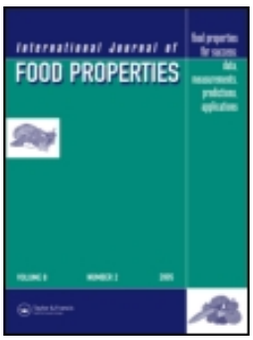

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# COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY OF THE FOOD FLOWERS OF WEST BENGAL, INDIA

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***In India, many flowers are consumed as food. During the present study, 37 flowers have been analyzed for their 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity and total antioxidant capacity. The flowers were found to have different levels of antioxidant properties in the systems tested. On the basis of the activity and availability, a few flowers were further studied for hydroxyl radical scavenging activity, superoxide radical scavenging activity, lipid peroxidation prevention, and DNA damage prevention properties. The correlations suggest that the total phenol contents of the flowers were responsible for the antioxidant properties of the aqueous extracts. The unweighted-pair group average dendrogram showing interrelationships between the investigated species grouped them into two high level clusters based on activity.***

**Keywords:** ***Anthocyanin, Antioxidant, Flavonoids, Food property, Flowers, Phenols.***

## INTRODUCTION

Studies to date have demonstrated that phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action, including scavenging oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects.[1] Flowers are consumed as food by many people of India. Flavonols, flavone, gallic acid, proanthocyanidins, and anthocyanins are the widely distributed group of polyphenols present in different flowers.[2,3] These compounds have been reported to have antitumor, anticancer, antifertility, and antioxidant activities.[4–11] During the present work, a comparative study of some flowers of West Bengal, India has been made for their free radical scavenging activity and property to prevent lipid peroxidation and DNA damage.

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## MATERIALS AND METHODS

### Plant Materials

Thirty-seven flowers, used as food by the santhals and other people of West Bengal, were collected from Sundarban to Chotanagpur plateau areas in the Western part of West Bengal and their ethnobotanical uses as food were recorded (Table 1). Cultivars were obtained from the Department of Agriculture, Sriniketan, Visva-Bharati, and experimental garden, Baruipur, University of Calcutta from naturally cultivated plants that were not treated with chemical fertilizers and pesticides. Vouchered specimens are deposited in the Herbarium, Surendranath College, Kolkata. The flowers or other floral parts were air-dried and then dried in an incubator at 40°C.

### Preparation of Extract

The dried powdered flowers (1–10 mg) were boiled for 2 min in double distilled water, centrifuged, and the supernatants were used for analyzing antioxidant activity *in vitro*.

### Chemicals

Chemicals, such as ethylenediamine tetra acetic acid (EDTA), trichloroacetic acid (TCA), butanol, ammonium molybdate, and sodium dodecyl sulphate, were purchased from E. Merck Ltd. (Mumbai, India). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was procured from Sigma (St. Louis, MO, USA). Thiobarbituric acid (TBA) was purchased from Spectrochem PVT. Ltd. (Mumbai, India). Nitro-blue-tetrazolium, Herring sperm DNA, and calf thymus DNA were obtained from Sisco Research Laboratories PVT. Ltd. (Mumbai, India). All other reagents were of analytical grade.

### DPPH Radical Scavenging Assay

The method described by Braca et al.[12] was followed for determining the antioxidant activity of the extracts on the basis of the scavenging activity of the stable DPPH free radical.

### Superoxide Radical ($O_2^{\bullet -}$) Scavenging Assay

The assay was based on the capacity of the extracts to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of riboflavin-light-NBT system.[13] $O_2^{\bullet -}$.radicals were generated by illuminating a solution containing riboflavin. The percentage inhibition of $O_2^{\bullet -}$ generation was measured by comparing the absorbance values of the control and those of the reaction mixture containing sample solution.[14]

### Assay of Hydroxyl Radical (•OH) Scavenging Activity

The assay was based on the benzoic acid hydroxylation method.[15] Benzoate is hydroxylated to hydroxybenzoates. Benzoate is weakly fluorescent, but after monohydroxylation forms highly fluorescent products.[16] The fluorescence was measured at 407 nm emission and excitation at 305 nm. Measurement of fluorometric changes has been used to detect damage by •OH radicals.

