

# ***In vitro* antioxidant potential and free radical scavenging activity of various extracts of pollen of *Typha domigensis* Pers.**

**Andleeb Anwar Sardar<sup>1\*</sup>, Zaheer-ud-Din Khan<sup>1</sup>, Anjum Perveen<sup>2</sup>, Sidra Farid<sup>3</sup> and Islam Ullah Khan<sup>3</sup>**

<sup>1</sup>Department of Botany, GC University Lahore, Katchery Road, Lahore, Pakistan

<sup>2</sup>Department of Botany, University of Karachi, Karachi, Pakistan

<sup>3</sup>Department of Chemistry, GC University Lahore, Katchery Road, Lahore, Pakistan

---

**Abstract:** Antioxidant potential of the pollen of *Typha domigensis* Pers. using Ferric Reducing power, Metal Chelating Activity and Trolox Equivalent Antioxidant Capacity (TEAC) assays has been carried out in the current research work. The antioxidant components were initially extracted from the pollen in methanol and were further fractionated in solvents of different polarity such as n-Hexane, Chloroform, Ethyl Acetate and Water. Methanol extract which was found to have high reducing power, total phenolic contents with high metal chelating activity, has considerable prospective to utilize as a natural antioxidant and be capable to link with the total phenolic contents of plant.

**Keywords:** *Typha domigensis* Pers., pollen, antioxidant, ethnopharmacological studies.

---

## **INTRODUCTION**

It has been well recognized that plants have natural antioxidants (Huda-Faujan *et al.*, 2009). Currently to a great extent consideration has been devoted to natural antioxidants and associated compounds due to their benefits towards human health (Arnous *et al.*, 2001). Traditional medicines are being used throughout the World. Plants are a prime foundation of natural antioxidants i.e. a good source of new drugs (Perry *et al.*, 1999). Pollen collected by bees are being used supplementary nutrition and in traditional medicines from several years mainly due to its health benefits (Serra and Escola, 1997; Isla *et al.*, 2001; Kroyer and Hegedus, 2001; Almeida-Muradian *et al.*, 2005). Its nutritional composition consists of sugars, lipids, proteins, mineral salts, fibers, vitamins and amino acids (Welsh and Marston, 1983; Marchini *et al.*, 2006). Moreover, pollen also contains polyphenolic substances, mainly flavonoids with antioxidant (Isla *et al.*, 2001; Campos *et al.*, 2003) and antimicrobial activity (Garcia *et al.*, 2001; Basim *et al.*, 2006). During the last decade, attention in the study of phenolic compounds has boosted greatly, mainly due to the antioxidant capacity of these substances in scavenging free radicals that are harmful to human health (Dorman *et al.*, 2003). The chemical structure of phenolic compounds is responsible for their varying antioxidant activity that can be derived by the action of the molecule as reducing agents. *In vitro* trials have revealed that some flavonoids have greater antioxidant activity than vitamins E and C (Almaraz-Abaraca *et al.*, 2007). Epidemiological studies have also shown positive correlation between the increase in phenolic compound consumption with antioxidant action (Javanmardi *et al.*, 2003) and reduction of the threat in different kinds of cancer and cardiovascular

diseases (Cook and Samman, 1996).

*Typha*, commonly known as 'Cat Tail' is a worldwide distributed genus of monocotyledons flowering plants with about eleven species of a monotypic family 'Typhaceae'. *Typha* are tall with submerged rhizomes forming dense stands near pond, canal and river banks. They are common wetland plants of shallow waters, wet soils, marshlands and bogs (Long and Lakela, 2004). *Typha* species are unisexual having monoecious wind pollinated flowers. Male flowers grow in a dense barb at climax of stem of the plant, whereas minute feminine flowers also grow in a dense barb beneath the male barb (Foulis, 2004). The Herbal medication community of Turkey used feminine flowers of different *Typha* species for stop of bleeding, devoid of distinguishing species (Sezik *et al.*, 1997). Other than this, *Typha* pollen are also used for cure of wound healing and smolder (Yesilada, 2002). *Typha* pollen are recognized for removing stasis and haematemeses, and are frequently recommended to cure nose and uterine bleedings (Gibbs *et al.*, 1983; Qin and Sun, 2005; Tao *et al.*, 2010). *Typha* pollen is being eaten orally to increase flow of urine, lessen fever, prevent bleedings as well as cure injuries in Pakistan.

