Phytochemical screening and antioxidant potential of *Parthenocissus quinquefolia* (L.) planch extracts of bark and stem

Summiya Faisal[1], Anjum Perveen[2], Zaheer-ud-Din Khan[1], Andleeb Anwar Sardar[1]*, Shabnum Shaheen[3] and Asma Manzoor[4]

[1]Department of Botany, GC University, Lahore, Pakistan
[2]Department of Botany, University of Karachi, Karachi, Pakistan
[3]Department of Botany, Lahore College for Women University, Lahore, Pakistan
[4]Institute of Biochemistry and Biotechnology, University of Punjab, Lahore, Pakistan

Abstract: The phytochemical screening and antioxidant potential of bark and stem of *Parthenocissus quinquefolia* (L.) planch was assessed in order to verify its ethnopharmacological significance. All major secondary metabolites e.g. alkaloids, flavonoids, saponins, terpenoids, tannins, reducing sugars, cardiac glycosides and anthraquinones were present. Antioxidant activity was analysed by using five techniques which included DPPH Free Radical Scavenging Activity, FRAP (Ferric Reducing Antioxidant Power), TAA (Total Antioxidant Activity, TPC (Total Phenolic Content and MC (Metal Chelating) Activity. Ethanolic extract of bark showed the highest scavenging effects of 90.01±0.01%, with IC_{50} value of 24.32mg/ml. Aqueous stem extract showed best activity with IC_{50} value of 13.6±0.34mg/ml. The significance antioxidant potential indicates the effectiveness of bark and stem of *P. quinquefolia* in treatment of many diseases.

Keywords: *Parthenocissus*, antioxidant activities, DPPH scavenging and pharmacology.

INTRODUCTION

Plants play an important role in our lives due to their extraordinary range of diverse class of biochemicals (Buckingham, 1999). They are rich source of secondary metabolites. They build up the immune system and help the body to combat infectious agents. Plant-derived antioxidants have become the major research concern for the past two decades. The U.S. market sales of medicinal plants have climbed up to U.S. $3 billion per year (Glaser, 1999).

Free radicals including the reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), peroxyl radicals (ROO), superoxide anion (O^{2-}) and nitric oxide (NO) are formed by both endogenous and exogenous factors in humans. Oxidative stress is amongst the main factors for stimulation of many diseases i.e. degenerative and chronic diseases counting *diabetes mellitus*, cardiac diseases, atherosclerosis, cancer, ischemic, immunosuppression and neurodegenerative diseases etc. Antioxidants are the most useful to eradicate free radicals that create oxidative stress (Metodiewa and Koska, 2000). Oxidation of lipids and other molecules is inhibited by antioxidants by restraining oxidizing chain reactions. Redox properties of polyphenols play a significant task to adsorb or neutralize free radicals and quenching oxygen and to decompose peroxides (Moosmann *et al*., 1999; Parr and Bolwell, 2000). The interest is increasing day by day to find out the importance of antioxidants occurring naturally in plants to reinstate synthetic antioxidants. Because natural antioxidants can be helpful to protect the human body from side effects of synthetic antioxidants diseases (Siddhuraju and Manian, 2007).

Antioxidant activities of *Parthenocissus tricuspidata* and *Ampelopsis brevipedunculata* has been studied by Kundakovic *et al*. (2008). Ibrahim *et al*. (2010) have done similar studies of different medicinally important plants. Therefore, the present study was planned to conduct the antioxidant potency of *P. quinquefolia*.

MATERIAL AND METHODS

Collection and peparation of samples

Parthenocissus quinquefolia (L.) planch was collected from Lahore District. 300grams of dried powered bark was successively extracted in non-polar solvents with the gradual shifting to the polar solvents. n-Hexane, chloroform, ethanol and double distilled water were used as solvents. The extraction was carried out by maceration of the powdered plant material for 8 days in each solvent.

