orbital shaker (Gallenkamp, UK) for 8h at room temperature. The extract was separated from the solid by filtration. The residue was extracted twice with the same solvent and then these extracts were combined. This procedure was repeated for aqueous extraction where same amount of plant material (40g) was taken. The extracts were concentrated under reduced pressure at 45°C, in a rotary evaporator (EYELA, Tokyo, Japan). The concentrated extracts were weighed and percentage of yield (w/w) was calculated.

Antioxidant activities

The assays which were employed for evaluation of antioxidant activity of aqueous and methanol extracts are given below:

Total phenols

The total phenol contents in the extracts were determined using the Folin-Ciocalteau reagent. The standard curve was made with Gallic Acid standard solution (fig. 1) (Albano and Miguel., 2011).

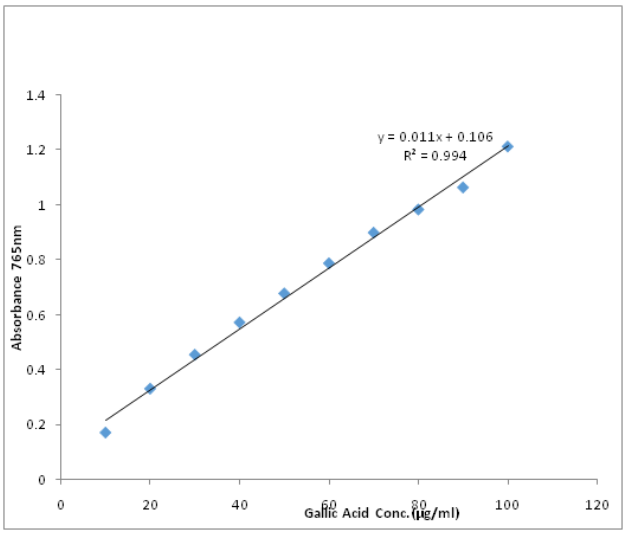

Fig. 1: Standard Curve For Total Phenols

Antioxidant activity against lipid per oxidation

The antioxidant activity of the 90% aqueous ethanol extracts against lipid peroxidation was measured using the thiocyanate method (Shi *et al*. 2011). Butylated hydroxyl toluene (BHT) was employed as a reference and the antioxidant activity was expressed as percent inhibition relative to control using the equation:

Lipid Peroxide Inhibition % =1-(sample absorbance at 48 h-sample absorbance at 0 hr/ Control absorbance at 48 hour-Control absorbance at 0 hr×100

DPPH radical scavenging assay

The free radical scavenging potential of extracts was evaluated by spectrophotometric assay, quantifying their capacity to bleach a purple solution of DPPH in methanol

(Shivhare *et al*. 2010). Different concentration of each plant extract were prepared in methanol and 3mL of each solution was mixed with 1mL of 0.1mM methanolic DPPH solution. After a 30 min incubation period at room temperature, each absorbance (A) was determined at 517 nm. Butylated hydroxyl toluene (BHT) was used as positive control. Inhibition percentage of DPPH (I %) was calculated as follows:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

EC_{50} of sample was obtained from a plot of percentage inhibition verses extract concentration.

Antiglycation activities

Normal human plasma was used as a protein and glycated albumin was prepared. In brief plasma was incubated at 37C° and 50C° for five weeks in a solution of D-glucose (25mM) in 75m M phosphate buffer (pH 7.4) in the presence or absence of plant extract. To prevent bacterial contamination 0.02%w/v sodium azide (NaN3) was added.

To prepare inhibitor 0.1g of each plant extract was added to 10mL of phosphate buffer. To study the effect of natural inhibitors from plants on non-enzymatic glycation, different combinations of plasma, inhibitor and glucose were made. Combination of plasma and glucose was used as control. Negative control was used by adding inhibitor to combination of buffer and plasma against test samples.

Aliquots (0.1mL sample was drawn and diluted with 3.9mL distilled water) from plasma-glucose solution after each incubation period (Zhang and Swaan 1999). Browning production was recorded by taking absorbance at 370nm. Samples were dialysed extensively to remove free glucose molecule and were further analyzed for glucose and protein concentration before and after dialysis to monitor the sensitivity and validity of test. Enzymatic glycation (EN) and non-enzymatic glycation was determined by TBA method (Furth 1988) to evaluate glycation level and percentage inhibition was calculated.

