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Research Article

EVALUATION OF EDIBLE FLOWERS FROM NORTHERN INDIA AS A RICH SOURCE OF PHYTOCHEMICALS WITH ANTIOXIDANT AND PHENOLIC PROPERTIES

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ABSTRACT

Herbal plants have been used in the traditional system of medicine, aiming to maintain health and to cure diseases. We investigated the phenolic compounds and antioxidant capacities of free and bound phenolics from 13 available edible flowers which have long been consumed as vegetable and used as ingredients in cooking. In this study, an attempt was made to estimate the phenolic level, carbohydrate level, protein level, ascorbic level, FRAP, modified FRAP, flavonoid test, antioxidant level, tannins test. Herbal flowers were chosen (e.g. *Chrysanthemum indicum*, *Vachellia nilotica*, *Butea monosperma*, *Salvia splendens*, *Rosa indica*, *Hibiscus rosasinensis*, *Dianthus pavonius*, *Calendula officinalis*, *Tropaeolum majus*, *Celosia argentea*, *Lantana camara*, *Viola tricolor*). The highest ascorbic acid content (164.44 \pm 5.09 mg/ml, phenolic levels (760.66 \pm 5.03 mg TAE/g) and antioxidant levels (39.03 \pm 0.50 mg AAE/g) was found in *Rosa indica* whereas highest flavonoid content was found in *Viola tricolor* (0.56 \pm 0.02 mg AAE/g. The antioxidant levels, total phenolic and total flavonoid content (correlation coefficient R² = 0.5851 for phenolic, R²= 0.456 for flavonoid, R²= 0.6639 for ascorbic acid) were correlated which showed significant correlation indicating that the phenolics and ascorbic acid are major components responsible for antioxidant activity of flowers.

Keywords: edible flowers, antioxidants, phenolics, flavonoids, ascorbic acid

INTRODUCTION

India is a rich source of edible flowers with medicinal uses and high nutritional value. Worldwide, around 4,22,000 flowering plants are reported, out of which more than 50,000 plants are used for medicinal purposes. India has a very rich flora with nearly 17,500 flowering plants which constitutes 12% of the recorded world flora. In India, more than 43% of the total flowering plants are reported to be of medicinal importance¹.

In Indian traditional systems of Medicine *Ayurveda* and *Siddha* system (prevalent mostly in South India), flowers are used in the treatment of various ailments. It has been reported that around 2500 species are used as medicinal herbs in India². The medicinally useful part may be whole or inflorescence as in *Kumbhi* (*Careya arborea* Roxb.), only petals as in *Shatapatra* (*Rosa centifolia*), stigmas and upper portion of the styles as in *Saffron* (*Crocus sativus* L.)

The flowers have phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, quinine, saponins, steroids, triterpenes and tannin as major phytochemical groups³. The main soluble phenolic acids in edible flowers were gallic acid, ferulic acid and sinapic acid. Quercetin is acommon flavonoid found in edible flowers and is usually present in glycosylated form⁴. These phyto constituents are used for the treatment of various human ailments and possesses wound healing, allelopathic, anthelmintic, anticancer, antifungal, nematostatic, insecticidal, and anti hyperglycemic activity⁵.

In countries like India, flowers are used in religious, cultural activities, worship places and various civilizing and sacred ceremonies, which make them a usual source of floral waste⁶. However, due to the presence of phytoconstituents, these floral wastes can be potentially considered as a major source of phytocompounds in pharma and nutraceutical industries.

Chandigarh is beautiful city in Northern India with a wide range of medicinal flowers in Kansal forests, Sukhna Lake, parks etc., The objective of the present study was to estimate phenolic, flavonoid and antioxidant properties of 13 edible flowers which were available locally. An attempt was also made to study the relationship between antioxidant capacity and total phenolic content which will help to throw light on their potential health benefits that could be useful for consumers and public health workers.

MATERIAL AND METHODS

Processing of flowers

The flowers of Chrysanthemum indicum, Vachellia nilotica, Butea monosperma, Salvia splendens, Rosa indica, Hibiscus rosasinensis, Dianthus pavonius, Calendula officinalis, Tropaeolum majus, Celosia argentea, Lantana camara, Tagetes patula and Viola tricolor were collected from parks of Chandigarh and dried under sun for 3-5 days. The dried flowers were powdered and stored at room temperature.

