International Journal of Pure and Applied Zoology
Volume 3, Issue 2, pp: 113-118, 2015
http://www.ijpaz.com

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585
Rishan Publications

Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF EXTRACT FROM *DAHLIA X HYBRIDA* SPECIES

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Article History: Received 17th January 2015; Accepted 12th April 2015; Published 8th May 2015

ABSTRACT

The fact that many health problems arise due to the use of synthetic ingredients has directed research to investigate the importance of natural products. Many fruits and vegetables are of nutritional value and considered as health promoters, due to the presence of various phytochemicals. It is known that Dahlia x hybrida contain secondary metabolites, including antioxidants that are important to human health. However, very little is known about nutritional and medicinal value of their leaves. In the present study, we have investigated the medicinal value of *Dahlia x hybrida* species. Antioxidant properties of the leaves were examined in the laboratory using different methanolic solvents by Soxhlet extractor. The total phenolic content of the extract was determined by Folin–Ciocalteu method. The dried fractions were screened for their antioxidant activity potential by 1diphenyl 2 picryl hydrazyl (DPPH), ferric reducing power (FRAP). The results showed that waste extract obtained from Dahlia x hybrida species exhibited remarkable antioxidant activity. Our conclusion, of the present study is that Dahlia x hybrida is possibly a vegetable bye product that has immense potential as sources of polyphenols. However, more research should be carried out to identify other possible antioxidants and bioactive compounds in these resources.

Keywords: Antioxidant activity, DPPH, *Dahlia x hybrid*, Medicinal value.

INTRODUCTION

The demand for natural sources of supplements is enhancing every day due to the more awareness about the dangers and side effects of synthetic counterparts. Natural food supplements are not only healthier, but they are also less contaminated with byproducts (Farag *et al.*, 1986). The presence of various antioxidants together with their composition has been reported in various fruits and vegetables (Singh *et al.*, 2002; Gorinstein *et al.*, 2001). The oxidative damage of various biomolecules (lipids, proteins, RNA and DNA) in the human body is associated with lipid peroxidation, cell structural injury, tissue impairment and gene mutation. Free radicals play a crucial role in aging, as well as many

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diseased conditions (cardiovascular disorders, cancer, neurodegenerative diseases, inflammation) (Bhalodia *et al.,* 2011). In addition, lipid oxidation initiated by free radicals is one of the major factors for food deterioration during processing and storage.

There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their role in health and disease and their nutritional value. Different aromatic herbs and spices have been investigated for their antioxidant activity. Some, particularly those belonging to the *asteraceae* have been found to be very effective with regard to natural antioxidants.

In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical p reparations of modern medicines. According to National Health Experts, 2000 different plants are used for medicinal preparations for both internal and external use in India alone. *Dahlia x hybrida* belongs to the family *asteraceae* and is a perennial growing to 1m. It is in flower from June to October. The flowers are hermaphrodite (has both male and female organs) and are pollinated by Insects. The flower petals are used in salads. Root cooked and used as a vegetable. Its have bitter flavour. A sweet extract of the tuber, called 'dacopa', is used as a beverage or as a flavouring. It is mixed with hot or cold water and sprinkled on ice cream. Its naturally sweet mellow taste is said to combine the characteristics of coffee, tea and chocolate. The root is rich in the starch insulin. Whilst not absorbed by the body, this starch can be converted into fructose, a sweetening substance suitable for diabetics to use. An orange dye is obtained from the flowers and seed heads. The aim of the present study is to determine the antioxidant activity of various extracts of *Dahlia x hybrid* (Wimmer *et al.,* 1998).

The aim of this work was to examine *asteraceae* species from Calicut for their *in vitro* possible antioxidant activity. Assays were carried out on methanolic extracts: DPPH (2,2-diphenyl-1-picrylhydrazyl) free - radical scavenging assay . The relationship between the antioxidant activity and the total content of polyphenols was also studied.

MATERIAL AND METHODS

Chemicals and apparatus

All chemicals and reagents were of analytical grade and were purchased from Sigma Aldrich Chemical Co. (Germany) and Merck (Germany).All spectrophotometric measurements were performed on ABLE-JASCO V 550 UV - VIS spectrophotometer.

Plant materials

The plant materials used in this study were flowers of *asteraceae* plants growing in Botanical Garden from Calicut, Kerala. Harvest was done in the first half of June 2012, at flowering stage.