Phytochemical screening

The phytochemical screening of extracts were performed using standard procedures by following Ayoola *et al.* (2008).

Antioxidant activities

DPPH free radical scavenging activity

This activity of the bark extract was done by comparison with the BHT (Butylated Hydroxytoluene) by following

**Corresponding author:* e-mail: andleebanwar@gcu.edu.pk

Lee *et al.* (1998). 3ml (0.1mM methanolic solution of DPPH) was merged into various concentrations of 60µg/ml, 125µg/ml, 250µg/ml and 500µg/ml. Then these solutions were incubated for 1 hour (25°C) after shaking. By using methanol as a blank the absorbance was taken at 517nm in the spectrophotometer.

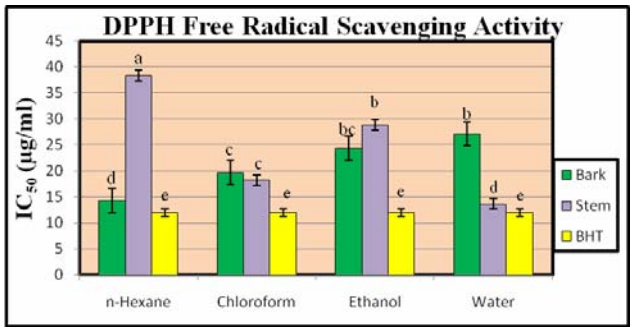

Fig. 1: Comparative analysis of DPPH free radical scavenging activity of bark and stem extracts of *P. quinquefolia*.

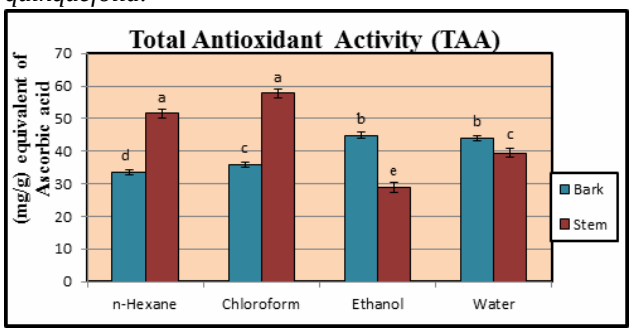

Fig. 2: Comparative analysis of Total Antioxidant Activity (TAA) of bark and stem extracts of *P. quinquefolia*.

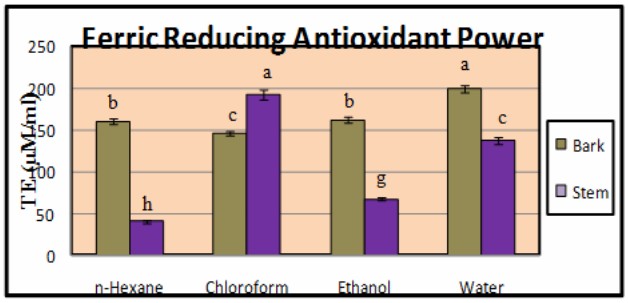

Fig. 3: Ferric Reducing Antioxidant Power (FRAP) bark and stem extracts of *P. quinquefolia*.

The DPPH discoloration percent was designed as per the given formula:

$$\%\,\text{scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Scavenging activity percent was then used in the calculation of IC_{50} value (Inhibitory concentration to produce 50% reduction of the DPPH).

TAA (total antioxidant activity)

Phosphomolybdenum complex formation method of Prieto *et al.* (1999) was used to evaluate the TAA. 4mM reagent solution was prepared by mixing 2.47g ammonium molybdate, 5.32g sodium phosphate and 16.7ml H_2SO_4 in 500ml final volume of distilled water. 4ml of reagent solution was then mixed with 500µg/ml of plant sample. 4ml reagent solution was also used as blank. All the test tubes were cotton plugged. After that incubated for 90 minutes at 95°C in a water bath and cooled at room temperature. The absorbance was measured at 695nm. The total antioxidant activity was calculated by using ascorbic acid (in milligram per gram) with the help of below mentioned equation.