Whereas some preliminary studies on antioxidant and antibacterial potential of the pollen of some plants have been carried out, but no comprehensive study on the radical scavenging and antioxidant capacity has been endeavored as yet. Moreover, most of the studies have been done on the bee pollen, not on the fresh pollen. The current study is carried out to assess antioxidant prospective and free radical scavenging activity of *T. domigensis* Pers pollen extracted in methanol via ABTS<sup>+</sup> colorization Assay, Ferric Reducing Antioxidant Power Assay, Total Phenolic Content assay (FRAP), Metal Chelating Assay, and Total Flavonoids contents.

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

### **Methodology**

Reagents used in all experimental assays were of purified grade purchased from Fluka and Merck. Trolox (Hoffman-La Roche) (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich Chemical Co., Gillingham, Dorset, UK) was made in ethanol and it was used as an antioxidant standard.

Ultra Violet-1700 Pharma-Spec Ultra Violet-Visible Spectrophotometer, Shimadzu, Japan equipped with CPS controller was used to carry out experimental work. All the experiments done repeatedly and final results were obtained by taking mean values at  $\pm$ SD ( $n=3$ ).

### **Sample preparation**

The pollen grains of *Typha domigensis* Pers. was collected in powdery form from different Ponds, Canals and River banks and extracted with methanol at room temperature in soxhelt apparatus for 10-12 hours. The methanol extract was made dense in rotary evaporator at 30°C. Then this extract was mixed in distilled water. This further extracted with n-hexane, chloroform and ethyl acetate to obtain fractions of different polarity.

The filtrate of each extract was measured and suspended in solvents of increasing polarity to get stock solution for use in following antioxidant assays:

### **ABTS<sup>•+</sup> decolorization assay**

ABTS radical cation protocol (Re *et al.*, 1999) used in present work. ABTS stock solution was dissolved in potassium persulfate (2.45/mM) and set aside (12-16 hours) without light to obtain desired ABTS<sup>•+</sup>. To analyze antioxidant activity of each pollen extract was diluted in relevant organic solvents from which they were extracted. Now 10/ $\mu$ L of this solution was mixed with diluted ABTS<sup>•+</sup> solution (2.99/mL) for measurement of absorbance (30°C) with one minute interval for a total duration of eight minutes. Solvent without pollen extract were also run parallel for accurate readings. The %age inhibition was computed with the help of following prescription:

$$I_{734} = \left(1 - \frac{A_f}{A_o}\right) \times 100$$

In above mentioned equation,

$A_o$  = absorbance of radical cation

$A_f$  = absorbance of the addition of sample/standard antioxidants in stock solution

Results were obtained by the comparison of antioxidants concentration and Trolox.

### **Total phenolic contents assay**

Total phenolic contents measured using a protocol (Slinkard & Singleton, 1999). To obtain a stock solution, 0.5 grams (gallic acid) were mixed in 10 mL (ethanol) and final volume was raised to 100mL by adding distilled water. Anhydrous sodium carbonate (200/grams) was

mixed with double distilled water (800/mL) in order to get Sodium carbonate solution. Few crystals of sodium carbonate were also added after boiling and cooling. The solution was set-aside for a day and night. It was then filtered and final volume was made upto one litre by adding double distilled water. The different volumes (0, 1, 2, 3, 5 and 10/mL) of stock solution of phenol diluted with double distilled water up to 100 mL. A volume of 40  $\mu$ L was taken into separate cuvettes from each dilution and further diluted with double distilled water up to 3.16 mL. A blank was also run in parallel. Then Folin-Ciocalteu's reagent (200/ $\mu$ L) was mixed and subsequently sodium carbonate solution (600/ $\mu$ L) also added, placed for half an hour at 40°C. Each solution and blank measured at 765 nm for absorbance.