Physical characteristics of the samples

Colour

Color is generally the first attribute, which influences acceptability. Colour of dried powders by visual observation was determined using Munsell colour charts (Macbeth, 2000).

Water absorption capacity (WAC)

WAC of the sample (mL/100g) was determined by the centrifuge technique (Janicki and Walczak, 1954). A 1.0 g sample taken in a centrifuge tube was mixed with 5.0 mL water. The slurry was weighed, kept aside for 30 min with gentle stirring with a glass rod every 5 min and centrifuged at 3000 rpm for 25 min. The amount of water retained was calculated by measurement of the difference in the weight of the sample before and after equilibration with water.

Preparation of methanolic Extract

Collected plant material was air-dried and stored at room temperature without exposure to direct sunlight. Air-dried flowers of *Dahlia x hybrida* were reduced to a fine powder (50 g),

and soxhlet extracted with absolute methanol (250 mL) for 24 h. The extract was filtered using Whatman No. 1 filter paper and the filtered methanolic extract was concentrated to dryness under vacuum desiccators and the solvent was removed by vacuum evaporation. The residues were kept at -20^{o}C.

Determination of DPPH free-radical scavenging activity

The antioxidant activity of the extracts based on the scavenging activity of the stable 1,1 - diphenyl-2-picrylhydrazyl (DPPH) free radical was determined by the method described by (Malterud *et al.,* 1993). Initially, four dilutions in DMSO (20 mg/mL; 10 mg/mL; 5 mg/mL and 2.5 mg/mL) were carried out with the dried extracts of *Dahlia* flowers. Briefly, an aliquot of each dilution (0.05 mL) was mixed with a solution of DPPH in methanol (4 mg %) (A517 = 1.02; 2.95 mL) and the absorbance was measured at 517 nm for 5 min. Ascorbic acid at various concentrations was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity.

Reducing power ability

In this assay, Fe3+ /ferricyanide complex was reduced to the ferrous form by antioxidants. The Fe2+ formed was monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986). Different amounts of sample in

1.0 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) were mixed and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

The data were analysed for mean and standard deviation. Student's t -test was used to determine significant differences in polyphenols and antioxidant activity in pulp wastes. Correlation coefficient test was applied to test the association between the antioxidant components and the antioxidant activity of the pulp wastes using a statistical package SPSS 10.0 for windows. Probability level was fixed to $P< 0.05$.

RESULTS AND DISCUSSION

Physical characteristics of the *Dahlia* flowers

The physical characteristics of the *Dahlia* flowers is given in the table 1.

Table 1. Physical characteristics of the flowers.

Samples	Colour	Yield		Physical characteristics	
	Dry	As g/100g whole flowers	As dry powder (g/100g)	Water absorption capacity of powder (ml/100g)	Bulk density of dry powder (ml/100g)
Orange colour *Dahlia*	2.5 YR 8/3 Pink	35.45	13.31	1020.00±0.00	54.54±0.00
Beetroot colour *Dahlia*	10 YR 3/3Dark brown	19.96	14.56	560.00±0.00	53.57±0.00

Total polyphenols and total antioxidant activity

Vegetable and flowers materials contain many compounds with antioxidant activity. Several plants have been studied as sources of potentially safe natural antioxidants for the food industry; various compounds have been isolated with many of them being polyphenols. Polyphenolic compounds affect the functional and nutritional values of vegetable proteins by reducing the nutritional values of foodstuffs and contributing to the sensory and organoleptic properties of fruits and vegetables (colour, taste, astringency) (Shyamala and Jamuna, 2010). Total phenolic contents (TPC) in orange colour *Dahlia* and maroon colour *Dahlia* are extracted in aqueous

and solvent medium are given in Table 2. Methanol extract had the highest polyphenol content in both orange (250 mg) and maroon (220 mg) colour flowers compared to aqueous extracts of the samples, which ranged from 67-110 mg TAE/100 g sample. The difference between extracts was marginally significant (P value = 0.02). The total antioxidant activity was higher in aqueous extracts than in solvent extracts.

The value ranged from 46788–91225µmoles of ascorbic acid/100 g of sample. Between the two flowers, there was no significant difference in total antioxidant activity as determined by Student t -test (P > 0.05).