$$X = \frac{Y + 0.0328}{0.0112}$$

Where, Y = Sample absorbance
X = mg/ml of ascorbic acid

FRAP (Ferric reducing antioxidant power assay)

The method of Benzie and Strain (1996) was followed to carry out FRAP assay. 25ml of 300mM acetate buffer (pH 3.6) was mixed with 10mM TPTZ solution prepared in 40mM HCl (2.5ml) and 20mM ferric chloride (2.5ml). 2990µl of FRAP reagent mixed in 10µl of plant extract solution. Then it was placed for 30 minutes in dark. The absorbance was noted in the spectrophotometer at 593nm. The results were expressed in Micromoles Trolox Equivalents (TE) per ml of the sample. Standard curve was made by using different concentrations of trolox. Frap values calculated by using following equation derived from standard calibration curve.

$$X = \frac{Y - 0.069}{0.002}$$

Where, Y = Sample absorbance
X = TE (µM /ml)

TPC (Total phenolic contents)

Total Phenolic ingredients of each extract of *P. quinquefolia* were estimated after the technique of Makkar *et al.*, (1993). 0.1ml of plant sample was mixed with 2.8ml of 10% Na_2CO_3 and 0.1ml of 2N Folin-Ciocalteu (FC) reagent. Absorbance was measured at 725nm in spectrophotometer after 40 minutes. Various concentrations of gallic acid in milligrams was used to construct standard curve for calculation of TPC with the help of following equation:

$$Y = 0.006X + 0.139$$

Where, Y = Sample absorbance
X = TPC value

Metal chelating activity

This activity of extracts measured after Dinis *et al.* (1994). 50µl of 2mM ferrous sulphate ($FeSO_4$) and 200µl of 5mM ferrozine solution were mixed in 100µl of sample and volume was raised up to 4ml with methanol. Then it was placed for 15 minutes at room temperature. Absorbance was noted at 562nm. The following formula was used to calculate the inhibition percent of Ferrozine-Ferrous Complex:

$$\%\,\text{Inhibition} = [(A_b - A_s)/A_b] \times 100$$

Where, Ab = Absorbance of the blank solution
As = Absorbance of the plant extract

STATISTICAL ANALYSIS

All parameters were done in a set of triplicates. The data was showed as mean value ± S.E. The final value, standard deviation and standard error were calculated using Microsoft excel. Duncan's Multiple Range Test (co-stat software version 3.03) and Analysis of Variance (ANOVA) were used to determine the significant value of analysis after Steel *et al*. (1997).

RESULTS

Phytochemical screening of crude extracts of bark and stem extracts of *P. Quinquefolia* revealed the presence of secondary metabolites given in table 1.

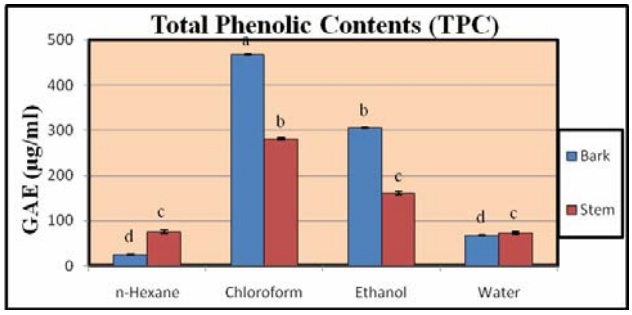

Fig. 4: Graphical representation of Total Phenolic Content (TPC) of different parts of *Parthenocissus quinquefolia* (L.) planch

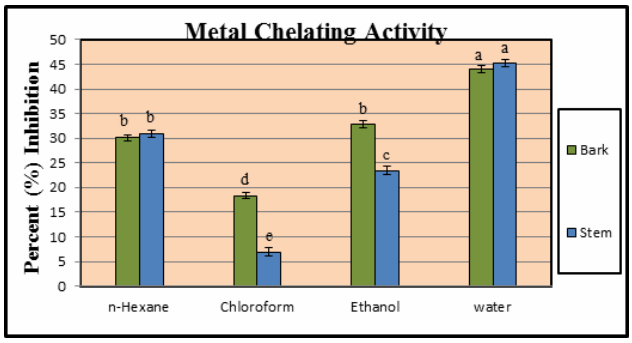

Fig. 5: Metal Chelating (MC) activity exhibited by *P. quinquefolia*.

