NBU Journal of
Plant Sciences

Vol. 5, No.1 March 2011 p. 41-45
ISSN No. 0974 6927

Free radical scavenging activity of ornamental and edible cultivars of Canna found in Eastern India

T Mishra, AK Goyal, P Mondal and A Sen*
Department of Botany, University of North Bengal, Siliguri 734013, India

Abstract
Canna, the only genus of family Cannaceae, found in eastern India have gained economic importance for their starchy rhizomes and attractive flowers. The rhizome of some edible cultivars are consumed as food and herbal medicines in rural areas. The presence of total phenols, flavonoids and DPPH scavenging activity of both cold and hot extracts and their correlations have been studied for eleven cultivars of *Canna* spp. Antioxidants, quercetin and gallic acid equivalence of phenolic compounds were determined spectrophotometrically. It has been found that total phenol, flavonoids and DPPH free radical scavenging activity were higher in hot extracts than in cold. Further studies gave an idea about the correlation between phenol and flavonoid contents of both cold and hot extracts, but both the above said parameters were not correlated with DPPH scavenging activity. Finally it can be concluded that higher DPPH scavenging activities in all these Canna cultivars may not be due to the presence of endogenous phenols and flavonoids but these may be due to any other phytochemicals.

Keywords: Antioxidant, *Canna*, phenol, flavonoids, DPPH

Canna is a genus of approximately twenty species of flowering plants having rhizomatous root stock (Tanaka, 2001; Tanaka, 2008). It is the only genus in the family of Cannaceae. The flowers are typically red, yellow, orange, pink or any combinations of those colors and are aggregated in inflorescences that are spikes or panicles. *Canna* is considered to be ornamental plant having bright and attractive flowers. But some of them like *Canna edulis* are described to be edible and found to be one of the richest sources of starch (Tanaka, 2004). The plant parts like rhizome, leaves and flowers acts as herbal medicine and is used as a remedy in various women complaints and it is also believed to be diuretic, demulcent and diaphoretic (Duke *et al.*, 1985).

Oxidation is a complex chemical reaction in which electron transfers from a substance to an oxidizing agent producing free radicals, leading to specific chain reactions. These chain reactions hazard different cellular and extracellular components and finally block or degenerate various metabolic processes involving fatal diseases in human body. Antioxidants can terminate these chain reactions by removing free radical intermediates by being oxidized themselves. These processes usually occur in human body and are constantly inhibited by an efficient network of antioxidant (Bagul *et al.*, 2005). Deficiency of these networks in the human body may lead to different fatal diseases like cancer, atherosclerosis, diabetes, premature aging etc. Plants have been considered to be the major source of natural antioxidants (Larson, 1988; Middha *et al.*, 2009). Uptake of phytochemicals has increased significantly since it might drift the balance towards a sufficient antioxidant status. Keeping this view in mind interest on natural antioxidants has increased manifolds (Goyal *et al.*, 2010). Many techniques have been developed for producing synthetic antioxidants by different industries; however natural antioxidants have the preference for consumption to those of synthetic ones.

Though some antioxidants work on *Canna indica* have been attempted (Atrooz, 2007; Vankar *et al.*, 2008), the presence of antioxidant in different cultivars of *Canna*, found in eastern India have not been done. Taking this into consideration, in the present study, the presence of total phenols, flavonoids and variation of antioxidant activity through DPPH scavenging assay of these plants present in the aforesaid regions have been performed.

Materials and methods

Chemicals Used

DPPH (2,2-diphenyl-1-picryl hydrazyl) and gallic acid were obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India. Methanol, quercetin, folin-Ciocalteu (FC reagent) reagent, sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$) and CH_3COOK were obtained from Merck, Mumbai, India. All chemicals and solvents were of analytical grade.

Plant material

Eleven cultivars of *Canna* were collected from different regions and commercials nurseries of West Bengal and Odisha and maintained in the experimental laboratory garden.

Rhizome extract

Ten grams of rhizome of each of these cultivars of *Canna* were taken and washed properly and dried. These

*Corresponding author:
E-mail: senarnab_nbu@hotmail.com

were then crushed in 20ml of ddH_2O (double distilled water) using mechanical grinder and finally 20ml of ddH_2O was added to get the suitable concentration of 1:4 (rhizome:H_2O w/v).

