Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 1261-1263
Received: 13-01-2018
Accepted: 14-02-2018

Jiji Allen J
Department of floriculture and landscaping, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Kannan
Department of floriculture and landscaping, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

SP Thamaraiselvi
Department of floriculture and landscaping, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

D Uma
Department of Biochemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Jiji Allen J
Department of floriculture and landscaping, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Extraction of Phenolic compounds and assessing antioxidant activity of *Malvaviscus arboreus Cav* flowers

Jiji Allen J, M Kannan, SP Thamaraiselvi and D Uma

Abstract
Malvaviscus arboreus Cav is an ornamental flowering shrub, which belongs to the family Malvaceae. The flowers of *Malvaviscus arboreus Cav* are a rich source of phytochemicals like phenols, flavonoids, tannins etc. These phytochemicals are responsible for the medicinal properties of these flowers. It has two different flowers colours like red and pink. Both the varieties were collected and they were screened for antioxidant potentials like ABTS, DPPH, Chelating potential, FRAP and CUPRAC. Phytochemicals like total phenol and total flavonoids was quantified. Based on the study it was observed that the red and pink coloured flowers possess antioxidant potential but the highest was found in the red coloured flower.

Keywords: *Malvaviscus arboreus Cav*, Antioxidants, Flowers, Phytochemicals

1. Introduction
Malvaviscus arboreus Cav *i*s an ornamental flowering shrub, which belongs to the family Malvaceae. Malvaceae is a family of flowering plants which consists of 244 genera and 4,225 species. Major economically important plants of this family are Okra, Hibiscus, Cotton and Durian. M. *arboreus*can produce flowers throughout the year in partial shaded and also in sunny conditions. It produces flowers in two different colours like red and pink. In landscaping these plants are used as hedge plants and also as potted plant.
M. arboreus contain Phytochemical compounds like gallic acid, protocatechuic acid, p-hydroxy benzoic acid, chlorogenic acid, p-coumaric acid and ferulic acid [9]. The flowers containtotal soluble flavonoids like rutin, myricetin, quercetin and kaempferol. Flavonoid aglycones found in the flowers of *M. Arboreus variety* drummondii were, total pigments 53 (mg per g fresh tissues), which comprises of quercetin 41 mg, cyanidin2 mg, and pelargonidin 10 mg, respectively [12].
Malvaviscus has been used in traditional medicine in Central America and Haiti. Costa Ricans use the leaf decoction for cystitis, diarrhoea and gastritis. Cubans use the flower decoction as gargle for sore throat. Dominicans apply the leaf juice to lice, seborrhoea and wounds, and flower decoction is given to nursing infants with cold. Haitians and Mexicans drink the flower decoction for bronchitis, diarrhoea, thrush and tonsillitis. Hondurans drink the leaf decoction for fever [8]. The bark infusion is used to treat Stomach trouble [4]. Hence in the present study two different coloured *M. arboreus* were selected to assess and compare their antioxidant potential and total phenol content.

2. Materials and methods
2.1 Preparation of extract
The flower materials of *M. arboreus* (red and pink flowers) were collected from The Botanical Garden, Tamil Nadu Agricultural University Coimbatore. The collected flowers were shade dried and powdered using a blender. 100 mg of the powdered petals were macerated with methanol and kept for shaking overnight. The solution was then filtered using whattman filter paper and concentrated using rotary evaporator. The final volume was made up to 10 ml. The concentrated extract was stored in deep freezer (-30 ºC) for further analysis.

Fig 1: Ac.1. *M arboreus* (Red flower)

Fig 2: Ac.2. M arboreus (Pink flower)

2.2 Estimation of total phenol content

The total phenol content was estimated based on Folin–Ciocalteu (FC) method [15] with some slight modification. 0.2 ml of the extract was diluted with 8.5 ml of water to which 0.5 ml of Folin–Ciocalteu (FC) was added and incubated for 3 minutes. After incubation, 1 ml of sodium carbonate (20%, w/v) was added. The solution was mixed well and incubated at room temperature for 60 minutes. After incubation, the absorbance was measured at 760nm. A suitable calibration curve was prepared using Gallic acid and the results are expressed in milligram per gram (mg/g) Gallic acid equivalent.

2.3 Estimation of total flavonoids

Total flavonoids in the sample were determined using the Aluminium chloride method [10]. 0.5 ml of the extract was added to 3 ml of Sodium nitrate (5%, w/v) and 2.5 ml of distilled water. It was incubated at room temperature for 3 minutes and then 0.3 ml of Aluminium chloride (10%, w/v) was added. The reaction mixture was allowed to stand for six minutes and then 2 ml of Sodium hydroxide (1 M) was added. The final volume was made up to 10 ml after 60 minutes and the absorbance was measured at 415nm.A suitable calibration curve was prepared using Quercetin and the results are expressed in milligram per gram (mg/g) Quercetin equivalent.