Five parameters were performed to investigate the antioxidant potential of bark and stem of *Parthenocissus quinquefolia* (L.) planch. All of the crude extracts showed DPPH scavenging activity more than 50%. Aqueous stem extract had shown best activity with IC_{50} value of 13.6±0.34 while n-hexane, chloroform and ethanol stem extracts had IC_{50} values 38.28±2.79, 18.24±1.43 and 28.84±2.20 respectively as shown in fig. 1. Among bark extracts, n-hexane exhibited best results with 14.25±0.65 IC_{50} value whereas, chloroform, ethanol, and aqueous extracts had IC_{50} values of 19.67±0.70, 24.32±1.02 and 27.08±3.41 respectively. Ethanolic extract of bark showed the highest scavenging effects of 90.01±0.01% at concentration of 500µg/ml, with IC_{50} value of 24.32mg/ml.

It was observed that chloroform stem extract possessed significant total antioxidant capacity equivalent to 57.75±1.70mg/g of ascorbic acid. n-hexane, aqueous and ethanol stem extracts had 51.5±0.50mg/g, 39.45±2.36 mg/g and 28. 82±1.94mg/g respectively. Likewise, among bark extracts, highest TAA was shown by ethanolic and aqueous extracts, i.e. 44±1.00mg/g and chloroform and n-hexane extracts 35.87±1.45mg/g and 33.55±0.73mg/g of Ascorbic acid as given in fig. 2.

The highest FRAP potential was exhibited by aqueous extract of bark, i.e. 199.5±1.1µM/ml while least activity was displayed by n-hexane stem extract, i.e. 40±0.50 µM/ml as shown in fig. 3. The results also depicted that chloroform bark extract had maximum TPC value, i.e. 468±1.7µg/mL while minimum GAE was exhibited by n-Hexane bark extract, i.e. 24.5±0.50µg/mL (fig. 4). Among metal chelating activity, maximum and the minimum % inhibition of of ferrozine-ferrous complex formation was reported by aqueous extracts of both stem and bark, and chloroform stem extract, i.e. 45.29±0.37% and 6.92±0.04 %, respectively as given in fig. 5.

DISCUSSION

The qualitative phytochemical screening of bark, stem, leaves and fruits extracts of *P. quinquefolia* revealed the presence of secondary metabolites like alkaloids, tannins, saponins, terpenoids, flavonoids, anthraquinones, cardiac glucosides and reducing sugars. All these chemicals were present in aqueous stem extract due to which it executed significant antioxidant activities in all tested parameters. Similar results were documented by Soladoye and Chukwuma (2012) while studying the phytochemical analysis of stem and root of *Cissus populnea* (Vitaceae). They reported the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides in stem and root of *C. populnea.*

The total antioxidant activity determined is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of green phosphate-Mo(V) complex at acid pH. It evaluates both water-soluble and fat-soluble antioxidants thus, called as total antioxidant capacity. In TPC, both chloroform and ethanolic extracts of bark and stem depicted good results, Likewise the highest % inhibition of of ferrozine-ferrous complex formation was reported by aqueous extracts of both stem and bark.

CONCLUSION

Results revealed that all extracts of bark and stem of *Parthenocissus quinquefolia* (L.) planch had significant antioxidant activity. The results were compared with standard antioxidants and it was found that *P. quinquefolia* have better antioxidant potential. Therefore, it can be used for treatment of cancer and aging in future.