Cold extract (CeC)

Half of the extract (20 ml) thus prepared was kept overnight for cold percolation. The sample was filtered using Whatman 2 filter paper. The filtrate was subjected to lyophilisation (EYELA FREEZE DRYER FDU -506) to form powdery mass. Then required amount of ddH_2O was added to those powdery extracts to make suitable concentrations of these samples and stored at -20°C for further use.

Hot extract (CeH)

The remaining extract was refluxed exhaustively at boiling temperature for 3 hours. The extract thus obtained was lyophilized as described previously.

DPPH scavenging activity

The radical scavenging activities of different extracts of all the 11 cultivars were tested against DPPH (2,2-diphenyl-1- picrylhydrazyl). Decrease in absorbance at 540 nm of methanolic solution of coloured DPPH was used as measure of antiradical activity (Vanni *et al.*, 1997; Ravishankara *et al.*, 2002). DPPH stock (60 mg /l) was prepared using methanol. To 1800 µl of DPPH stock solution, 200 µl of the sample of different concentrations were added and incubated in room temperature for 30 minutes. Decreases in the absorbance for different fractions were noted at 540 nm using UV visible spectrophotometer using methanol (200µl) and DPPH as control. DPPH scavenging activity (%) was measured using the following equation:

$$\%scavenging = \frac{A_o - A_1}{A_o} X100$$

Where Ao is the absorbance of the control and A_1 is the absorbance in the presence of the sample (aqueous rhizome extract of *Canna*). The actual decrease in absorption induced by the test compounds was compared with the positive controls.

Determination of Total Phenolic Content

Total phenolic content of both cold and hot extracts of all the 11 cultivars were determined spectro-photometrically according to Folin-Ciocalteu (FC) reagent method (Singleton *et al.*,1999) with slight modification. The rhizome extracts (1 ml) was mixed with 1 ml of FC reagent (previously diluted 1:1 with ddH_2O) and 10 ml of 7% sodium carbonate. This mixture was incubated for 90 min at room temperature. The absorbance was measured at 765 nm. The tests were performed for three concentrations of extracts i.e. 1 mg/ml, 0.5 mg/ml, 0.2 mg/ml. Gallic acid monohydrate was used as standard. The total phenolic content was expressed as gram of gallic acid equivalents (GAE) per 100 g extract.

Determination of total flavonoid content

The total flavonoid content was determined according to aluminium chloride ($AlCl_3$) method (Lin *et al.*,2007) with slight modification using quercetin as a standard. Both the cold and hot rhizome extract (2 ml each) were mixed with 0.1 ml of 10% aluminium chloride which was followed by the addition of 0.1 ml of 1M of CH_3COOK. Then the reaction mixture was incubated at room temperature for 40 min and the absorbance was measured at 415 nm. The tests were performed for three concentrations of extracts i.e. 1 mg/ml, 0.5 mg/ml, 0.2 mg/ml. The flavonoid contents were calculated from a quercetin standard curve.

Statistical Analysis

Results were expressed as mean ± S.E.M. of triplets and then graphically placed. The groups were compared by two-way ANOVA using Graph Pad Prism, Version 5.0

Table1: Morphological information of different cultivars of *Canna*

Canna cultivars	Plant Height (inches)	Morphological Features
C_1	64	Broad green leaves with deep violet sheds. Small red coloured flower (edible)
C_2	36	Big yellow flower with orange spots or sheds on the petal.
C_3	41	Small red coloured flower
C_4	36	Big orange coloured flower
C_5	40	Medium sized pinkish red flower
C_6	28	Big yellow flower with red dots and stripes in the middle of the petal
C_7	12	Variegated leaves. Medium sized flowers having light yellow colour in the upper and lower base portion with off-white in the middle of the petal
C_8	24	Broad light green leaves without any sheds with small yellow flowers (edible)
C_9	16	Small yellow coloured flower
C_{10}	62	Big red coloured flower
C_{11}	24	Big yellow flower