### **FRAP (Ferric Reducing Antioxidant Power) assay**

Ferric Reducing capacity analyze by a reported method (Benzie & Strain, 1999). Freshly prepared FRAP solution contained 25ml (pH 3.6) of acetate buffer (300 mM), TPTZ solution (2.5/mL of 10/mM) in 40/mM of HCl solution, ferric chloride solution (2.5/mL of 20/mM). Then FRAP reagent (3/mL) was added in the sample (100/ $\mu$ L) and distilled water (300/ $\mu$ L) and incubated (37°C) during monitoring period. Absorbance was measured after one minute for a total duration of six minutes at 593 nm. The obtained readings were contrasted with ferrous sulphate standard curve.

### **Metal chelating activity**

The method developed by Dinis *et al.* (1994) is utilized for determination of chelation of ferrous ions and standards. Aliquots (1/ml) of the plant extracts and control separately taken in test tubes, followed by mixing with 50  $\mu$ l (2mM FeSO<sub>4</sub>.7H<sub>2</sub>O) and 150. $\mu$ l (5.mM ferrozine). The mixtures were shook well and placed for 10min at room temperature. Each solution was measured in a spectrophotometer at 562 nm for absorbance. The %age inhibition measured with the help of following equation:

$$\% \text{age Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Here,

$A_0$  = absorbance of control

$A_1$  = absorbance in presence of the plant extracts or standards.

EDTA was utilized as a reference compound.

### **Total Flavonoid Content**

Total Flavonoid contents determined with the help of colorimetric method modified by Jia *et al.* (1999). Distilled water (1.25/ml) was mixed in sample extract and then Sodium nitrite solution (0.075/ml, 5%) was also added and incubated for five minutes. 0.15 ml of 10% aluminum chloride was added after incubation and one Molar sodium hydroxide (0.5/ml) was finally mixed after an interval of six minutes. This was diluted distilled water of with 0.275ml and measured at 510 nm instantly for absorbance by comparing with standard curve of quercetin.

## RESULTS

In this research work antioxidant potential of *Typha domingensis* Pers. Pollen has been exploited by three basic working mechanisms of antioxidants. For this purpose, we employed ABTS<sup>+</sup> assay to determine free radical scavenging ability, FRAP assay to identify ferric reducing ability and metal chelating ability to find its chelating potential. Moreover to correlate this antioxidant ability with its phytochemical composition, we estimate its total phenolic and flavanoid contents. All the results of antioxidant activity are summarized in table 1.

### ABTS<sup>+</sup> decolorization assay protocol

Trolox Equivalent Antioxidant Capacity (TEAC) values were obtained by measuring the percentage inhibition values of pollen extracts with Trolox curve. TEAC values ranges from 3.94-5.96 mM of trolox equivalents. The graphical representation of results of ABTS<sup>+</sup> assay is shown in fig. 1.