**Table 1** Flowers used as food in West Bengal.

| Plant material | Vernacular names | | Uses |
|---|---|---|---|
| | Bengali | Santhali | |
| *Allium cepa* (peduncles) | *piyanj kali* | *piyanj kati* | Taken as a vegetable |
| *Amaranthus viridis* L. (Amaranthaceae) | *no te* | *gandheri* | Flowers along with leaves are cooked as a vegetable, useful for colds and coughs |
| *Azadirachta indica* A. Juss. (Meliaceae) | *neem* | *neem* | Flowers and tender leaves with rice (neem dak' mandi/neem suruk') taken as a ritual food by santhals. Applied with mustard oil as a skin protector, mosquito repellent, spermacidal, and for insomnia. Carminative, useful for dyspepsia |
| *Basella rubra* L. (Basellaceae) | *puin* | *puin* | Taken as a vegetable, laxative |
| *Bauhinia acuminata* L. (Caesalpiniaceae) | *kanchan* | *kachnar* | Buds and leaves are consumed as a vegetable, antidysenteric, antidiabetic |
| *Bombax ceiba* L. (Bombacaceae) | *shimul* | *aedel* | Calyx cooked as a vegetable. Infusion of petals taken as contraceptive and for uterine infections |
| *Brassica campestris* (Brassicaceae) | *sarson* | *sarson* | Flowers are consumed as vegetables and prescribed as tonic for colds and coughs |
| *B. oleracea* brocoli | *phulkapi* | *phulkapi* | Cultivated and consumed as a vegetable |
| *Cassia tora* (L.) Roxb. (Caesalpiniaceae) | *kal kasunda* | *bheda deren* | Consumed as a vegetable by santhals |
| *Colocasia esculenta* (L.) Schott. (Araceae) | *kochu phul* | *kocho baha* | Yellow bracts are cooked as a vegetable, laxative, and tonic |
| *Commelina benghalensis* L. (Commelinaceae) | *kansira kanayia* | *kania* | Flowers along with leaves are cooked as a vegetable |
| *Crotalaria retusa* L. (Fabaceae) | *jhunjhun* | *jhunjhunia* | Consumed as a vegetable |
| *Cucurbita maxima* Duch (Cucurbitaceae) | *kumra* | *andia kundri* | Male flowers are cooked or fried as a vegetable |
| *Curcuma longa* L. (Zingiberaceae) | *kalud* | *kalud* | Spike cooked/raw consumed as a vegetable |
| *Digera muricata* (L.) Mart. (Amaranthaceae) | *maori* | *maori* | Spikes are cooked as a vegetable, used for lung and kidney infections, and as a laxative |
| *Dillenia indica* L. (Dilleniaceae) | *chalta* | *chalta* | Ripe calyces used as sour food |
| *Dregea volubilis* (L. f) Benth. ex Hook. f (Asclepiadaceae) | *jukti* | *jukti* | Flowers are cooked as vegetables, considered immunostimulant, aphrodisiac |
| *Euphorbia hirta* L. (Euphorbiaceae) | *dudhi* | *dudhi* | Cooked with leaves as a vegetable |
| *Ficus racemosa* L. (Moraceae) | *dumur* | *dumur* | Hypanthodiums are cooked as vegetables |
| *Hibiscus mutabilis* L. (Malvaceae) | *sthal padma* | *bhera* | Whole flowers, fried, or fresh petals consumed as food; useful for colds and coughs |
| *H. sabdariffa* cv. *archer H. sabdariffa* cv. *victor* | *chukoir* | *bombara* | Archer variety with petals red and greenish calyx. Victor with petals yellow and red ripe calyx, consumed as sour food and health drink |
| *Ipomoea aquatica* F. (convolvulaceae) | *kolmi phul* | *kurbi baha* | Cooked with the leaves. Juice given in snake bite, dog bite, scorpion sting, and as nerve tonic |

(*Continued*)