STATISTICAL ANALYSIS

Data was entered and analyzed by SPSS-21. Quantitative variables were expressed as the mean ± standard error of the mean (SEM). one-way ANOVA was applied to find out level of significance. $P<0.05$ was considered to be statistically significant.

RESULTS

Qualitative analysis

Preliminary analysis of *B. repens* indicated appreciable amounts of various phytoconstituents including steroids, saponnins, alkaloids and tannins (table 1) with weak presence of flavonoids and glycosides.

Quantitative analysis

Quantitatively *B. repens* expressed high contents of alkaloids (4.22%) and flavonoids (3.58%) with low saponin contents (0.16%) (table 2).

Table 1: Qualitative analysis of crude extract of *Boerhavia repens*

Phytoconstituents	Qualitative Analysis
Tannins	+++
Saponins	+++
Alkaloids	+++
Flavonoids	+
Glycosides	+
Steroids	+++

+++ indicates strong presence of constituent + indicates weak presence of constituent

Table 2: Quantitative (%) analysis of crude extract of *Boerhaviarepens*

Phytoconstituents	Quantitative (%)
Saponins	0.16±0.02
Alkaloids	4.22±0.23
Flavonoids	3.58±0.08

Extracts yield

The aqueous and methanolic extracts of *B. repens* were weighed for dry matter yields (g/100g of dry material) and ranged from 4.59%-7.85%g/100g of dry material (table 3).

Table 3: Percentage Yield (g/100g) and Total Phenolics expressed as GAE from aqueous and methanolic extract of *Boerhaviarepens*

Boerhavia repens	Aqueous extract	Methanol extract
Yield (%)	4.59 ± 0.02^{b}	7.85 ± 0.05^{a}
Total Phenolics (µg/mL)	47.91 ± 2.51^{b}	190.77 ± 4.63^{a}

ab, similar alphabet do not differ significantly at $P>0.05$

Total phenolic contents

Data for total phenolic contents of *B. repens* was assimilated in table 3 and varied from 47.9- 190.77mg /GAE per g for aqueous and methanol extract respectively depicting substantial levels of methanolic extract

Inhibition of peroxidation

Inhibition of linoleic acid of aqueous and methanolic extracts was found to be varied in relation to extraction solvents (table 4). Considerable % age inhibition of per oxidation was shown by methanol extract (86.11%, EC_{50}=0.99) than aqueous extract (71.71%, EC_{50}=1.37) at maximum concentration of 2.5mg/mL expressing greater efficacy of methanolic extract.

Table 4: Mean Inhibition of peroxidation (%) of aqueous and methanolic extract of *Boerhavia repens* at different concentrations in linoleic acid system

Concentration (mg/mL)	Aqueous Extract	Methanolic extract	BHT
0.5	33.64 ± 1.25^{op}	41.15 ± 0.27^{m}	31.79 ± 0.78^{o}
1	43.72 ± 0.54^{m}	48.97 ± 0.27^{l}	52.78 ± 0.89^{k}
1.5	50.21 ± 0.63^{l}	57.61 ± 0.27^{j}	61.93 ± 1.07^{gh}
2	62.57 ± 0.70^{i}	73.35 ± 0.45^{ef}	70.68 ± 0.53^{f}
2.5	71.71 ± 0.54^{fgh}	86.11 ± 0.36^{b}	88.99 ± 0.98^{b}
EC_{50}	1.37	0.99	1.07
Mean of DPPH radical scavenging activity (%) of aqueous and methanol extract of *B. repens*			
Concentration (µg/mL)	Aqueous Extract	Methanolic extract	BHT
100	$23.76\pm1.23l^{m}$	7.61 ± 0.20^{pq}	12.61 ± 0.75^{no}
200	33.57 ± 0.93^{j}	37.52 ± 0.52^{j}	32.14 ± 0.76^{k}
300	44.10 ± 1.02^{gh}	43.45 ± 0.63^{hi}	40.90 ± 0.83^{ij}
400	57.09 ± 0.99^{ef}	69.27 ± 0.55^{d}	55.21 ± 1.57^{e}
500	76.49 ± 0.87^{c}	88.65 ± 0.89^{ab}	85.63 ± 0.38^{b}
EC_{50}	323.19	212.33	263.54

a-q, similar alphabet do not differ significantly at P>0.05

Table 5: Mean inhibition of browning of aqueous and methanolic extract of *B.repens* at different weeks at 37°C and 50°C