Sample extraction

1 g dried flowers was weighted into a beaker and 100 ml of boiling distilled water was added. After brewing for 5 min, the blend was removed and the extract was cooled down. All analyses of aqueous tea extracts were done in triplicate

Estimation of total phenol content (TPC)

The total phenol content (TPC) was determined by spectrophotometer using tannic acid as standard with some modifications⁷. 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of tannic acid equivalent per g dry weight (mg TAE/g).

Determination of Total flavonoid content

Total flavonoid content (TFC) was measured by the modified aluminium chloride colorimetric assay⁷. The reaction mixture consisted of 1 ml of extract and 4 ml of distilled water taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was added and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was added and final volume of the mixture was brought to 10 mL with double-distilled water. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve and was expressed as mg Ascorbic acid equivalent AAE/g of extract.

Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant capacity was determined spectrophotometrically using ascorbic acid as standard by modified FRAP assay⁷. 0.1 ml of extract was taken and to it 0.9 ml of ethanol, 5 ml of distilled water, 1.5 ml of HCl, 1.5 ml of potassium ferricyanide, 0.5 ml of 1 % SDS and 0.5 ml of 0.2 % of ferric chloride was added. This mixture was boiled in water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 750 nm to measure the reducing power of the tea extract. The antioxidants in samples were derived from a standard curve of ascorbic acid and were expressed as mg ascorbic acid equivalent (AAE)/g.

Statistical analysis

The assays were carried out in triplicate and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one way ANOVA ($p \le 0.05$). Correlation coefficient (R) and coefficient of determination (R²) were calculated using Microsoft Excel 2000.

RESULT AND DISCUSSION

The ascorbic acid levels in various flower ranged from 33.11 \pm 3.00 mg/ml to $164.44 \pm 5.09 \text{ mg/ml}$ (Table 1). The highest ascorbic acid content was found in Rosa indica (164.44 \pm 5.09 mg/ml) whereas the lowest was found in the Tropaeolum majus $(33.11 \pm 3.00 \text{ mg/ml})$. Among the 13 flowers studied the phenolic levels ranged from 760.66 ± 5.03 mg TAE/g to 37.20 \pm 5.01 mg TAE/g (Table 1). The highest phenolic levels were found in Rosa indica (760.66 \pm 5.03 mg TAE/g) followed by Tagetes patula (594.00 \pm 4.16 mg TAE/g) and *Chyransethemum indicum* (432.66 ± 5.03 mg TAE/g), *Hibiscus* rosasinensis (406.00 \pm 7.21 mg TAE/g), Viola tricolor (340 \pm 8.00 mg TAE/g), Celosia argentea (200 \pm 6.00 mg TAE/g), Dianthus pavonius (174.66 \pm 6.11 mg TAE/g) and lowest were found in Tropaeolum majus, Vachellia nilotica, Salvia splendens, Lantana camara, Butea monosperma, Calendula officinalis (54.73 \pm 4.51 mg TAE/g to 37.20 \pm 5.01 mg TAE/g). The flavonoid contents in various flower teas ranged from 0.1 \pm 0.005 mg AAE/g to 0.56 \pm 0.02 mg AAE/g (Table 1). The highest flavonoid content was found to be present in Viola $tricolor (0.56 \pm 0.02 \text{ mg AAE/g})$ whereas the lowest were found in Butea monosperma (0.01 \pm 0.005 mg AAE/g). Among the 13 flowers studied the antioxidant levels ranged from (39.03 ± 0.50) mg AAE/g) to $(6.99 \pm 0.51 \text{ mg AAE/g})$ (Table 1). The highest antioxidant levels were found in Rosa indica (39.03 \pm 0.50 mg AAE/g) whereas the lowest levels were found in Salvia splendens (6.99 \pm 0.51 mg AAE/g). The levels of antioxidants in decreasing order are Tagetes patula (30.76 ± 0.40), Viola tricolor (24.69 ± 0.45 mg AAE/g), Celosia argentea and $\textit{Vachellia nilotica}\ (18.97 \pm 0.66\ mg\ AAE/g,\ 18.07 \pm 0.73\ mg$ AAE/g), Butea monosperma (16.14 \pm 0.97 mg AAE/g) and Dianthus pavonius and Chrysanthemum indicum (11.49 \pm 0.58 mg AAE/g and 11.39 ± 0.892 mg AAE/g respectively), Calendula officinalis and Hibiscus rosasinensis (9.83 \pm 0.97 mg AAE/g, 9.25 ± 0.43 mg AAE/g), Lantana camara and Tropaeolum majus $(8.86 \pm 0.54 \text{ and } 8.38 \pm 0.35 \text{ mg AAE/g})$ and Salvia splendens (6.99 \pm 0.51 mg AAE/g). The correlations between antioxidant activity and total phenolics content, ascorbic acid and total flavonoid content were determined by linear regression analysis in 13 flowers studied. The coefficient of determination was highest between total antioxidant levels and ascorbic acid ($R^2 = 0.6639$) (Figure 1) followed by total antioxidant levels and TPC ($R^2 = 0.5851$) and total antioxidant levels and TFC ($R^2 = 0.456$) (Figure 2).