2.4 DPPH radicle scavenging activity assay

DPPH assay was carried out using the method of Brand-Williams *et al.* (1995) [5] with some modifications. A stock solution of 0.1mM DPPH in methanol was prepared and then it was diluted to get an absorbance of about 0.980 (±0.02) at 517 nmusing the spectrophotometer.3ml aliquot of this solution was taken and 100µl of the extract at different concentration (100 – 500 µg/ml) was added. The reaction mixture was mixed well and then incubated in dark for 15 min. Their absorbance at 517nm was measured after incubation. Ascorbic acid was used a standard. The percent inhibition was calculated using the following formula.

$$\%\ inhibition = \left(\frac{\text{initial absorbance} - \text{final absorbance}}{\text{initial absorbance}}\right) \text{x } 100$$

The antioxidant capacity of test samples was expressed as EC_{50}, the concentration necessary for a 50% reduction of DPPH.

2.5 ABTS radicle scavenging activity assay

ABTS radicle cation mixture was prepared by mixing ABTS (7mM) with potassium persulphate (2.45mM) and kept in dark overnight. The working solution is then diluted with methanol to get an absorbance of 0.700 (±0.02). 3ml aliquot of this solution was taken and 100µl of the extract at different concentration (100 – 500 µg/ml) was added. Their absorbance at 734nm was measured after 6 minutes of incubation [14]. Ascorbic acid was used a standard and the antioxidant capacity of the extract was expressed as EC_{50} i.e. the concentration required to inhibit 50% of the ABTS free radicle. The percent inhibition was measured using the following formula.

$$\%\ inhibition = \left(\frac{\text{initial absorbance} - \text{final absorbance}}{\text{initial absorbance}}\right) \text{x } 100$$

2.6 Ferric reducing antioxidant potential assay

100µL of the extract was added to 1.9ml of FRAP reagent (25 ml of 300 mM Acetate buffer pH3.6, 2.5 ml of 10mM TPTZ in 40 mM HCL and 2.5 ml of 2.mM $Fecl_3$). The reaction mixture was incubated in water bath at 36ºC. The increase in absorbance at 593nm after 30 minutes of incubation[3]. The Ferric reducing antioxidant potential of the Malvaviscus extract was expressed in µM Ascorbic acid equivalent.

2.7 CUPRAC

100 µl of the extract was added to 1ml of CUPRAC reagent. (1mL of 1.0×10^{-2}M copper (II) chloride, 1mL of 1M ammonium acetate buffer at pH 7.0, and 1mL of 7.5×10^{-3}M neocuproine solution). The reaction mixture was then incubated at room temperature for 30 minutes and their absorbance was recorded at 450nm [2]. A standard calibration curve was developed using ascorbic acid and CUPRAC reagent. The cupric ion reducing antioxidant capacity of the extract was expressed in µM Ascorbic acid equivalent

2.8 Chelating power

An aliquot of each sample (200 µl) was mixed with 100 µl of $FeCl_2.2H2O$ (2.0 mM) and900 µl of MeOH. After 5 min incubation, the reaction was initiatedby the addition of 400 µl of ferrozine (5.0 mM). After 10 min incubation,the absorbance at 562 nm was recorded[7]. The chelatingactivity (%) was calculated using the following equation.

$$\%\ inhibition = \left(\frac{\text{initial absorbance} - \text{final absorbance}}{\text{initial absorbance}}\right) \text{x } 100$$

Ascorbic acid was used as standard. The chelating power of the extract was expressed as EC_{50} i.e. effective concentration that chelates 50% of iron (II).

2.9 Statistical analysis

All statistical analysis were carried out in triplicates and the values were expressed in Mean ± SD. EC_{50} value was calculated using the statistical software, Graph pad prism5.0.

3. Results and discussion

3.1 Total Phenol and Flavonoid content

The phenolic compounds are the secondary produced by plants through pentose phosphate, shikimate, and phenylpropanoid pathway [13]. The Phenolic compounds occur widely in plants and have their role in growth and reproduction, providing protection against pathogens and predators [6], besides contributing towards the colour and sensory characteristics of fruits and vegetables [1].

In the present study the total phenol content found higher in the Accession number 1. Which is the red coloured flower. The total flavonoid content was expressed in mg/g Quercetin equivalent and it was found high in the Accession number 1. The total phenol and flavonoid content of M. *arboreus Cav.* Flowers were expressed in Table 1.

Table 1: Total Phenol and flavonoid content of the methanolic extract of M. *arboreus Cav.* Flowers

Accession number	Flower colour	Total phenol (mg/g Gallic acid equivalent)	Total flavonoid (mg/g Quercetin equivalent)
1.	Red	42.57 ± 0.68	13.38± 0.46
2.	Pink	37.53± 1.52	11.86± 0.26

Values were expressed as Mean ± SD (n=3)

3.2 DPPH radicle scavenging activity

DPPH (2, 2 – diphenyl- 1 –picryl hydroxyl) is a stable free radicle and has been widely used to determine the primary antioxidant potential of plants, flowers, fruits and food products. The reduction in absorbance at 517 nm indicates the presence of antioxidant activity in the flower extract. The EC_{50} value for the DPPH antioxidant potential was presented in Table.2. The study revealed that the flowers of *M. arboreus* possess free radicle scavenging or inhibiting activity but the highest was found in the red coloured flower.