Weeks	37°C		50°C	
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
1	16.66 ± 0.29^{pq}	55.55 ± 0.96^{g}	7.33 ± 0.13^{m}	41.33 ± 0.72^{jk}
2	31.66 ± 0.55^{ijk}	62.22 ± 1.08^{f}	36.15 ± 0.63^{i}	46.53 ± 0.81^{hi}
3	53.07 ± 0.92^{de}	81.50 ± 1.41^{b}	43.87 ± 0.76^{gh}	54.51 ± 0.94^{efg}
4	22.70 ± 0.39^{mno}	43.24 ± 0.75^{k}	50.92 ± 0.88^{def}	70.37 ± 1.22^{bc}
5	14.80 ± 0.26^{q}	-----	18.33 ± 0.32^{l}	38.33 ± 0.66^{kl}

a-o, similar alphabet do not differ significantly at P>0.05

DPPH radical scavenging assay

Free radical scavenging activity of aqueous and methanolic extracts was found to be 76.49% and 88.65% respectively at maximum concentration in the present analysis. In term of EC_{50} methanol extract (EC_{50}=212.33µg/mL) exhibited greater efficacy than aqueous extract (EC_{50}=323.19µg/mL).

Browning determination

Analysis of aqueous and methanolic extract for browning production exerted by *B. repens* was found to be increasing with increase in incubation temperature and attained maximum level in 5th week. In term of percentage inhibition maximum antiglycation potency (81.50%) was observed at 3rd week of incubation (table 5) by methanol extract at 37°C as compared to aqueous extract .In similar manner browning production was found to be inhibited (54.51%) at 3rd week of incubation (table 5) by methanol extract at 50°C as compared to aqueous extract.

Glycation level by TBA method

Dialyzed samples were estimated for glycation level by TBA method. Glucose concentration of 25mM (hyperglycemic condition) was incubated with plasma to produce glycation for a time period of 5 weeks. Results of present analysis indicated maximum glycation (3.73mole/mole) at 1st week of incubation attaining minimum level (0.63mole/mole) at 3rd week of incubation by aqueous extract at 37°C with comparable values for methanol extract (table 6). At 50°C minimum glycation level (1.56) was expressed at 2nd week of by aqueous extract whereas for methanol extract, it was obtained at 3rd week of incubation. Maximum percentage inhibition was observed at 3rd week of incubation by all the extracts except aqueous extract at 50°C, which showed significant %age inhibition (P>0.05) at 2nd week of incubation (table 7).

DISCUSSION

The whole plant of *B. repens* L. was subjected to preliminary phytochemical screening, expressing strong presence of steroids, saponnins, alkaloids and tannins. Quantitatively *B. repens* showed considerable amount of alkaloids and flavonoids with comparatively low saponin contents (table 2). Alkaloidal crude percentage was 4.22% (table 2) in contrast to another study who found only 0.15% in dried root powder of *B. diffusa* (synonym of *B. repens*)(Rachh *et al*., 2009). Difference in yield may be attributed to the difference in plant part.

Table 6: Mean of Glycation Level of aqueous and methanolic extract of *B.repens* at different weeks at 37°C and 50°C mole/mole