**Table 1** (Continued).

| Plant material | Vernacular names | | Uses |
|---|---|---|---|
| | Bengali | Santhali | |
| *Madhuca longifolia* (L.) Macbride (Sapotaceae) Village *matkom dihi* is named after this | *mahua* | *matkom* | The deciduous petals (*teke*) are collected, dried in the sun, boiled/raw eaten with rice or wheat flour and consumed at the time of scarcity. Wine (*duhlia* and *paure*) made from the petals |
| *Millettia auriculata* Baker ex Brand. (Fabaceae) | *farash* | *hehel* | Flowers consumed as food, fried, cooked, or eaten raw |
| *Moringa oleifera* Lam. (Moringaceae) | *sajne* | *munga* | Cooked flowers are favorite food, cholagogue, diuretic, tonic, immunoprotective |
| *Musa paradisiaca* Lam. (Musaceae) | *kola mocha* | *kola baha* | Cooked flowers and apical bud are favorite health food, tonic, astringent, antidiabetic |
| *Nelumbo nucifera* Gaertn (Nymphaeaceae) | *padma* | *padma* | Peduncles and thalamus consumed as vegetable, diuretic, soothing |
| *Nymphaea alba* L. (Nymphaeaceae) | *saluki/sapla* | *sapla* | |
| *N. nouchali* Burm f. | *lal saluk* | *sapla* | |
| *Phyllanthus emblica* Gaertn (Euphorbiaceae) | *amlaki* | *amla* | In addition to the popular fruit; leaves and minute deciduous flowers are also eaten |
| *Rosa centifolia* L. (Rosaceae) | *golap* | *golap baha* | Petals are used to garnish sweets and to prepare jelly, flower water in refreshing drinks |
| *Sesbania grandiflora* L. (Fabaceae) | *bak* | *bak* | Vegetables, fried or cooked, used for diarrhea and dysentery |
| *Tamarindus indica* L. (Caesalpiniaceae) | *tentul* | *jojo* | Sour tasting flowers, eaten raw or cooked, astringent and sedative |
| *Typha angustata* Bory et Chaub. (Typhaceae) | *hogla renu* | *hogla baha* | Pollen cooked with molasses and consumed as food |
| *Zea mays* L. cv. (Poaceae) | *bhutta mocha* | *mokai mocha* | Bud/young inflorescence/baby corn eaten raw or cooked as health food, cultivated |

### Lipid Peroxidation Assay

A modified[14] thiobarbituric acid reactive species (TBARS) assay[17] was used to measure the lipid peroxide formed using egg yolk homogenates as lipid-rich media.[18] Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm, which was measured.[17] In materials containing anthocyanin that also absorbs strongly at 532 nm, the method described by Banerjee et al.[19] was followed to eliminate the non-MDA interference.

### Determination of Total Antioxidant Capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH.[20] The antioxidant capacity is expressed as ascorbic acid (μg) equivalent per mg plant material.

### DNA Damage Prevention Assay

An *in vitro* assay[21] was followed by the Fenton reaction leading to formation of TBA-reactive substance and chromogen with a maximum absorption at 532 nm.[21–23] The reaction mixture (1.5 ml in a tube) contained 0.75 mg of Herring sperm DNA, 0.5 mM EDTA in 25 mM phosphate buffer, pH 7.4. The TBARS was measured by the addition of 1.5 ml of TBA (0.7% in 0.05 N KOH) and 1.5 ml of 2.5% TCA to the mixture. After heating at 100°C for 8 min in boiling water, the mixture was cooled and centrifuged and the supernatant was read at 532 nm. Another set of samples was treated in the same way, incubating without TBA to subtract the absorbance of anthocyanin (for the materials containing anthocyanin). Protection of DNA damage by aqueous extracts of flowers at different concentrations was calculated.

### Determination of Total Phenol Content

Total phenol content was determined by Folin-Ciocalteau reagent in alkaline medium[24] and was expressed as gallic acid equivalent.

### Determination of Total Flavonoid Content

Total flavonoid content was measured following Kim et al.[25] and expressed on a fresh weight basis as μg/100 mg catechin equivalent.