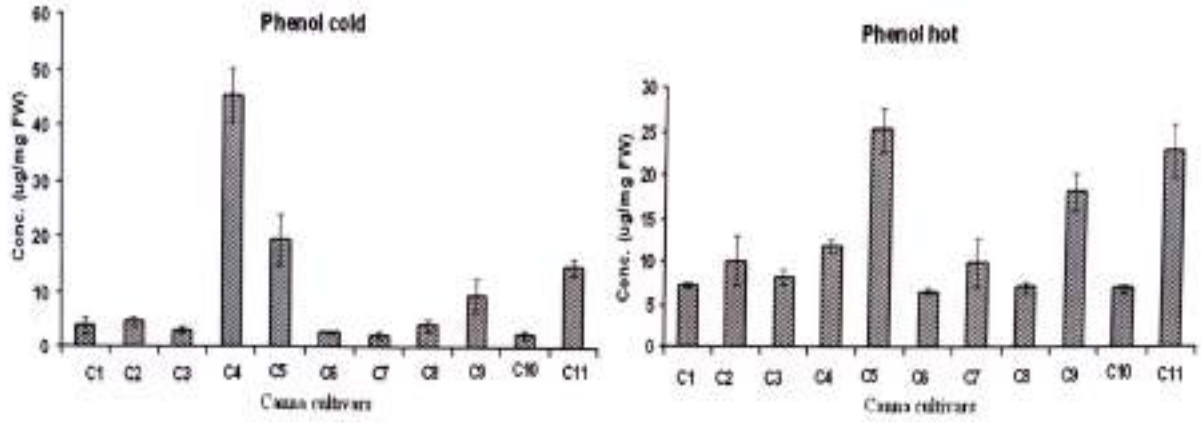

Fig 1: Total phenol of cold and hot extract of cultivars of Canna.

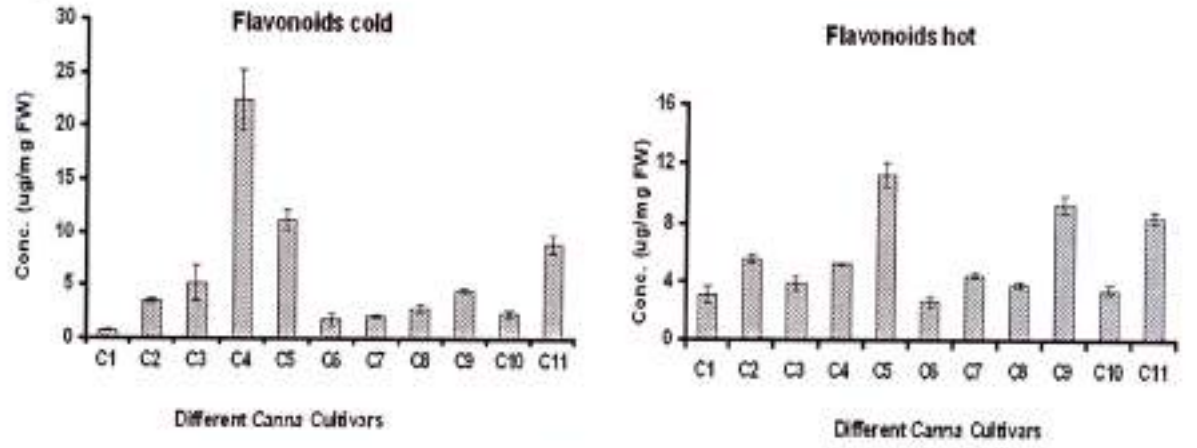

Fig 2: Flavonoid contents of cold and hot extracts of different cultivars of Canna

