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Research Article

Antioxidant activity and phytochemistry of various flowers from Tamil Nadu, India

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ABSTRACT

It is a known fact that plants are widely used in pharmaceutical and food industries due to their biological importance. Among the plant parts, leaves, stems, roots and bark are being widely studied for their biological properties. However, flowers are almost neglected and are not much probed for their importance. In this study, the methanolic and aqueous extracts of 25 commonly available flowers belonging to 16 different families from the state of Tamil Nadu are probed for their antioxidant property and their phytochemical constituents respectively. Methanolic crude extracts of the flowers at the concentrations of 200, 400, 600, 800 and 1000µg/ml were studied against DPPH to know their antioxidant potential. The presence or absence of phytochemicals like cardiac glycosides, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids was detected. The flowers of *Couroupita guianensis* and *Gerbera sp.* recorded EC_{50} values of <200 µg/ml. However, the flower of *Gomphrena globosa* has not recorded any value even at the concentration of 1000 µg/ml. Among the phytochemicals, flavonoid was recorded from almost all the floral extracts except that of *Gomphrena globosa.* Phlobatannins and Steroids were not recorded in any of the floral extracts studied. As few flowers showed the presence of either flavonoid and tannin or flavonoid and saponin, they may better exploited for pharmaceutical and nutraceutical applications.

Keywords: Antioxidant; DPPH; Phytochemistry; Flavonoids; Flowers; Tamil Nadu; Pharmacy; Nutraceuticals

INTRODUCTION

Many of the major human diseases like Alzheimer's, Cancer, Diabetes, Sclerosis, Parkinson's, etc. are due to the presence or generation of free radicals resulting in oxidative stress (Ozgen et al., 2006). Thus, the maintenance of oxidative stress in balance is necessary and intake of anti-oxidant rich nutrition is important (Tiwari et al., 2001). It is also noticed that interest on finding natural source for antioxidants to expand the storage value of food as an alternate to BHA and BHT is on the rise (Kumar et al., 2011). Thus, the interest on natural medicinal sources in the form of nutraceuticals is attracting many researchers (Firdaus et al., 2013) and plants are considered as a major source for this.

Plants are described as organic chemical producing factory (Egwaikhide and Gimba, 2007) and as pharmaceutical sleeping giants (Hamburger and Hostettman, 1991). Plants are widely used in Naturopathy, Homeopathy and serve as an origin for present day Allopathic field of medicine. Ancient medicinal system in several countries including India use plants. Siddha, Ayurvedha and Unani are famous systems of medicine which use plants or active ingredients of plants as a major therapeutic agent. Above all, the usage of plants in the system of medicine is widely accepted by nearly 80% of the people throughout the world (Rajasekharan, 2002). Hence, research on identifying specific plants for their bioactivity and bioefficacy is being conducted widely. Among the plant parts, leaves, stems, roots and bark are widely studied for their biological properties than flowers which are nearly neglected or rarely studied.

In countries like India, flowers are always used in all its cultural activities (Jeeva et al 2011). Flowers are widely used for their beauty and the color they radiate (Joselin et al., 2013). Flowers which serve their purpose usually wilt and are thrown as trash. However, due to the possession of phytoconstituents, they can be potentially considered as a major source of phytocompounds in pharma and nutraceutical industries. Antioxidant potency and bioactivities of metabolites from flowers have been reported already (Ebrahimzadeh et al., 2009). In this study, a total of 25 species of flowers belonging to 16 different families collected from Koyambedu flower market in Chennai, Tamil Nadu and flowers of local flora were evaluated for their antioxidant potency and their phytoconstituents.

MATERIALS AND METHODS

Plant Source

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The flowers of 25 plant species belonging to sixteen different families were collected from Koyambedu Flower Market, Chennai. Few of the fresh flowers were collected from common plants present in and around Chennai, the state capital of Tamil Nadu in India. The flowers were collected during May to September 2013. The species were identified using Gamble (1915) and Mathew (1983).

Preparation of Extract for Phytochemical evaluation

Aqueous extracts were obtained by mixing 5g of the dried flowers with 100 ml of distilled water at the ratio of 1:20 and allowed to boil for 2 minutes in microwave oven. This was filtered using Whatman No.1 filter paper and the analysis was carried out immediately without storage.

Phytochemical Analysis

The aqueous extracts of the flowers were evaluated for the presence of phytochemicals like cardiac glycosides, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids as described by Evans (1996) and Udayaprakash et al., (2011, 2012a, 2013a, b and c).

Cardiac Glycosides

Two ml of glacial acetic acid containing a drop of ferric chloride was added to 5 ml of the aqueous extract of the flowers. To this, 1ml of concentrated sulphuric acid was added. The presence of brown ring indicates positive result.