Weeks	37°C			50°C		
	Control	Aqueous extract	Methanolic extract	Control	Aqueous extract	Methanolic extract
1	4.12±0.07^{e}	3.73±0.06^{f}	3.31±0.06^{ef}	5.19±0.09^{e}	4.67±0.08^{fgh}	3.49±0.06^{h}
2	5.75±0.10^{b}	2.65±0.05^{ij}	1.70±0.03^{jk}	7.84±0.14^{c}	1.56±0.03^{rst}	5.20±0.09^{e}
3	8.70±0.15^{a}	0.63±0.01^{o}	0.68±0.01^{op}	14.02±0.24^{a}	3.05±0.05^{j-m}	2.80±0.05^{jk}
4	5.38±0.09^{c}	2.43±0.04^{jk}	2.37±0.04^{g}	10.77±0.19^{b}	6.85±0.12^{d}	4.80±0.08^{ef}
5	4.23±0.04^{k}	2.18±0.02^{m}	2.21±0.02^{mn}	8.89±0.05^{k-n}	6.74±0.05^{lmn}	6.83±0.04^{h}

a-p, similar alphabet do not differ significantly at P>0.05

Table 7: Mean of inhibition (%) of glycation level of aqueous and methanolic extract of *B.repens* at different weeks at 37°C and 50°C

Weeks	37°C		50°C	
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
1	9.46±0.16^{l}	19.66±0.34^{kl}	10.01±0.17^{qr}	32.75±0.57^{ij}
2	53.91±0.93^{c-f}	70.43±1.22^{e}	80.10±1.39^{cde}	33.67±0.58^{ij}
3	92.75±1.61^{a}	92.18±1.60^{a}	78.24±1.36^{def}	80.02±1.39^{cde}
4	54.83±0.95^{cde}	55.94±0.97^{fg}	36.39±0.63^{o}	55.43±0.96^{h}
5	48.46±0.82^{ghi}	47.75±0.79^{ij}	24.18±0.09^{k}	23.17±0.35^{k}

a-o, similar alphabet do not differ significantly at P>0.05

Methanolic extract showed greater yield than aqueous extract with highest total phenolics (table 3). Effectiveness of extracting solvent is an important factor to dissolve varying plant components endogenously. Phenolics has been proved to be extracted by methanol effectively (Al-Temimi and Choudhary 2013). Depending upon solvent, phytochemical analysis of *Blepharis repens (vahl) Roth* (Rajan *et al*., 2013) exhibited maximum %age yield for the ethanolic extract ranging from 1.11-4.5%. Phenolics have acquired great significance due to their ability to scavange free radicals (Sultana *et al*., 2007). Difference in total phenolic contents is attributed to varying plant species, maturity at harvest, growing conditions, post harvesting treatment and soil conditions as well as extraction techniques (Pak-Dek *et al*., 2011). In another study methanolic extract was determined for total phenolics for 24 different plants of Nepal (Ghimire *et al*., 2011) ranging from 28.87±2.36 to 156±0.62 except one plant which has highest total phenolic contents (321.23±1.06mg GAE/g of extract) as compared to current findings of phenolic contents in methanolic extract 190.77±1.13mg GAE /g of extract.

Determination of antioxidant activity can be done by determining the ability of medicinal plants to prevent lipid peroxidation from oxidation (Kotebagilu *et al*., 2015). Aqueous extract showed less inhibition of peroxidation (71.17±0.54%) than methanolic extract (86.11±0.36%) and butylatedhydroxytoluene (BHT) 88.99±0.98% (table 3). In term of EC_{50}methanolic extract (0.99mg/mL) was more effective than aqueous extract (1.37mg/mL) and BHT (1.07mg/mL) (table 4). These findings were comparable with another study who reported I/EC 50=0.97±0.01mg/mL in lipid per oxidation inhibition assay (Konan *et al*., 2013).

DPPH assay is a remarkable method to determine *in vitro* antioxidant activities due to its sensitivity and is found to be adjustable for many samples at the same time (Guleria *et al*., 2013; Shekhar and Anju, 2014). Profound inhibition of free radical scavenging by DPPH method was determined for aqueous (76.49±0.87) as well as methanolic extract (88.65±0.89) of *B. repens* as compared to control (85.63±0.38). Methanolic extract was found to be more effective than aqueous extract (table 4). In another study (Gopal *et al*., 2010) roots of *B. Diffusa* exerted comparable antioxidant potential by the ethanol extract (81.94% inhibition) further supporting our results.