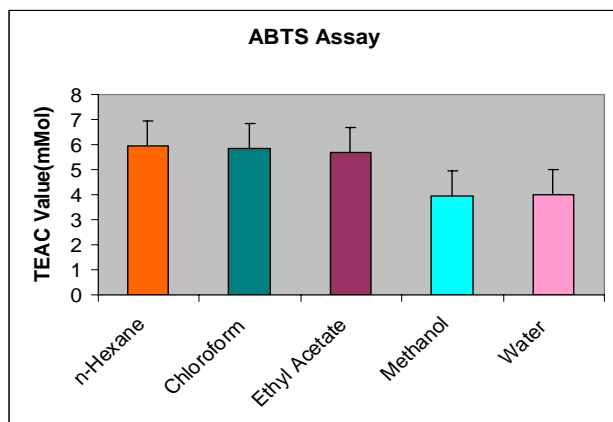


Fig. 1: Graphical representation of results of ABTS<sup>+</sup> assay

### Total phenolic contents

Phenolic compounds due to hydroxyl groups in their structures are very powerful antioxidants (Robbins, 2003). All the fractions showed high values of Total Phenolic Contents. Total phenolic content values vary from 262-354 mg/1 of Gallic Acid. The graphical representation of total phenolic contents of total phenolic contents is shown in fig. 2.

### FRAP (Ferric reducing antioxidant power) Assay

The FRAP developed by Benzie and Strain, 1999 which engrosses a single electron reduction of the Fe(TPTZ)<sub>2</sub> (III) complex (pale yellow) to the Fe(TPTZ)<sub>2</sub> (II) complex (blue) by sample antioxidants at acidic pH. Any antioxidant species with lower reduction potential than that of Fe (III) TPTZ salt (0.7 V) be able to reduce Fe<sup>3+</sup>. TPTZ to Fe<sup>2+</sup>.TPTZ causative to FRAP value (Dejianet al., 2005). This reduction is observed spectrophotometrically at 593 nm. Appearance of deep blue coloration

is a sign of presence of reducing components in the sample. The FRAP values of the fractions of Pollen extracts were calculated by way of comparison with a calibration curve obtained using iron (II) sulfate as the standard reductant. Ferric reducing antioxidant power values for different fraction varied from 1.312-5.944 mg/L of FeSO<sub>4</sub> equivalents. The graphical representation of results of FRAP assay are shown in fig. 3.