Flavonoids

Formation of yellow colour when aqueous extract of flowers were added to 1% liquor ammonia taken in test tubes confirms the presence of flavonoids.

Phlobatannins

Deposition of red precipitate when 10 ml of floral extract was boiled with 1% HCl in a test tube gives positive reaction for the presence of phlobatannins.

Saponins

Three ml of distilled water was added to 10 ml of the plant extract and shaken well. Formation of froth resulting in emulsion when added with a few drops of Olive oil confirms the presence of saponins.

Steroids

To two grams of dry flower powder, 10 ml of chloroform was added, boiled and filtered. To 2ml of the filtrate obtained, 2 ml acetic anhydride and a few drops of concentrated sulphuric acid were added. Presence of stable blue-green ring confirms the presence of steroids.

Test for Tannins

Ferric chloride at the concentration of 0.1% was added to 5ml of the floral extract. The presence of brownish green or blue black color indicates positive reaction for Tannin.

Terpenoids

Consecutively add 2 ml of chloroform and 3 ml of concentrated sulphuric acid in 5ml of aqueous solution of floral extract. Formation of reddish brown interface in the solution denotes the presence of terpenoids.

Antioxidant assay

Preparation of plant extracts

Undamaged or uninfected flowers were chosen. The fresh flowers collected were cleaned using running tap water and dried in shade for 4-6 days. The dried material was pulverized and stored in an airtight container. Methanol was added to the plant material at the ratio of 1:10 and stirred in temperature-controlled shaker at room temperature of 28 ± 2^{o}C. The extracts of plant materials were filtered and concentrated using rotary evaporator. These extracts were reconstituted for evaluating free radical scavenging activity.

Free radical scavenging assay

Free radical scavenging assay was performed as stated by Udayaprakash (2013d and e). The methanolic extracts obtained from the flowers of 25 species belonging to 16 different families were studied against DPPH (2, 2 diphenyl-1-picryl hydrazyl) to evaluate their free radical scavenging ability. Extracts of plant crude prepared at various concentrations (200, 400, 600, 800 and 1000µg/ml) in methanol were analysed against 1 ml of 0.01 mM DPPH. The same solution of DPPH was used as control and Butylated hydroxyanisole (BHA) as reference. After half an hour of incubation time in dark at room temperature, the absorbance was read at 517 nm using UV-Visible Spectrophotometer (Cyberlab, USA). The percent inhibition was calculated as:

$$Effective\ Concentration\ \% = \frac{Control\ Absorbance - Test\ Absorbance}{Control\ Absorbance} \times 100$$

The concentration which inhibits the formation of DPPH radicals by 50 % is termed as EC_{50} value (Seal, 2011).

RESULTS AND DISCUSSION

The current study provides the detail on the phytochemical compounds present and the antioxidant potency of flowers of individual species. A total of 25 flowers belonging to 16 different families of Angiosperms were evaluated for their antioxidant property and phytochemical constituents. Among the families, Apocynaceae are represented by maximum number of species (5) followed by Fabaceae (3 species). The following families, i.e. Amaranthaceae, Asteraceae and Bignoniaceae are represented by 2 species. The families Acanthaceae, Agavaceae, Cannaceae, Lecythidaceae, Malvaceae, Moringaceae, Nymphiaceae, Oleaceae, Rosaceae, Rubiaceae and Verbenaceae are represented by single species each.

Table 1: List of floral species and their presence of Phyto constituents

No.	Species	Tannins	Flavo-noids	Sapo-nins	Terpe-noids	Cardiac Glycosides	Ster-oids	Phloba Tannins
1.	*Bauhinia purpurea*	+	+	-	+	-	-	-
2.	*Bougainvilla spectabilis*	-	+	-	-	-	-	-
3.	*Calendula officinalis*	+	+	-	-	-	-	-
4.	*Calotropis gigantea*	+	+	+	-	+	-	-
5.	*Canna indica*	+	+	-	+	-	-	-
6.	*Cassia alata*	-	+	-	-	-	-	-
7.	*Celosia argentea*	+	+	+	+	-	-	-
8.	*Couroupita guianensis*	+	+	+	+	-	-	-
9.	*Crossandra infundibuli-formis*	-	+	+	-	+	-	-
10.	*Delonix regia*	+	+	+	-	+	-	-
11.	*Ervatamia coronaria*	+	+	-	-	+	-	-
12.	*Gerbera* sp.	+	+	+	+	-	-	-
13.	*Gomphrena globosa*	-	-	+	-	-	-	-
14.	*Hibiscus rosa-sinensis*	+	+	+	-	-	-	-
15.	*Ixora coccinea*	+	+	+	-	-	-	-
16.	*Jasminum officinale*	-	+	+	-	-	-	-
17.	*Lantana camara*	-	+	+	-	-	-	-
18.	*Moringa oleifera*	+	+	+	-	-	-	-
19.	*Nerium oleander*	+	+	-	-	+	-	-
20.	*Plumeria obtusa*	+	+	+	-	-	-	-
21.	*Plumeria rubra*	+	+	+	-	-	-	-
22.	*Polianthes tuberosa*	-	+	+	-	-	-	-
23.	*Rosa sp.*	+	+	+	-	-	-	-
24.	*Stenolobium stans*	-	+	+	-	-	-	-
25.	*Tabebuia rosea*	+	+	-	-	-	-	-