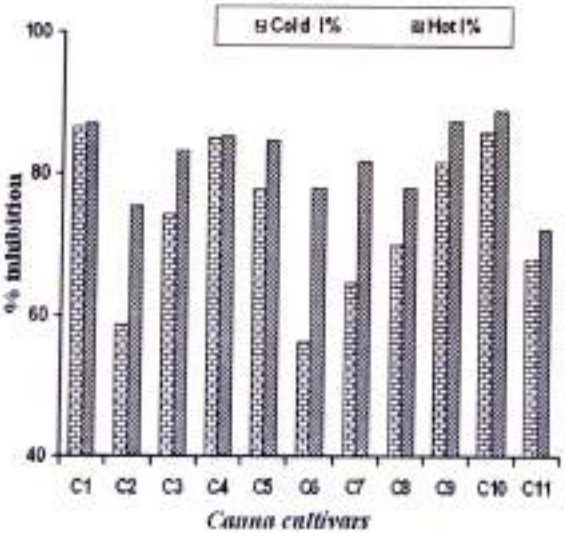

Fig 3: DPPH antiradical activity of cold and hot extracts of different cultivars of *Canna*

(Graph Pad Software, San Diego, CA, USA). P-values < 0.001 were considered significant. Correlation coefficient of all the data matrices were measured through SPSS Ver. 12.0.

Results and Discussion

Morphological characteristics like plant height, leaf feature and specifically the colour and size of the flowers of 11 different cultivars of *Canna* are described in table-1.

Extractive Yield

Total extractive yield was found to be in the range of 3.0 - 4.0% in both cold and hot extract of all the cultivars.

Determination of total phenolic contents

In our study we performed the total phenolics content of both cold and hot extracts of rhizomes of *Canna* cultivars. The total phenol was found to be highest in hot rhizome extract of C_3. Interestingly, it was found that, hot extracts of almost all the cultivars have more phenolics than that of cold extracts except in case of C_6 (Figure-1) The release of low molecular weight phenolic compound due to heat treatment may be responsible for increase in the total phenol in hot extract (Xu *et al.*, 2007).

Determination of flavonoids content

We also determined the flavonoid contents of both cold and hot extracts of rhizomes of all the 11 *Canna* cultivars(Figure-2). Here also we found that hot extracts

Table 2: Correlation Co-efficient of different parameters of hot and cold extracts of *Canna* cultivars

	DPPH Hot	Flavonoids Hot	Phenolics Hot	DPPH cold	Flavonoids Cold	Phenolics Cold
DPPH Hot	1	-0.019	-0.132	**0.815(**)**	0.071	0.128
Flavonoids Hot	-0.019	1	**0.964(**)**	0.139	0.396	0.368
Phenolics Hot	-0.132	**0.964(**)**	1	0.103	0.443	0.410
DPPH Cold	**0.815(**)**	0.139	0.103	1	0.324	0.377
Flavonoids Cold	0.071	0.396	0.443	0.324	1	**0.981(**)**
Phenolics Cold	0.128	0.368	0.410	0.377	**0.981(**)**	1

exhibit higher flavonoids than cold extracts, though it was seen in case of C_4 that cold extract had higher flavonoids as compared to hot extract. The increase in flavonoids of rhizome extracts after heat treatment may be due to disruption of cell wall, which helps in release of flavonoids from the cell matrix (Choi *et al.*, 2005)

DPPH scavenging activity

The rhizome extracts of all the *Canna* cultivars were tested for their antioxidant properties to determine their power to scavenge reactive oxygen species (ROS) (Figure-3). Quite interestingly here also we found that the hot extract exhibit higher antioxidant than the cold extract. High levels of DPPH scavenging activity in hot extract of all the studied cultivars of *Canna* may be due to the presence of large amount of polyphenols (Rice-Evens *et al.*, 1997).

Correlation Co-efficient

In our study we found a close linear correlation between different parameters of hot and cold extract of all the 11 cultivars of *Canna* (Table-2). It was found that total phenol of both hot and cold extracts were highly correlated with that of flavonoids and *vice versa*. DPPH scavenging activity of hot and cold extracts of all the 11 cultivars were positively linked with each other. But surprisingly, DPPH scavenging activity of both these extracts was not correlated with that of phenols and flavonoids. This lack of correlation is in accordance with other literature (Heinonen *et al.* 1998). The specific structure of the polyphenols having hydroxyl position in the molecule is known to act as proton donor showing radical scavenging activity (Mensor *et al.* 2001, Hou *et al.* 2003). As the extracts are very complex mixture of different compounds, it can be inferred that DPPH inhibitory activities in these *Canna* cultivars were not due to the presence of phenols and flavonoids rather these may be due to the presence of steroids or terpenoids or glycosides or any other phytochemicals (Haraguchi *et al.*, 1997).