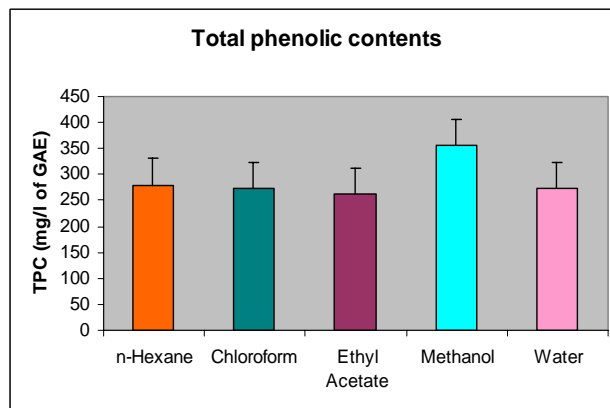


Fig. 2: Graphical representation of Total phenolic contents

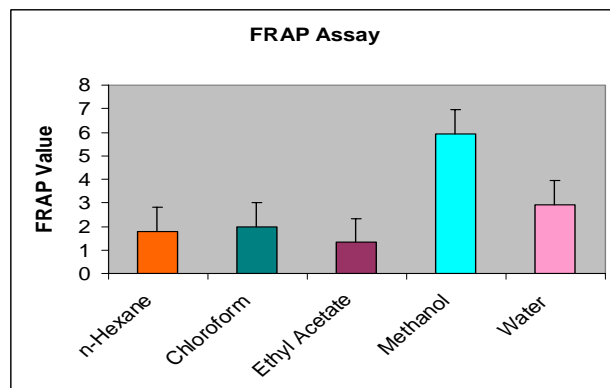


Fig. 3: Graphical representation of results of FRAP Assay

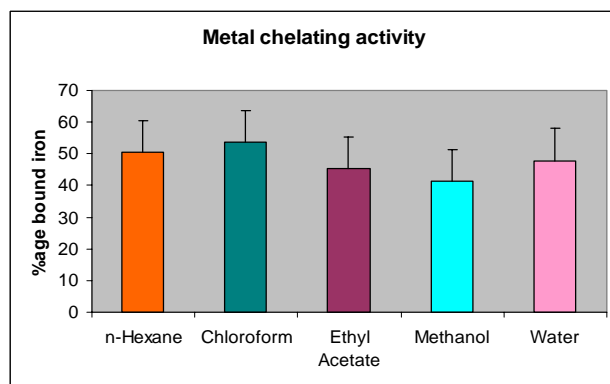
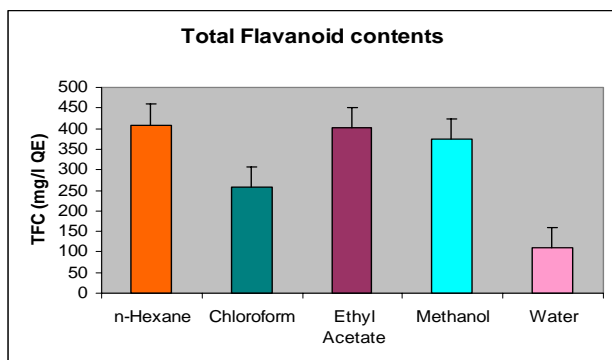


Fig. 4: Graphical representation of results of metal chelating activity

**Table 1:** All the results of antioxidant activity of *T. domigensis* Pers. pollen

Sample	TEAC Value mMol	FRAP Value (mMol)	%age bound iron	TPC (mg/L of GAE)	TFC (mg/L of QE)
n-Hexane	8.96±0.02	1.80±0.76	50.40±0.98	289.99±0.32	409.18±0.91
Chloroform	6.82±0.04	2.80±0.86	58.80±0.15	271.49±0.86	258.27±0.05
Ethyl Acetate	5.70±0.10	1.71±0.93	45.20±0.06	262.49±0.92	401.90±0.88
Methanol	3.94±0.25	5.94±0.48	41.40±0.05	354.99±0.75	372.82±0.16
Water	4.00±0.98	3.98±0.72	47.90±0.76	272.94±0.66	110.09±0.17

Results were recorded with ±SD when n=3



**Fig. 5:** Graphical representation of results of total flavanoid contents

**Metal chelating activity**

The different fractions of *Typha* pollen and reference compounds (EDTA) were found to have impeded in the presence of ferrozine and ferrous complex, indicating that pollen contain chelating activity and are able to detain ferrous before ferrozine. Graphical representation of Metal chelating capacities of different pollen extracts are shown in fig. 4. Among these, Chloroform and n-Hexane fractions owned higher metal chelating activities in contrast to the other solvents. The metal chelating activity of the fractions depends upon the solvent due to the presence of different antioxidant potentials of complex. The compounds having high polarity were responsible for metal chelating activity. This proposes that the compounds, representing that there were many phenolic compounds in the extracts that possessed different activities. However results demonstrate that all extracts had useful ability to bind iron. Therefore, as peroxidation inhibitors they can be correlated for iron binding capacity.

**Total flavanoid contents**

Flavonoids being most varied general groups of natural compounds that are important phenolic compounds occurred naturally (Agrawal, 1989). Therefore, the contents of flavonoids in the extracts were also evaluated. The graphical representation of results of total flavanoid contents were shown in fig 5.