### Determination of Total Anthocyanin Content

Anthocyanin in the dried powdered material was extracted with methanol containing 1% HCl for 24 h at 4°C and was estimated spectrophotometrically at 535 nm using the molar extinction coefficient 29,500 $M^{-1}$ $cm^{-1}$ for cyanidin-3-monoglucoside (molecular weight, 445.2 g $mole^{-1}$).[26]

### Statistical Analysis

Correlation coefficients to determine the relationship between two variables (concentrations and percent inhibition; different radical scavenging tests, content of total phenolic compounds) were calculated using MS Excel software (Hyderabad, India) (CORREL statistical function). In all of the tests, *r* varies from 0.78 to 1, which is a considerable value.[27] Unweighted-pair group average (UPGA) dendrogram was prepared using STATISTICA 6.0 (Statsoft, New Delhi, India).

### Determination of $IC_{50}$ Value

Taking 0% inhibition in the mixture without plant extract, regression equations were prepared from the concentrations of the extracts and percentage inhibition of free radical formation/prevention of lipid peroxidation in different systems of assay. $IC_{50}$ values (concentration of sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%) were calculated from these regression equations. A lower $IC_{50}$ value indicates stronger antioxidant activity.

## RESULTS AND DISCUSSION

A large number of flowers are consumed as food in West Bengal, India. Thirty-seven flowers were tested for DPPH radical scavenging activity and total antioxidant capacity. On the basis of the activity and availability, a few food flowers were further studied for hydroxyl radical scavenging activity, superoxide radical scavenging activity, and prevention of lipid peroxidation and DNA damage prevention properties. Table 2 shows the comparative data of DPPH radical scavenging activity and total antioxidant capacities (ascorbic acid equivalent) of the flowers. Superoxide and hydroxyl radical scavenging activities, lipid, and DNA damage preventing properties of selected flowers are shown in Table 3.

The aqueous extracts of the flowers scavenged DPPH radical in a dose dependent manner (correlation coefficient (*r*) values between 0.85–1). The highest activity was observed in *E. officinalis* flower extract. The total antioxidant capacities (equivalent to ascorbic acid) of the food flowers were measured. The strongest activity was observed in *E. officinalis.* The UPGA dendrogram showing interrelationships between the investigated species (Fig. 1) reveals that the varieties are grouped into two high-level clusters. The cluster I brought together *C. maxima* and *F. racemosus* receptacle with very low DPPH-RSA and very low to low total antioxidant capacity (TAC). Cluster II included two lower-level clusters (IIA and IIB). IIA included flowers/flower parts with low DPPH-RSA and low TAC. IIB is again segregated into two groups: IIB1 and IIB2. IIB1 includes the single flower *E. officinalis* with very high DPPH-RSA and TAC. Cluster IIB2 is again segregated into IIB2a and IIB2b. IIB2a includes the flowers with high DPPH-RSA and medium TAC (except *R. centifolia* with very high DPPH-RSA; *B. campestris* with medium DPPH-RSA and low TAC). IIB2b brings the flowers with medium DPPH-RSA and low to medium TAC into a group. This group again is divided into two lower groups. IIB2b/1 includes the flowers with DPPH-RSA in between 300–415 μg/ml together. Flowers with DPPH-RSA in between 416 to 600 μg/ml are grouped into IIB2b/2.

The super oxide radical ($O_2^{\bullet -}$) is a highly toxic species that is generated by numerous biological and photochemical reactions. Photochemical reduction of flavins generates $O_2^{\bullet -}$, which reduces NBT, resulting in the formation of blue formazan.[13] The formation

**Table 2** Comparative DPPH radical scavenging activity* and total antioxidant capacity* of the aqueous extracts of the food flowers ($n = 5$).