Conclusion

The above studied *Canna* cultivars showed different levels of phenols, flavonoids and DPPH scavenging activity. This report also indicated that hot rhizome extracts of *Canna* possessed high antioxidant activity as compared to cold extracts. The above *in vitro* assay showed significant linear correlation between phenols and flavonoids, whereas DPPH scavenging activity was neither correlated with phenols nor with flavonoids. So it can be concluded that high DPPH scavenging activity was not due to the presence of high levels of phenolics but because of steroids or terpenoids or glycosides or any other phytochemicals. Further investigation is needed to isolate the active phytochemicals responsible for high radical scavenging activity.

References

Atrooz OM 2007. The incorporation effects of methanolic extracts of some plant seeds on the stability of phosphatidylcholine liposomes. Pak J Biol Sci. 10: 1643-1648.

Bagul MS, Kanaki SN and Rajani M 2005. Evaluation of free radical scavenging properties of two classical polyhedral formulations. Indian J Exp Biol. 43: 732-736.

Choi Y, Lee SM, Chun J, Lee HB and Lee J 2005. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. J. Food Chem. 99: 381-387.

Duke JA and Ayensu ES 1985. Medicinal plants of *China*. Vol I, Reference Publications, Algonac, Michigan, pp 232.

Goyal AK, Middha SK and Sen A, 2010. Evaluation of the DPPH radical scavenging activity, total phenols and antioxidant activities in Indian wild Bambusa vulgaris "Vittata" methanolic leaf extract. J. Nat. Pharm. 1: 40-45.

Haraguchi H, Ishikawa H, Sanchez Y, Ogura T, Kubo Y and Kubo I 1997. Antioxidative constituents in *Heterotheca inuloides*. Bioorganic and Medicinal Chemistry. 5: 865-871.

Heinonen M, Meyer AS and Frankel EN 1998. Antioxidant activity of Berry phenolics on human low density lipoprotein and liposome oxidation. J. Agric. Food Chem. 46: 4107-4112.

Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY and Lee MH 2003. Free radical scavenging activity of Taiwanese native plants. Phytomedicine. 10: 170-175.

Larson RA 1988. The antioxidants of higher plants. Phytochemistry. 27: 969-973.

Lin JY, Tang CY 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 101: 140-147.

Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS and Leitao SG 2001. Screening of Brazilian

plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 15: 127-130.

Middha SK, Mittal Y, Talembedu U, Kumar D, Srinivasan R, Vashisth L, Bhattacahrgae B and Nagaveni MB 2009. Phyto-mellitus: A phyto-chemical database for diabetes. Bioinformation, 4: 78-79.

Ravishankara MN, Shrivastava L, Padh H. and Rajani M 2002. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus*. Phyomedicine. 9: 153-157.

Rice-Evens CA, Miller NJ and Paganga G 1997. Antioxidant properties of phenolic compound. Trends Plant Sci. 2: 152-159.

Singleton VL, Orthofer R and Lamuela –Raventos RM 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth. Enzymol. 299: 152-178.

Tanaka N 2001. Taxonomic revision of the family Cannaceae in the New World and Asia. Makinoa N. Ser. 1: 1–75.

Tanaka N 2004. The utilization of edible *Canna* plants in southeastern Asia and southern China. Econ Bot. 58: 112-114.

Tanaka N 2008. A new species of the genus Canna (Cannaceae) from eastern Honduras. J. Jpn. Bot. 83: 7–10.

Vankar PS and Srivastava J 2008. Comparative Study of Total Phenol, Flavonoid Contents and Antioxidant Activity in *Canna indica* and *Hibiscus rosa sinensis*. Prospective Natural Food Dyes. Int J Food Eng. 4. DOI: 10.2202/,2008,1556-3758.1232.

Vanni T, Rajani M, Sarkar S and Sishoo CJ 1997. Antioxidant properties of the ayurvedic formulations Triphala and its constituents. Int. J. Pharmacol. 35: 313-317.

Xu G, Ye X, Chen J and Liu D 2007. Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. J Agric Food Chem. 55: 330-335.