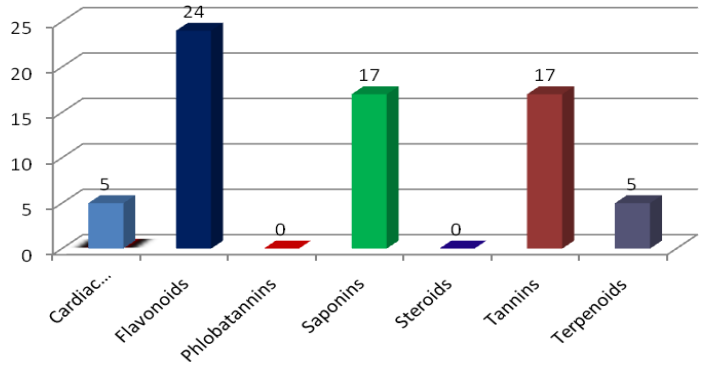

Figure 1: Number of floral species showing their presence of Phyto constituents

The evaluation on the presence or absence of phyto-chemicals like cardiac glycosides, flavonoids, phloba-tannins, saponins, steroids, tannins and terpenoids showed that flavonoids was almost recorded in all flor-al species other than *Gomphrena globosa* which proves that flowers are potential source of antioxidants. Simi-lar result of recording the presence of flavonoid in 14 out of 15 flowers studied has been already reported (Britto and Gracelin, 2011).

Similarly, large numbers of flowers have recorded the presence of both tannins and saponins. The biological importance of tannins and saponins are already dis-cussed (Udayaprakash, 2013a). However, none of the flower has shown the presence of steroids and phloba-tannins. Further, the absence of steroids in all the flowers studied reduces the risk on secondary effects or side effects compared with other plant parts when consumed.

The presence of flavonoid and tannin alone in the flowers of *Calendula officinalis, Ervatamia coronaria* and *Tabebuia rosea* and the presence of flavonoid and saponin in the flowers of *Crossandra infundibuliformis, Jasminum officinale, Lantana camara, Polianthes* tube-rose and *Stenolobium stans* make the species as the most preferable species for the isolation of flavonoids. Isolation of flavonoids from these species is easy as they possess only few other secondary metabolites other than complex compounds usually present in oth-

Table 2: List of plant species, their family and Percent inhibition of free radical recorded for each flower against DPPH

No.	Species	Family	% Inhibition					EC_{50}
			200	400	600	800	1000	
1.	*Bauhinia purpurea*	Fabaceae	0	17.7	36.6	73.3	82.2	672.8
2.	*Bougainvilla spectabilis*	Nyctaginaceae	46.6	47.7	47.7	48.8	63.3	815.3
3.	*Calendula officinalis*	Asteraceae	46.6	58.8	60	63.3	74.4	254.5
4.	*Calotropis gigantea*	Apocynaceae	28.8	54.4	60	62.2	87.7	365.2
5.	*Canna indica*	Cannaceae	22.2	37.7	81.1	84.4	86.6	456.4
6.	*Cassia alata*	Fabaceae	0	13.3	13.3	45.5	83.3	823.5
7.	*Celosia argentea*	Amaranthaceae	15.5	36.6	45.5	48.8	60	820
8.	*Couroupita guianensis*	Lecythidaceae	54.4	63.3	74.4	84.4	87.7	<200
9.	*Crossandra infundibuliformis*	Acanthaceae	0	18.8	44.4	82.2	86.6	629.4
10.	*Delonix regia*	Fabaceae	0	37.7	81.1	82.2	86.6	456.4
11.	*Ervatamia coronaria*	Apocynaceae	44.4	55.5	66.6	71.1	72.2	300
12.	*Gerbera sp.*	Asteraceae	58.8	60	63.3	74.4	87.7	<200
13.	*Gomphrena globosa*	Amaranthaceae	0	0	11.1	27.7	38.8	ND
14.	*Hibiscus rosa-sinensis*	Malvaceae	15.5	21.1	24.4	26.6	51.1	990.9
15.	*Ixora coccinea*	Rubiaceae	22.2	46.6	47.7	78.8	84.4	614.2
16.	*Jasminum officinale*	Oleaceae	17.7	21.1	37.7	47.7	53.3	880
17.	*Lantana camara*	Verbenaceae	21.1	44.4	47.7	78.8	83.3	614.2
18.	*Moringa oleifera*	Moringaceae	36.6	48.8	65.5	74.4	88.8	421.4
19.	*Nerium oleander*	Apocynaceae	33.3	48.8	51.1	60	66.6	500
20.	*Plumeria obtusa*	Apocynaceae	20	22.2	51.1	54.4	64.4	592.3
21.	*Plumeria rubra*	Apocynaceae	15.5	21.1	52.2	55.5	66.6	585.7
22.	*Polianthes tuberosa*	Agavaceae	36.6	38.8	42.2	47.7	53.3	880
23.	*Rosa* sp.	Rosaceae	37.7	37.7	45.5	60	62.2	661.5
24.	*Stenolobium stans*	Bignoniaceae	17.7	33.3	65.5	73.3	87.7	503.4
25.	*Tabebuia rosea*	Bignoniaceae	0	11.1	27.7	38.8	50	1000