Protein AGE adducts are measured by determining browning production (Miroliaei *et al*., 2011). A significant decrease of glycation reaction (% age inhibition) explore the potential of plant to act as natural inhibitor. *Boerhaviarepens* was treated for two temperatures 37C° and 50C°. Increased temperature accelerated the glycation level *in vitro* that takes months or years to complete *in vivo* (Matsuura *et al*., 2002). Mean browning of B*. repens* was attained with increase in incubation period and raised temperature. At 3rd week of incubation significant inhibition was found by aqueous as well as methanolic

extract at 37°C but more profoundly exerted by methanolic extract (81.50±1.41%). At 50°C formation of browning products was accelerated but inhibition of glycation was decreased, exhibiting low antiglycation potential of plant at this temperature (table 5). Methanolic extract showed maximum inhibition 70.37% at 4th week of incubation as compared to aqueous extract (50.92%). Vhangani and Wyk 2013 detrmined the browning intensity as a function of temperature and time supporting present study where browning is found to be increasing with increase in time and incubation period.

Minimum glycation level was attained by aqueous (0.63 mole/mole) as well as methanolic extract (0.68 mole/mole) at 3rd week of incubation (table 6) with maximum inhibition of about 92% (table 7) by these extract at 37°C.

At 50°C minimum mean glycation (1.56 mole/mole) was exhibited by aqueous extract at 2nd week of incubation and for methanolic extract minimum glycationlevel (2.8 mole/mole) was attained at 3rd week of incubation exerting maximum effectiveness of aqueous extract at 50°C. Glycation level was found to be decreasing after attaining maximum level in 3rd week of incubation and further decreased till 5th week of incubation due to formation of advanced glycation end products (Kousar *et al*., 2012). These results agree with the study of another group (Aslam *et al*., 2014) who found natural Inhibitor *"Malusdomistica"* to inhibit glycation. Change in concentration of glucose and inhibitors, was found to exert different fluorescence measurements with change in incubation time. *B. repens* has exerted its antiglycation potential in 2nd,3rd or 4th week depending upon solvent extract or temperature (table 7).

The results obtained from antioxidant and antiglycation assays prove the ethno pharmacological use of *B. repens*. Furthermore the methanol fraction showed comparatively higher activity than aqueous extract. This indicate that methanol solvent system contain chemical compounds that exert antioxidant and antiglycation activities as in another study (Al-Harrasi *et al*., 2013) who found higher antioxidant and antiglycation activities in polar fractions.

CONCLUSION

Keeping in mind the *in vitro* antiglycation and antioxidant potential of *B. repens* the therapeutic implication of this plant in traditional indigenous system of medicine can be well understood. *In vivo* studies are required to apprehend these activities using a rat as model. It is further suggested that active ingredients isolation with characterization of bioactive components from plant extract and structure elucidation will enable us to know the mechanism of action.

ACKNOWLEDGEMENT

The authors of this publication are highly obliged to the staff of the Department of the Biochemistry and Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad for their kind help. The authors are obliged to Mr. Amanat Ali for his technical and statistical help.

REFERENCES

Albano SM and Miguel MG (2011). Biological activities of extracts of plants grown in Portugal. *Ind . Crops Prod.*, **33**: 338-343.

Al-harrasiA, Ali L, Ceniviva E, Al-Rawahi A,Hussain J, Hussain H, Rehman N, Abbas G, and Al-Harrasi R (2013). Antiglycation and antioxidant activities and HPTLC analysis of Boswellia sacra Oleogum resin: The Sacred Frankincense. *Trop. J. Pharm. Res*., **12**: 597-602.

Al-temimi A and Chaudhary R (2013). Determination of antioxidant activity in different kinds of plants *In vivo* and *In vitro* by using diverse technical methods. *J. Nutr. Food. Sci.,* **3**: 184.

Aslam N, Ahmad R and Sheikh MA (2014). Malusdomestica as an inhibitor of glycation. *Indian J. Pharmaceutical Sci. Res*., **4**: 26-33.

ChenYF,Roan HY, Lii CK, Huang YC and Wang TS (2011). Relationship between antioxidant and antiglycation ability of saponins, polyphenols and polysaccharides in Chinese herbal medicines used to treat diabetes. *J. Med. Plants Res*., **5**: 2322-2331.