Table 1: Phytochemical tests of bark and stem of *Parthenocissus quinquefolia*

Plant parts	Solvents	Presence/absence of phytochemical constituents							
		Reducing sugars	Anthra-quinones	Terpenoids	Flavonoids	Saponins	Tannins	alkaloids	Cardiac glycolsides
Bark	n-Hexane	+	-	-	-	-	-	+	+
	Chloroform	-	-	+	-	+	-	+	+
	Ethanol	-	-	+	+	+	-	-	+
	Water	+	+	+	-	+		-	+
Stem	n-Hexane	+	-	+	-	+	-	+	-
	Chloroform	+	-	+	+	+	-	+	-
	Ethanol	+	-	+	+	+	-	+	+
	Water	+	+	+	+	+	+	+	+

REFERENCES

Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC and Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for Malaria therapy in Southwestern Nigeria. *Trop. J. Pharm. Res.*, **7**(3): 1019-1024.

Benzie IEF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power, the FRAP assay. *Anal. Biochem.*, **239**: 70-76.

Buckingham J (1999). *Dictionary of Natural Compounds*. Chapman and Hall, U.K. pp.14-20.

Dinis TCP, Madeira VMC and Almeida MLM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.*, **315**: 161-169.

Glaser V (1999). Billion-dollar market blossoms as botanicals take root. *Nat. Biotechnol.*, **17**: 17-8.

Ibrahim TA, El-Hefnawy HM and El-Hela AA (2010). Antioxidant potential and phenolic acid content of certain cucurbitaceous plants cultivated in Egypt. *Natural Product Res*., **24**(16): 1537-1545.

Kundakovic T, Stanojkovic T, Milenkovic M, Grubin J, Juranic Z, Stevanovic B and Kovacevic N (2008). Cytotoxic, antioxidant and Antimicrobial activities of *Ampelopsis brevipedunculata* and *Parthenocissus tricuspidata* (Vitaceae). *Arch. Biol. Sci*., **60**(4): 641-647.

Lee SR, Mbwambo ZH, Chung HS, Luyengi L, Games EJC and Mehra RG (1998). Evaluation of antioxidant potential of natural products. *Combination Chem. & High Throughput Screening*, **1**(1): 35-46.

Makkar HPS, Blummel M, Borowy NK and Becker K (1993). Gravimetric determination of tannins and their correlation with chemical and protein precipitation methods. *J. Sci. Food and Agric.*, **61**: 161-165.

Metodiewa D and Koska C (2000). Reactive oxygen species and reactive nitrogen species: Relevance to cyto(neuro)toxic events and neurologic disorders. An overview. *Neurotox. Res.*, **1**: 197-233.

Moosmann B and Behl C (1999). The antioxidant neuroprotective effects of estrogens and phenolic compounds are independent from their estrogenic. *Proc. Natl. Acad. Sci. USA.* **96**: 8867-8872.

Parr AJ and Bolwell GP (2000). Phenols in plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *J. Sci. Food Agric.*, **80**: 985-1012.

Prieto P, Pineda M and Agular M (1999). Spectrophotometric quantitation antioxidant capacity through the formation of a phosphomolybdenum complex. *Anal. Biochem.*, **269**: 337-341.

Siddhuraju P and Manian S (2007). The antioxidant activity and free radical scavenging capacity of dietary phenolic extract from horse gram (*Macrotyloma uniflorum* (Lam.) Verde seeds. *Food Chem.*, **105**: 950-958.

Soladoye MO and Chukwuma EC (2012). Phytochemical analysis of the stem and root of *Cissus populnea* (Vitaceae) an important medicinal plant in Central Nigeria. *Phytologia. Balcanica.*, **18**(2): 149-153.

Steel RGD, Torrie JH and Discky DA (1997). *Principles and Procedures of Statistics: A Biometrical Approach*. 3rd ed. McGraw Hill Book Co., New York. pp.248-263.