| Plant material | DPPH radical scavenging activity (DPPH-RSA) $IC_{50}$ value (μg/ml) | Total antioxidant capacity (TAC) (AAE) |
|---|---|---|
| *A. cepa* (peduncle) | 511.4 ± 20.5 | 73.8 ± 7 |
| *A. indica* | 365.08 ± 11.5 | 91.72 ± 8.5 |
| *B. rubra* | 582.03 ± 19.6 | 42.1 ± 7 |
| *B. acuminata* | 358.9 ± 18 | 122.71 ± 5 |
| *B. ceiba* calyx | 761.04 ± 13.6 | 22.6 ± 11 |
| *B. campestris* | 485.04 ± 18.4 | 68.73 ± 3.5 |
| *B. oleracea* var. broccoli | 590.05 ± 15 | 101.09 ± 8.5 |
| *C. tora* | 405.1 ± 22.5 | 101.7 ± 6 |
| *C. esculenta* | 1083.54 ± 2.4 | 46.31 ± 4.5 |
| *C. benghalensis* | 415.07 ± 8.6 | 64.4 ± 5 |
| *C. juncea* | 916.84 ± 23.5 | 33.1 ± 4.6 |
| *C. maxima* | 1820.22 ± 31 | 8.89 ± 3 |
| *C. longa* | 107.88 ± 6.5 | 146.57 ± 5.5 |
| *D. muricata* | 516.2 ± 18.4 | 67.98 ± 6 |
| *D. volubilis* | 694.6 ± 14.5 | 62 ± 3 |
| *D. indica* calyx | 460.8 ± 11.4 | 120.95 ± 8.6 |
| *E. officinalis* | 20.37 ± 3.5 | 415.5 ± 12.5 |
| *E. hirta* | 112.3 ± 5.5 | 131.17 ± 5.3 |
| *F. racemosus* flowers | 538.5 ± 21 | 132.89 ± 3.6 |
| *F. racemosus* receptacle | 1342.57 ± 12 | 54.6 ± 4.5 |
| *G. arborea* | 415 ± 12.5 | 125.01 ± 4 |
| *H. mutabilis* morning | 163 ± 9.5 | 108.68 ± 3.4 |
| *H. mutabilis* afternoon | 158.68 ± 3.3 | 107.80 ± 3.8 |
| *H. sabdariffa archer* | 174.22 ± 5.5 | 124.03 ± 6 |
| *H. sabdariffa victor* | 166.55 ± 8.5 | 138.9 ± 6.4 |
| *I. aquatica* | 164 ± 6.5 | 117.66 ± 4.5 |
| *M. longifolia* | 134.65 ± 11 | 117.33 ± 9.5 |
| *M. auriculata* | 335.2 ± 14.6 | 120.51 ± 6.5 |
| *M. oleifera* | 216.5 ± 11.5 | 147.93 ± 4.5 |
| *Musa x paradisiaca* cv. *kanchkela* | 393.86 ± 9.6 | 95.23 ± 6 |
| *M. x paradisiaca* v. *sapientum* cv. *sabri* | 375.96 ± 8 | 98.57 ± 4.8 |
| *N. nouchali* pedicel | 263.6 ± 11 | 118.8 ± 9 |
| *O. tenuiflorum* syn. *O. sanctum* | 213.94 ± 13.4 | 88.3 ± 7.5 |
| *O. tenuiflorum* v. *krishnae* | 192 ± 5 | 88.43 ± 6 |
| *R. centifolia* | 46.91 ± 5 | 151.8 ± 12.5 |
| *S. alata* | 452.76 ± 19.5 | 80.89 ± 6.5 |
| *S. grandiflora* | 288.11 ± 7.5 | 124.83 ± 6 |
| *S. grandiflora* purple *vexillum* | 311.8 ± 8.5 | 104.33 ± 4.8 |
| *T. indica* | 258.5 ± 10.4 | 125.72 ± 7 |
| *T. elephantina* | 948.07 ± 23.4 | 58.33 ± 2.5 |
| *Z. mays* (young green corn silk) | 765.04 ± 15 | 81.11 ± 3 |

| *Activity considered as | Very Low | Low | Medium | High | Very high |
|---|---|---|---|---|---|
| DPPH-RSA | >1100 | 600–1100 | 300–599 | 100–299 | 100 |
| TAC | 20 | 20–85 | 86–160 | 161–300 | >300 |

of blue formazan was inhibited by the flower extracts in a dose-dependent manner (correlation coefficient ($r$) values ranged from 0.9–1.0). Of the food flowers analyzed, the highest activity was found in *H. mutabilis* (Table 3).