Darsini DTP, Sasikumar JM and Kulandhaivel M (2009). *In Vitro* Antioxidant and cytotoxic analysis of *Boerhaaviadiffusa* L inn. *Ethnobotanical Leaflets*. **13**: 263-68.

Dey A, Abdur Rahman SM, Chowdhury RN, Hamiduzzaman MD and Alvi MR (2013). *In Vitro* pharmacological investigations of the plant *Boerhaviarepens* (Family Nyctaginaceae). *International Res. J. Pharmacy,* **4**: 36-38.

Edeoga HO, Okwu DE and Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*., **4**: 685-688.

Elosta, A, Ghous T and Ahmad N (2012). Natural products as antiglycation agents: Possible Therapeutic potential for Diabetic Complications. *Current Diabetes Reviews*. **8**: 92-108.

Esmaeili MA (2014). Advanced Glycation End Product Inhibitory Effect of Quercetin-3-ORutinoside,Isolated from *Teucriumpolium*. *Pharm Sci.,* **19**: 125-134.

Furth AJ (1988). Method for assays non enzymatic glycation: A review. *Anal. Biochem*., **175**: 347-360.

Ghimire BK, Seong ES, Kim EH, Ghimeray AK, Yu CY, Ghimire BK and Chung IM (2011). A comparative evaluation of the antioxidant activity of some

medicinal plants popularly used in Nepal. *J. Med. Plants Res*., **5**: 1884-1891.

Golon A, Kropf C, Vockenroth I and Kuhnert N (2014). An investigation of the complexity of maillard reaction product profiles from the thermal reaction of amino acids with sucrose using high resolution mass spectrometry. *Foods*, **3**: 461-475.

Gopal TK, Harish G, Chamundeeswari D and Umamaheswara RC (2010). *In vitro* Anti-Oxidant Activity of Roots of Boerhaaviadiffusa Linn. *Res. J. Pharm. Biol. Chem. Sci*., **1**: 782-788.

Guleria S, Tiku AK, Singh G, Koul A, Gupta S and Rana S (2013). *In vitro* antioxidant activity and phenolic contents in methanol extracts from medicinal plants. *J. Plant Biochem. Biotech*., **22**: 9-15.

Harborne JB (1998). Phytochemical methods A guide to modern techniques of plant analysis. 3rd Edition. New Delhi: Springer Pvt. Ltd. pp.40-137.

KousarS, Sheikh MA and Asghar M (2012). Antiglycation activity of Thiamin-HCl and Benfoitiamine in diabetic condition. *J. Pak. Med. Assoc*. **62**: 1033-1038.

Khan N, Gothalwal R, Shrivastava NM and Shrivastava S (2014). Evaluate the effect of withania somnifera methanolic extracts as *in vitro* Anti-glycating agents. *octa. J. Biosci*., **2**: 13-17.

Konan K, David NJ, Souleymane M, Ahoua Y, Félix Yh and Joseph DA (2013). *In vitro* antioxidant activity and phenolic contents of the leaves of *Olaxsubscorpioidea* and *Distemonanthusbenthamianus*. *Res. J. Pharm. Biol. Chem. Sci*., **4**: 1419-1430.

Kotebagilu NP, Palvai VR and Urooj A (2015). Protective effect of selected medicinal plants against hydrogen peroxide induced oxidative damage on biological substrates. *International J. Medicinal Chemistry*. 2014: 1-7

Laroque D, Inisan C, Berger C, Vouland E, Dufosse L and Guerard F (2008). Kinetic study on the Millard reaction. Consideration of sugar reactivity. *Food Chem*., **111**: 1032-1042.

Maridass M and Britto JD (2008). Origins of plant derived medicines. *Ethnobotanical Leaflets.* **12**: 373-387.

Matsuura N, Aradate T, Sasaki C, Kojima H, Ohara M, Hasegawa J and Ubukata M (2002). Screening System for the Millard reaction inhibitor from natural product extracts. *J. Health Sci*., **48**: 520-526.

Miroliaei M, Khazaei S, Moshkelgosha Sand Shirvani M (2011). Inhibitory effects of Lemon balm (*Mellisaofficinalis* L.) extract on the formation of advanced glycation end products. *Food Chem*., **129**: 267-271.