Antioxidant activity by free radical scavenging activity

Free radical scavenging activity was high in methanol extract compared to aqueous extracts in both the samples. At 40 mg concentration, orange and maroon flower extracts had 78.8% and 65.6% activities respectively (Figures 1 and 2).

Both the flowers showed significant activity in methanol extract and lesser activity in aqueous extract at 5 mg concentration. Statistically, there was no significant difference observed between the free radical scavenging activity of the two pulp wastes (< 0.05 ns).

Table 2. Total polyphenols and total antioxidant activity of the *Dahlia x hybrida* flower extracts.

Extracts	Orange colour dahlia	Maroon colour dahlia	P-value
Total polyphenols (mg tannic acid equivalent/100 g of sample)			
Methanol	250.00±0.00	220.00±0.00 0.	020*
Aqueous	110.00±0.00	67.50±0.00	
Total antioxidant activity (µmoles of ascorbic acid/g of sample)			
Methanol	46788.92±200.	88 15784.67±100. 54 0.	244 ns
Aqueous	75048.92±211.45	91225.58±422.	64

* - P value < 0.05 = marginally significant ns - P value > 0.05 = not significant.

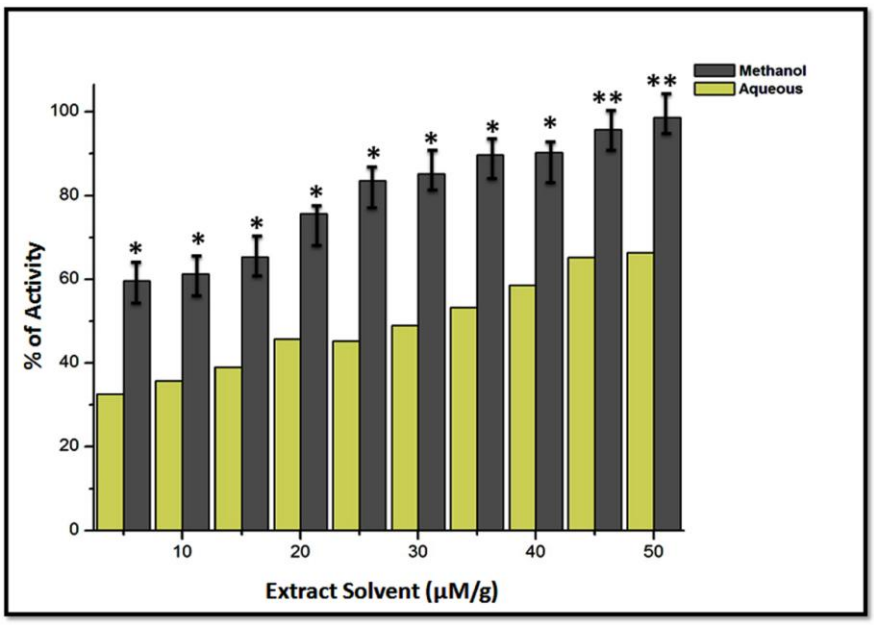

Figure 1. Free radical scavenging activity of orange dahlia flower.