Among the reactive oxygen species, the •OH radical is the most reactive and induces severe damage to the adjacent biomolecules.[28] •OH radicals are generated by direct

**Table 3** Comparative antioxidant activity of the aqueous extracts of selected food flowers ($n = 5$).

| Plant material | Superoxide radical scavenging activity $IC_{50}$ value (μg/ml) | Hydroxyl radical scavenging activity $IC_{50}$ value (μg/ml) | Prevention of lipid peroxidation $IC_{50}$ value (μg/ml) | Prevention of DNA damage $IC_{50}$ value (μg/ml) |
|---|---|---|---|---|
| *A. cepa* (peduncle) | 808.91 ± 31.4 | 1146.87 ± 23.33 | 478.15 ± 21.6 | — |
| *A. indica* | 452.39 ± 23.7 | 1015.08 ± 28.09 | 391.94 ± 19.3 | 453.6 ± 12.25 |
| *B. rubra* | 475.34 ± 14.4 | — | — | — |
| *B. acuminata* | 182.8 ± 10.4 | 303.84 ± 29.64 | 175.9 ± 15.5 | 305.84 ± 14.6 |
| *B. campestris* | 269.3 ± 10.24 | 814.33 ± 21.4 | 312.6 ± 23.5 | — |
| *C. tora* | 262 ± 10.67 | 471 ± 7 | 208.8 ± 14.8 | — |
| *C. juncea* | 352.35 ± 17.9 | 834.6 ± 17.4 | 524 ± 17.4 | 622.4 ± 31.7 |
| *D. volubilis* | 497.9 ± 18.6 | 844.7 ± 21.5 | 397.64 ± 15.4 | — |
| *D. indica* calyx | — | 385.5 ± 12 | 326.6 ± 22.8 | — |
| *E. hirta* | 123.67 ± 9 | 315.83 ± 5.83 | 222 ± 18 | — |
| *G. arborea* | 320.84 ± 8.2 | 558.6 ± 35.8 | 472 ± 6.7 | — |
| *H. mutabilis* | 87.8 ± 6.3 | 291.1 ± 18.4 | 158.42 ± 12.8 | 296.79 ± 12.9 |
| *H. sabdariffa archer* | 160.5 ± 17.4 | 509.38 ± 10.4 | 272.89 ± 15.9 | 365.7 ± 15.6 |
| *H. sabdariffa victor* | 147.6 ± 9.8 | 459.64 ± 25.66 | 239.2 ± 18.4 | 350.9 ± 13.08 |
| *M. longifolia* | 206.8 ± 12.95 | 459.3 ± 20.9 | 192 ± 15.8 | 280.0 ± 20.4 |
| *M. auriculata* | 281.5 ± 10.7 | 695.7 ± 27.6 | 288.15 ± 19.35 | — |
| *M. oleifera* | 141.1 ± 11.34 | 585.88 ± 29.57 | 234.35 ± 19.6 | 386.69 ± 21.6 |
| *Musa x paradisiaca* cv. *kanchkela* | 312.25 ± 14.3 | 885.11 ± 21.1 | 351.53 ± 12.6 | — |
| *M. x paradisiaca* v. *sapientum* cv. *sabri* | 325.18 ± 22.4 | 723.95 ± 28.35 | 322.2 ± 15.4 | 430.0 ± 19.8 |
| *N. nouchali* pedicel | 170.11 ± 4 | 572.35 ± 12.6 | 266.44 ± 7.08 | — |
| *O. tenuiflorum* | 317.04 ± 11.7 | 538.13 ± 28.8 | 214.3 ± 5.8 | 367.4 ± 17.5 |
| *R.. centifolia* | — | 235.6 ± 8.5 | — | — |
| *S. alata* | 326.5 ± 11.22 | 766.87 ± 22.5 | 415.24 ± 23.56 | 382.6 ± 17.3 |
| *S. grandiflora* | 198.42 ± 8.31 | 447.37 ± 24.66 | 171.2 ± 11.9 | 283.75 ± 14.8 |

—: Not done.

addition of iron (II) salts to a reaction mixture containing phosphate buffer.[28] All the flowers showed good correlation between activity and concentration (correlation coefficient [$r$] value ranged between 0.85–1). The highest activity was recorded in *R. centifolia* (Table 3). The lowest activity was recorded in *A. cepa* peduncle.