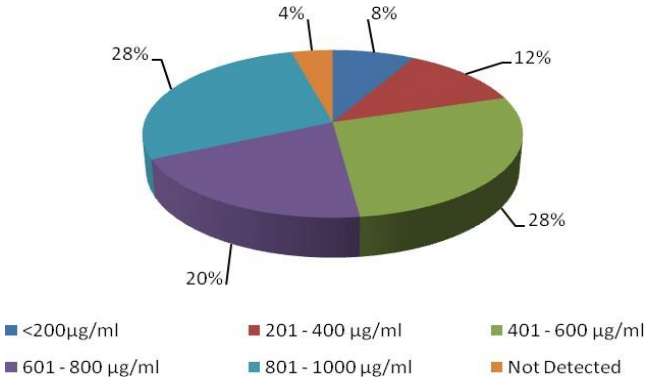

Figure 2: Antioxidant activity recorded for different concentrations of floral extracts (In %)

er plant parts. These specific characters of the flowers can be exploited by pharmaceutical and nutraceutical industries, whenever they are looking for the isolation of flavonoids.

The detail on the presence and absence of different phyto constituents is presented in Table 1. and the number of floral species showing the presence of different phytoconstituents is presented in Figure 1.

Methanolic extracts of the flowers at the concentration of 200, 400, 600, 800 and 1000µg/ml were studied against DPPH to determine their antioxidant potential. The details of the effective concentration recorded for each concentration of individual floral species is provided in Table 2. The flowers of *Couroupita guianensis* and *Gerbera sp.* recorded the EC_{50} value of <200 µg/ml. However, the flower of *Gomphrena globosa* has not recorded any value even at the concentration of 1000 µg/ml. Nearly 28% of flowers studied recorded the EC_{50} value at the range of 401-600 µg/ml and at the value of 801 – 1000 µg/ml. Twenty percent of flowers studied recorded their EC_{50} value at the range of 201 – 400 µg/ml. The percentage of flowers recording the concentration range of their EC_{50} value is represented in Figure 2.

Antioxidants play a major role in preventing many degenerative diseases and the intake of antioxidant rich

plant parts will reduce stress induced pathogenesis. Based on the results obtained, the study proves that flowers, possessing significant antioxidant compounds can be favoured as one among the consumables in human diet. The carotenoid, anthocyanin, flavonoid and vitamin rich flowers can be potentially exploited by nutraceutical industries for the production of value added foods. Further, it is recommended that the flowers rich in antioxidants can be used as an alternate for Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) used in food industry for food storage. BHA and BHT are already reported as carcinogenic compounds (Madavi, 1995).. The studies similar to this line was conducted on different floral types, i.e. flowers of medicinal plants (Jeeva et al., 2011), flowers of aromtic plants (Nisar et al., 2011), ornamental flowers (Bungihan et al., 2011), edible flowers (Kaisoon et al., 2011), flowers of family Apocynaceae (Joselin et al., 2012) and Bignoniaceae (Joselin et al., 2013).

CONCLUSION

The current study on the antioxidant ability and phytoconstituents of flowers from Tamil Nadu provides the result supporting usage of few flowers in isolation of flavonoids and antioxidants. The results revealed the presence of flavonoids in most of the flowers confirming that flowers are the potential source of flavonoids when compared with other plant parts. Flowers, which possess high antioxidant activities are recommended for usage in pharmaceutical and nutraceutical industries.

CONFLICT OF INTEREST

No Conflict of Interest lies between Authors

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