**DISCUSSION**

In this study amongst different fractions, n-hexane,

chloroform and ethyl acetate showed higher TEAC values. The fractions of increasing polarity extractive solvents showed higher value of TEAC indicating high solubility of phenolic and other antioxidant components. However, the remaining polar fractions showed small TEAC values indicating relatively low free radical scavenging ability. High Total Phenolic Content values are due to the presence of sterols, terpenoids, flavones, gallic acid, long chain hydrocarbons, caffeic acid and *p*-coumeric acid in pollen of *Typhae* (Esraet *al.*, 2011). The crown FRAP values were obtained in more polar solvents e.g. methanol. It is apparent that the polarity of the extractive solvent has vast influence on the extraction of antioxidant compounds. Antioxidant activity is important due to chelate/deactivate transition metals which have hydroperoxide decomposition catalyzing capacity and Fenton-type effects (Quang-Vinh and Jong-Bang, 2011). Chelating agents can serve as secondary antioxidants due to their capacity to reduce redox potential and to stabilize them in oxidized forms of metal species. So, the %age bound iron capabilities of the extracts were monitored. Complex formation between ferrozine and Fe<sup>2+</sup> is interrupted in the presence of chelating agents that consequence the formation of the red colour of the complex. Change in colour is measured to evaluate chelating activity of the co-existing chelator (Yamaguchi *et al.*, 2000). The yield of flavonoids depended on not only upon plant type but also upon the extraction solvent The Pollen of *Typha* extract with n-Hexane contained higher flavonoid contents compared to the other solvents. This means that the highest polar solvent (water) is not appropriate to extract flavonoid compounds by *Typha* pollen.

**CONCLUSION**

In general, it is concluded that *T. domigensis* pollen have a high antioxidant potential, as all the extracts shows a distinct antioxidant activity in all *in-vitro* working mechanisms of antioxidant activity. The results show that methanol extract displayed the highest total phenolic contents and also possessed the strongest reducing power. Moreover the highest metal chelating activity was found in the chloroform extract and total flavanoid content in *n*-hexane fraction. Therefore, it is suggested that methanolic extracts of *T. domigensis* pollen may be used for pharmaceutical uses.