Egg yolk lipid undergoes rapid non-enzymatic peroxidation when incubated in the presence of ferrous sulphate. All the flowers analyzed inhibited lipid peroxidation in a dose dependent manner (correlation coefficient [$r$] ranged between 0.94–0.99). Of the food flowers analyzed, the highest activity was observed in *H. mutabilis* (Table 3).

The Fenton reaction system caused damage to DNA as measured by production of TBARS (possibly from degradation of deoxyribose). The ROS involved in the damage to DNA by Fenton reagents were mainly •OH.[21] The DNA damage protective property of plant extracts is probably due to their •OH scavenging property. The *in vitro* assay using Herring sperm DNA shows significant protection of DNA by aqueous extracts of the flowers (Table 3). In all of the methods, percent inhibition of free radicals by the aqueous extracts were dose dependent with linear correlation (correlation coefficient [$r$] ranged between 0.83–1). Among food flowers studied, *M. longifolia* exhibited the highest protection followed by *S. grandiflora.* The activities in these two flowers were not significantly different.

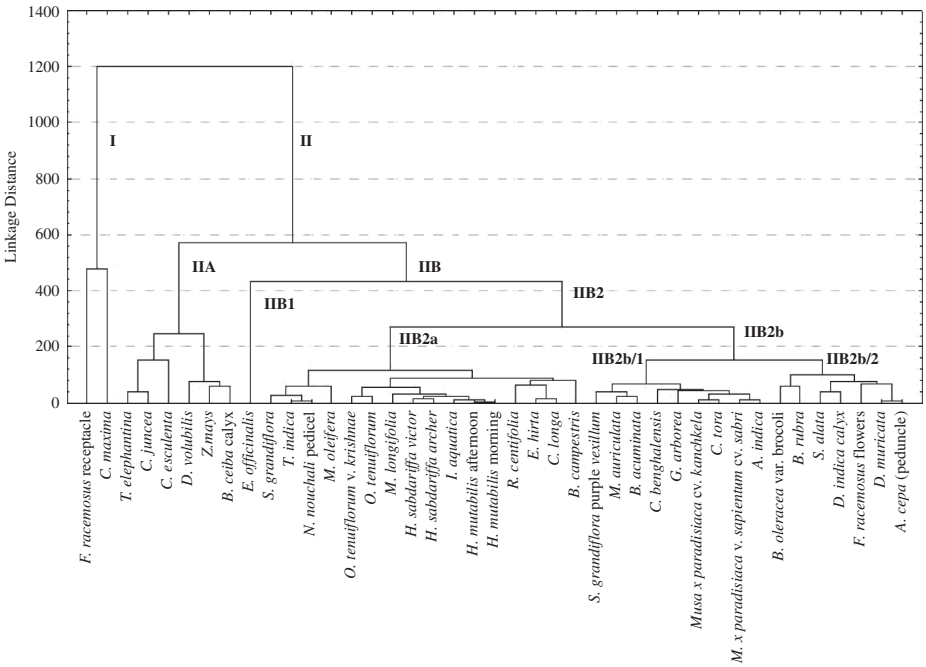


**Figure 1** Tree diagram for variables unweighted pair-group average Euclidean distances.

The total anthocyanin content, phenol content, and flavonoid content in different food flowers are presented in Table 4. Flowers and ripe calyces of *H. sabdariffa* are reported to contain flavonoids, anthocyanin, and organic acids.[29] *H. sabdariffa archer* where anthocyanin is absent also showed good activity. The result can be rationalized by taking into account the presence of organic acids and other phenols. In *H. mutabilis*, flavonoids change from morning to evening flowers. Cream-white morning flowers of *H. mutabilis* become red due to production of anthocyanin in the evening. However, the differences in the antioxidant activities, as measured by DPPH radical scavenging activity and total antioxidant capacity, of the morning and evening flowers of *H. mutabilis* (Table 2) are not statistically significant. The total phenol contents were similar in the morning and evening flowers (Table 4). *O. tenuiflorum*, which is reported to contain eugenol, anthocyanin, and $\beta$-caryophyllene[30] and *O. sanctum* containing phenols and essential oils,[31] were shown to have similar antioxidant activity (Table 2). Correlations of antioxidant activities (as determined by $IC_{50}$ values) and phenol content (Table 5) were compared. The correlations suggest that the total phenol contents of the flowers were responsible for the antioxidant properties of the aqueous extracts.