Ascorbic acid vs Antioxidants

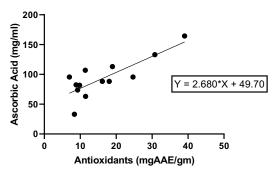


Figure 1: Linear correlations between ascorbic acid and antioxidants levels; Coefficient of determination $(R^2) = 0.6639$

Phenolics vs Antioxidants

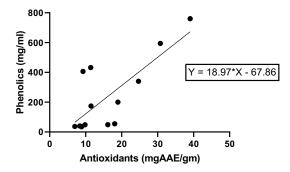


Figure 2: Linear correlations between total phenolic content (A) values and antioxidants levels; Coefficient of determination $(R^2) = 0.5851$

Table 1: Total Phenolic content, Total Flavonoid content, Total Antioxidant and ascorbic acid in edible flowers

S. No.	flowers	Total Phenolic content (mg TAE/g)	Total Flavonoid content (mg AAE/g)	Total Antioxidant (mg AAE/g)	Ascorbic Acid (mg/ml)
1	Rosa indica	760.66 ± 5.03	0.41 ± 0.01	39.03 ± 0.50	164.44 ± 5.09
2	Chrysanthemum indicum	432.66 ± 5.03	0.33 ± 0.02	11.39 ± 0.892	106.88 ± 9.00
3	Calendula officinalis	48.66 ± 7.02	0.2 ± 0.01	9.83 ± 0.97	81.77 ± 7.12
4	Hibiscus rosasinensis	406 ± 7.21	0.2 ± 0.02	9.25 ± 0.43	73.77 ± 6.01
5	Lantana camara	35.33 ± 7.02	0.36 ± 0.03	8.86 ± 0.54	82.44 ± 4.07
6	Dianthus pavonius	174.66 ± 6.11	0.17 ± 0.01	11.49 ± 0.58	63.11 ± 3.00
7	Butea monosperma	49.2 ± 5.01	0.1 ± 0.03	16.14 ± 0.97	88.27 ± 6.01
8	Vachellia nilotica	54.73 ± 4.51	0.1 ± 0.02	18.07 ± 0.73	88.27 ± 2.05
9	Celosia argentea L.	200 ± 6	0.42 ± 0.02	18.97 ± 0.66	113.11 ± 5.00
10	Viola tricolor	340 ± 8	0.56 ± 0.02	24.69 ± 0.45	95.55 ± 5.09
11	Tropaeolum majus	40.66 ± 7.02	0.125 ± 0.005	8.38 ± 0.35	33.11 ± 3.00
12	Salvia splendens	37.20 ± 5.01	0.125 ± 0.002	6.99 ± 0.51	95.55 ± 5.09
13	Tagetes patula L.	594 ± 4.16	0.32 ± 0.02	30.76 ± 0.40	133 ± 12.12

DISCUSSION

Ascorbic acid (AsA) is an antioxidant molecule and a key substrate for the detoxification of reactive oxygen entities. The high ascorbic acid content was found in various flowers studied e.g. Rosa indica, Hibiscus rosasinensis, Tagetes patula L., Calendula officinalis, Chrysanthemum indicum as reported in literature⁸. There was significant positive correlation between antioxidant levels and ascorbic acid in flowers. Thus, ascorbic acid present in flowers could a natural source of vitamin C and antioxidants.