## REFERENCES

- Agrawal PK (1989). Carbon-13 NMR Flavonoid., Elsevier, New York, USA, pp. 444-446
- Almaraz-Abaraca N, Campos MG, Avila-Reyes JA, Jimenez NN, Corral JH and Gonzalez-Valdez LS (2007). Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (*Prosopis juliflora*, Leguminosae). *J. Food Comp. and Anyl.*, **20**: 119-124.
- Almeida-Muradian LB, Pamplona LC, Coimbra S and Barth OM (2005). Chemical composition and botanical evaluation of dried bee pollen pellets. *J. Food Comp. and Anyl.*, **18**: 105-111.
- Arnous A, Makris DP and Kefalas P (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *J. Agric. Food Chem.*, **49**: 5736-5742.
- Basim E, Basim H and Ozcan M (2006). Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens. *J. Food Engg.*, **77**: 992-996.
- Benzie IFF and Strain JJ (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version of simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method Enzymol.*, **299**: 15-27.
- Campos MG, Webby RF, Markham KR, Mitchell KA and Cunha AP (2003). Age-induced diminution of free radical scavenging capacity in Bee pollens and the contribution of constituent flavonoids. *J. Agri. Food Chem.*, **51**: 742-745.
- Cook NC and Samman S (1996). Flavonoids-Chemistry, metabolism, cardioprotective effects and dietary sources. *J. Nutrlnl. Biochem.*, **7**: 66-76.
- Dejian H, Boxin O and Prior RL (2005). The chemistry behind antioxidant capacity assays. *J. Agri. Food Chem.*, **53**: 1841-1856.
- Dinis TCP, Madeira VMC and Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. BioPhy.*, **315**: 161-169.
- Dorman HJD, Kosar M, Kahlos K, Holm Y and Hiltunen R (2003). Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *J. Agri. Food Chem.*, **51**: 4563-4569.
- Esra AK, Ipek S, Hikmet K and Erdem Y (2011). The potential role of female flowers inflorescence of *Typha domingensis* Pers. in wound management. *J. EthnoPharm.*, **133**: 1027-1032.
- Foulis L (2004). Botanica. Italy, p.905.
- Garcia M, Perez-Arquillue C, Juan T, Juan MI and Herrera A (2001). Pollen analysis and antibacterial activity of Spanish honeys. *Food Sci. and Tech.*, **7**: 155-158.
- Gibbs A, Green C and Doctor VM (1983). Isolation and anticoagulant properties of polysaccharides of *Typha Augustata* and *Daemonorops* species. *Thromb Res.*, **32**: 97-108.
- Huda-Faujan N, Noriham A, Norrakiah AS and Babji AS (2009). Antioxidant activity of plants methanolic extracts containing phenolic compounds. *J. Afri. Biotech.*, **8**: 484-489.
- Isla MI, Moreno MIN, Sampietro AR and Vattuone MA (2001). Antioxidant activity of Argentine propolis extracts. *J. Ethno. Pharmacol.*, **76**: 165-170.
- Javanmardi J, Stushnoff C, Locke E and Vivanco JM (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chem.*, **83**: 547-550.
- Jia Z, Tang M and Wu J (1999). The determination of flavonoids content in Mullberry and their scavenging effects on superoxide radicals. *Food Chem.*, **64**: 555-559.
- Kroyer G and Hegedus N (2001). Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. *Inno. Food Sci. and Emerg. Tech.*, **2**: 171-174.
- Long RW and Lakela O (1976). A Flora of Tropical Florida, Banyan Books, Miami Fl.
- Marchini LC, Reis VDA and Moreti ACCC (2006). Composiçãofísico-química de amostras de pólencoletadoporabelhas africanizadas *Apismellifera* (Hymenoptera: Apidae) em Piracicaba, Estado de São Paulo *Cienc. Rur.*, **36**: 949-953.
- Perry EK, Pickering AT, Wang WW, Houghton PJ and Perry NS (1999). Medicinal plants and Alzheimer's disease: from Ethnobotany to Phytotherapy. *J. Pharm. Pharmacol.*, **51**: 527-534.
- Qin F and Sun HX (2005). Immunosuppressive activity of Pollen *Typhae* ethanol extract on the immune responses in mice. *J. Ethno. pharm.*, **3**: 424-429.
- Quang-Vinh N and Jong-Bang E (2011). Antioxidant activity of solvent extracts from Vietnamese medicinal plants. *J. Med. Plant Resr.*, **5**: 2798-2811.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radi Biol. Med.*, **26**: 1231-1237.
- Robbins RJ (2003). Phenolic acids in foods: An overview of analytical methodology. *J. Agric. Food Chem.*, **51**: 2866-2887.
- Serra JB and Escola JR (1997). Nutrient composition and microbiological quality of honeybee-collected pollen in Spain. *J. Agri. Food Chem.*, **45**: 725-732.
- Sezik E, Yesliada E, Tabata M, Honda G, Takaishi Y, Fujita T, Tanaka T and Takeda Y (1997). Traditional medicine in Turkey VIII. Folk medicine in East Anatolia; Erzurum, Erzincan, Agri, Kars, Igdir Provinces. *Econ. Bot.*, **51**: 195-211.

*In vitro antioxidant potential and free radical scavenging activity*

- Slinkard K and Singleton VL (1977). Total phenol analyses: Automation and comparison with manual methods. *Ami. J. Eno. Viticult.*, **28**: 49-55.
- Tao WW, Yang NY, Duan JA, Wu DK, Shang EX, Qian W and Tang YP (2010). Two new nonacosanetriols from the pollen of *Typhaangustifolia*. *Chin. Chem. Lett.*, **2**: 209-212.
- Welsh SO and Marston RM (1983). Nutritional Bioavailability of Zin. Amer Chem Soci. Washington DC., pp. 15-30
- Yamaguchi F, Ariga T, Yoshimura Y and Nakazawa K (2000). Antioxidative and antiglycation activity of garcinol from *Garciniaindica* fruit rind. *J. Agric. Food Chem.*, **48**: 180-185.
- Yesilada E (2002). Biodiversity in Turkish Folk Medicine. Kluwer Academic/Plenum Publishers, London, England, pp. 119-135