## CONCLUSION

From the present study, it can be concluded that the flowers consumed as food in West Bengal, India, have antioxidant properties. Because of such properties it is concluded that different edible flowers could be attractive as a novel food to the world population.

**Table 4** Total phenolic compounds in some food flowers.

| Flower | Total phenol content (GAE) | Total flavonoid content (CE) | Total anthocyanin content | |
|---|---|---|---|---|
| | | | % Dry wt. | % Fresh wt. |
| *A. indica* | 53.0 ± 3.5 | 88.0 ± 8.0 | — | — |
| *B. acuminata* | 96.9 ± 4.5 | — | — | — |
| *B. campestris* | 69.7 ± 4.4 | — | — | — |
| *C. bengalensis* | — | — | 0.09 ± 0.02 | 0.08 ± 0.02 |
| *D. volubilis* | 51.0 ± 3.0 | 75.0 ± 6.0 | — | — |
| *H. mutabilis* Pink (at 12 PM) | 122.3 ± 3.4 | 101.7 ± 11.0 | 0.25 ± 0.02 | 0.08 ± 0.03 |
| Red (at 5 PM) | 120.6 ± 2.4 | — | 0.35 ± 0.02 | 0.20 ± 0.03 |
| *H. sabdariffa* var. *victor* | 98.3 ± 5.0 | 83.6 ± 8.0 | 0.08 ± 0.06 | 0.16 ± 0.04 |
| *H. schulli* (mauve) | — | — | 0.04 ± 0.02 | 0.05 ± 0.02 |
| *I. aquatica* (mauve) | — | — | 0.12 ± 0.01 | 0.05 ± 0.005 |
| *M. longifolia* | 120.8 ± 8.0 | — | — | — |
| *M. auriculata* mauve | 83.3 ± 5.0 | 88.0 ± 7.5 | 0.08 ± 0.02 | 0.09 ± 0.02 |
| *M. oleifera* | 106.5 ± 9.5 | 131.5 ± 8.0 | — | — |
| *Musa x paradisiaca* v. *sapientum* cv. *sabri* | 76.5 ± 5.0 | — | 0.10 ± 0.02 | 0.05 ± 0.04 |
| *N. nouchali* pedicel | 89.3 ± 11.0 | 84.1 ± 5.3 | — | — |
| *O. tenuiflorum* (purple) | 72.0 ± 5.0 | 90.5 ± 6.6 | 0.20 ± 0.04 | 0.12 ± 0.02 |
| *R. centifolia* (red) | 133.9 ± 14.0 | — | 0.15 ± 0.03 | 0.20 ± 0.05 |
| *S. alata* | 81.8 ± 7.0 | 89.1 ± 12.0 | — | — |
| *S. grandiflora* | 99.8 ± 8.0 | 121.8 ± 5.0 | 0.08 ± 0.02 | 0.09 ± 0.02 |

—: Not done.

**Table 5** Correlation coefficient ($r$) between total phenol and antioxidant activity.

| | Total phenol content | Total flavonoid content |
|---|---|---|
| DPPH scavenging | 0.7868 | 0.5927 |
| •OH scavenging | 0.8905 | 0.5952 |
| $O_2^{\bullet -}$ scavenging | 0.9194 | 0.6641 |
| Lipid peroxidation inhibition | 0.8293 | 0.6722 |
| DNA damage prevention | 0.8407 | 0.6774 |
| Total antioxidant capacity | 0.9348 | 0.8712 |

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