In the present study, the highest flavonoid levels were observed in Viola tricolor which could be due to presence of flavonols such as quercetin and isorhamnetin glycosides, flavones such as apigenin glycosides and anthocyanins such as cyanidin and delphinidin glycosides⁹. The carotenoids such as lutein along with phenolic substances contribute to the antioxidant capacity of Viola tricolour. In Celosia argentea L., luteolin-7-O-glucoside is the most common flavonoid¹⁰. In Rosa indica, quercetin is the most abundant flavonoid that forms glycosides, such as rutin, with a great variety of sugars. Quercetin and its derivative quercetin-3-O-glucuronide help in inhibiting overproduction and thus help in chemoprotection of mitochondrial function through antioxidative actions¹¹. The flavonoids such as luteolin-7-O-β-glucoside apigenin-7-O-βglucoside, linarin, acacetin-7-O-β-glucoside, luteolin and apigenin are present in Chrysanthemum indicum¹².

Rosa indica have high level of phenolics like flavonols such as kaempferol and quercetin¹³. In *Hibiscus rosasinensis*, phenolic

compounds include phenolic acids, flavonoids and anthocyanins which contribute to the antioxidant levels also. In *Chrysanthemum indicum*, phenols such as chlorogenic acid and 3, 5-dicaffeoylquinic acid and flavonoids such as luteolin-7-*O*-β-glucoside apigenin-7-*O*-β-glucoside, linarin, acacetin-7-*O*-β-glucoside, luteolin and apigenin are present¹². In *Celosia argentea*, 1-(4-hydroxy-2-methoxybenzofuran-5-yl)-3-phenylpropane-1, 3-dione is most common phenolic compound found¹⁰. There was a positive correlation between total antioxidant levels and the total phenolic contents which indicated that polyphenol compounds largely contributed to the antioxidant capacities of the selected flowers as reported earlier¹⁴.

The high antioxidant activity in various flowers could be due to the presence of phenolic, ascorbic acid and secondary metabolites such as alkaloids, terpenoids etc¹¹. In addition, the anthocyanins present in various flowers such as anthocyanin -5-o glucoside-6"malonyl transferase, dimalonated anthocyanins glucoside-5-dimalonyl (Pelargonidin-3-caffeoyl glucoside, pelargonidin-3-p-coumaroyl glucoside-5-dimalonyl glucoside) and malonated anthocyanins which also contribute to total antioxidant activity. The high antioxidant activity of the Rosa indica could be due to phenolics like flavonols such as kaempferol and quercetin mainly in glycoside-bound form¹³. Delphinidin-3-sambubioside and cyanidin-3-sambubioside are the major anthocyanins present in Hibiscus rosasinensis which also contributes to its antioxidant capacity¹⁵. Various antioxidant compounds present in Tagetes patula are Lutein esters, tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol which contribute to its high antioxidant activity¹⁶. The antioxidant activity of Salvia splendens in our study could be due the presence of flavonoids (flavones of apigenin and luteolin and their hydroxylated derivates), phenolic glycosides, alkaloid, tannin, saponin, terpenoids, reducing sugar and steroids¹⁷. Various phyto constituents of *Butea monosperma* such as flavonoids like dihydrochalcone and dihydromonospermoside along with Butin, isobutrin, butein, monospermoside and isoliquiritigenin contributes not only to its antioxidant capacity but also antimycobacterial, anti-inflammatory and antidiabetic activity¹⁸. Various phytochemicals such as luteolin-7-*O*-β-glucoside, 3, 5-dicaffeoylquinic acid, apigenin-7-*O*-β-glucoside and apigenin are present in *Chrysanthemum indicum*¹². The antioxidant activity reported in *Salvia splendens* in our study could be due the presence of flavonoids (flavones of apigenin and luteolin and their hydroxylated derivates), phenolic glycosides, alkaloid, tannin, saponin, terpenoids, reducing sugar and steroids¹⁷.

CONCLUSION

The present study concludes that flowers have rich antioxidant efficacy with promising nutraceuticals potential and can have immense pharmacological application. They can also be used as potent antimicrobial herbal drug against clinical and drug-resistant pathogens. The flowers are a promising source of potential antioxidants and its extracts can be used in the pharmaceutical industry, as well as in food products and cosmetics. These antioxidants help in maintaining health and protect against atherosclerosis, stroke, diabetes, neurodegenerative diseases and cancer.

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