Mishra S, Aeri V, Gaur PK and Jachak SM (2014). Phytochemical, Therapeutic and Ethnopharmacological overview for a traditionally important Herb: Boerhavia Diffusa Linn. *Bio Med. Res. Int*., 2014: 808302.

Nadeem S, Ahmad Z and Malik A (2012). Mini review: Chemical constituents from Boerhavia species. *Student's J. Chem*., **1**: 45-56.

Najam A, Singh AKand Verma HN(2008). Ancient and modern medicinal potential of *Boerhaaviadiffusa* and *Clerodendrumaculeatum*. *Res.Envir. Life Sci*.1: 1-4.

Neelofar K and Ahmad J (2015).Amadorialbumin in diabetic nephropathy. *Indian J. Endocrinol. Metab.,* **19**: 36-46.

Obadoni BO and Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci.,* **8**: 203-208.

Pak-Dek MS, Osman A, Sahib NG, Saari N, Markom M, Hamid AA and Anwar F (2011). Effects of extraction techniques on phenolic components and antioxidant activity of Mengkudu (*Morindacitrifolia* L.) leaf extracts. *J. Med. Plant Res*., **5**: 5050-5057.

Rachh PR, Rachh MR, Modi DC, Shah BN, Bhargava AS, Patel NM and Ruparelia MT (2009). In vitro Evaluation of Antioxidant Activity of Punarnava (*Boerhaviadiffusa* Linn). *Int. J. Pharm. Res*., **1**: 36-40.

Rajan M, Senthilkumar N and Jeyabal G (2013). Antidiabetic, antihyperlipidaemic and hepatoprotective activity of whole plant ethanolic extract of *Blepharisrepens* (vahl) Roth in normal and allaxon induced diabetic rats. *Int. J. Biol. Pharm. Res*., **4**: 802-808.

Rajpoot K and Mishra RN (2011). *Boerhaaviadiffusa* roots (Punarnavamool) Review as Rasayan (Rejuvenator / Antiaging). *Int. J. Res. Pharma. Bio. Sci*., **2**: 1451-1457.

Riaz H, Raza SA, Hussain S, Mahmood Sand Malik F (2014). An overview of ethno pharmacological properties of *Boerhaaviadiffusa*. *Afr. J. Pharm. Pharmacology*, **8**: 49-58.

Sen S, Chakaborty R, Sridhar C, Reddy YSR and De B (2010). Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. *Int. J. Pharma. Sci. Rev. Res.,* **3**: 91-100.

Shekhar TC and Anju G (2014). Antioxidant activity by DPPH radical scavenging method of ageratum conyzoides Linn. Leaves. *Am . J. Ethnomed.,* **1(4)**: 244-249.

Shi YX, Xu YK, Hu HB, Na Z and Wang WH (2011). Preliminary assessment of antioxidant activity of young edible leaves of seven Ficus species in the ethnic diet in Xishuangbanna, Southwest China. *Food Chem.*, **108**: 889-894.

Shivhare Y, Singh P, Upadhyay U, Sharma S, Shukla S, Singhai AK and Soni P (2010). Determination of physicochemical parameters and DPPH radical scavenging activity of Chenopodium album Linn. *Phcog J.,* **2(14)**: 7-10.

Siham L, Saida O, Moha T, Nadia S and Hakima A (2014). Chemical analysis and antioxidant activity of "Nerium oleander" leaves. *On line J. Biol Sci.,* **14**: 1-7.

Sofowara A (1993). Medicinal Plants and Traditional Medicine In Africa. *Spectrum Books Ltd Ibadan Nigeria*. P.289.

Sultana B, Anwar F and Przybylski R (2007). Antioxidant activity of phenolic components present in barks of Azadirachta indica, Terminalia arjuna, Acacia nilotica and Eugenia Jambolana Lam. Trees. *Food Chem*., **104**: 1106-1114.

Vhangani LN and Wyk JV (2013). Antioxidant activity of millard reaction products (MRPs) derived from fructose-lysine and ribose lysine model systems. *Food Chemistry*. **137**: 92-98.

Zhang EY and Swaan PW (1999). Determination of membrane protein glycation in diabetic tissue. *AAPS Pharm. Sci*. **1**: 20-36.