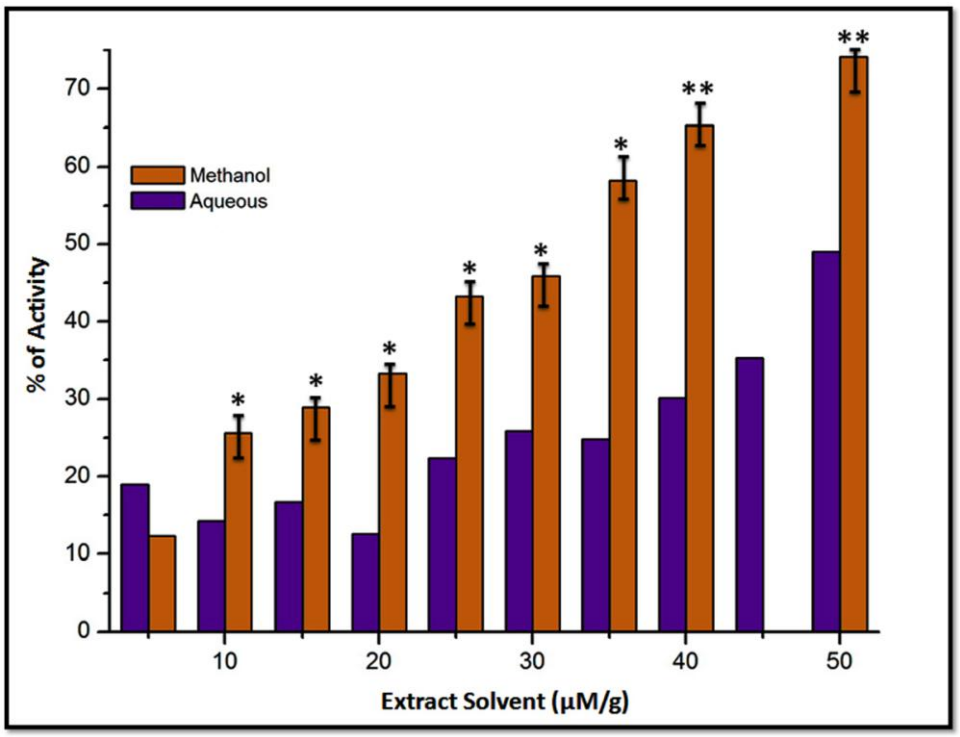

Figure 2. Free radical scavenging activity of maroon dahlia flower.

Antioxidant activity by reducing power

The reducing power of both the flowers had a similar trend in methanol extract (Figure 3). But maroon flower did not show any reducing power in methanol and aqueous extracts. Orange flower had almost similar reducing power in both methanol and aqueous extracts. There was a significant difference observed between reducing power of samples ($P \leq 0.01$) by this assay. Correlation coefficient was determined to see whether there existed any relationship between antioxidant activity and antioxidant components. In free radical scavenging activity, only tannins were well correlated (R 2 =1) and polyphenols were negatively correlated (R 2 = -1). But in the case of reducing power, polyphenols were positively correlated and tannins were negatively correlated (R2 = -1). In the case of total antioxidant activity, positive correlation was seen with tannins in aqueous extract (R2 =1) and polyphenols in methanol extract.

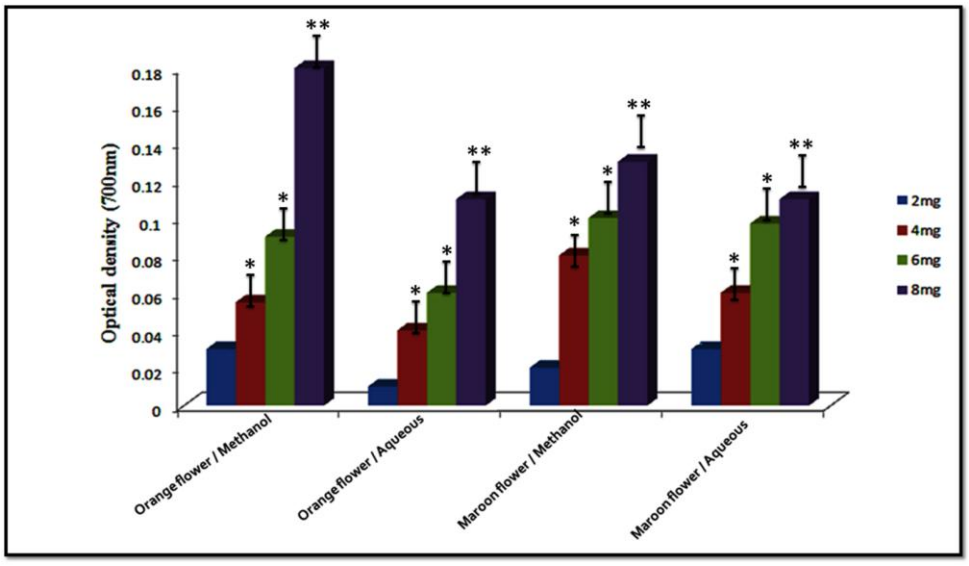

Figure 3. Reducing power of flowers in methanol solvent and aqueous extracts.

CONCLUSION

The hypothesis of obtaining plant based medicine is beneficial to human health based on the active profile exposed through various *in vitro* assays proved that the methanolic extract of flowers of *Dahlia x hybrida* species showed significant antioxidant activity. Further investigations on the isolation and identification of Bio active components on the plant would help to ascertain its potency.

ACKNOWLEDGMENT

The work was financially supported by UGC-Rajav Gandhi National Fellowship (RGNF)(No:F.14-2(SC)/2010(SA-III) University Grant Commission New Delhi, India, to the Second author (K.V.).

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