3.3 ABTS radicle scavenging activity

ABTS (2, 2' –azino-bis (3-ethylbenzthiazoline -6- sulphonic acid)) can be oxidised by potassium persulphate or manganese dioxide and will give rise to ABTS cation radicle. Their absorbance at 743 nm decreases in the presence of antioxidants due to the loss of electron from the nitrogen atom of ABTS. The result obtained clearly implied that the samples inhibit or scavenge the radical in a concentration dependen tmanner. The red coloured flower of *M.arboreus* has the highest ABTS radicle scavenging activity than the pink flowered type. The antioxidant effect of ascorbic acid was followed by the red flower and then the pink flowered cultivar.

3.4 Chelating potential

An important mechanism of antioxidant activity is the ability to chelate or deactivate transition metals, which possess the ability to catalyse hydrogen peroxide decomposition. Hence it is considered important to screen the iron (II) chelating ability of extract [11]. The chelating potential of the flower extracts were expressed in table.2. Accession number.1 which is a red flowered one showed the highest chelating potential (27.30 ± 0.11 µg/ml) when compared to the pink coloured one. Standard, Ascorbic acid showed the highest chelating potential (7.17± 0.08µg/ml) than the flower samples.

Table 2: Antioxidant effect (EC_{50}) on DPPH radicals, ABTS radicals and chelating power

Accession number	Scavenging ability on DPPH radicals (EC_{50} µg/ml)	Scavenging ability on ABTS radicals (EC_{50} µg/ml)	Chelating power (EC_{50} µg/ml)
1.	552.70 ± 9.60	905.42± 6.95	27.30± 0.11
2.	615.56 ± 10.94	970.99± 4.38	33.00± 0.12
Standard (Ascorbic acid)	54.23 ± 2.10	81.26± 0.81	7.17± 0.08

3.5 Ferric reducing antioxidant power (FRAP)

FRAP method relies on the reduction of Ferric ion-TPTZ (2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine) by the antioxidant compound, which leads to the development of intense navy blue colour. The intensity of blue colour was measured and was correlated with the amount of antioxidant. The values were expressed in (µM Ascorbic acid equivalent) and were presented in table 3. The results revealed that the Accession number 1, i.e. the red coloured cultivar possess the highest antioxidant value when compared to that of the pink coloured flowers.

3.6 Cupric reducing antioxidant power (CUPRAC)

The CUPRAC is based on the principle that**,** Cu(II) is reduced to Cu (I) through the action of electron donating antioxidants. The analysed flower samples possessed copper deducing potential and were expressed in µM Ascorbic acid equivalent in Table 3.

Table3: Antioxidant effect (EC_{50}) on DPPH radicals, ABTS radicals and chelating power

Accession number	FRAP(µM Ascorbic acid equivalent)	CUPRAC (µM Ascorbic acid equivalent)
1.	0.578± 1.72	0.309± 4.22
2.	0.433± 2.66	0.299± 6.75

4. Conclusion

Examination of both the cultivars of *Malvaviscus arboreus Cav* proved that the methanolic extract of the flowers possess antioxidant potential. Among the two coloured flowers, red coloured flower possess the highest antioxidant potential. Even though these flowers possess antioxidant potential lower than the standard ascorbic acid, it can be used as primary antioxidants.

5. References

1. Alasalvar C, Grigor JM, Zhang D, Quantick PC, Shahidi F. Comparison of volatiles, phenolics, sugars, antioxidant vitamins, and sensory quality of different colored carrot varieties. Journal of Agricultural and Food Chemistry. 2001; 49:1410-1416.
2. Bora D, Mehmud S, Das KK, Medhi H. Report on folklore medicinal plants used for female health care in Assam (India). Int. J. Herbal. Med. 2016; 4:4-13.
3. Brand-Williams W, Cuvelier M-E, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 1995; 28:25-30.
4. Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition reviews, 1998; 56:317-333.
5. Duke JA. Duke's handbook of medicinal plants of Latin America, CRC Press, 2008.
6. Kaisoon O, Siriamornpun S, Weerapreeyakul N, Meeso N. Phenolic compounds and antioxidant activities of edible flowers from Thailand. Journal of functional foods.2011; 3:88-99.
7. Liu X, Zhao M, Wang J, Yang B, Jiang Y. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthusemblica* L.) from six regions in China. Journal of Food Composition and Analysis. 2008; 21:219-228.
8. Manian R, Anusuya N, Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, Ficusbengalensis L. and Ficusracemosa L. Food Chemistry, 2008; 107:1000-1007.
9. Puckhaber LS, Stipanovic RD, Bost GA.Analyses for flavonoid aglycones in fresh and preserved Hibiscus flowers. Trends in new crops and new uses. ASHS Press, Alexandria, 2002, 556-563.
10. Randhir R, Lin Y-T, Shetty K. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pacific journal of clinical nutrition. 2004; 13.
11. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free radical biology and medicine, 1999; 26:1231-1237.
12. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965; 16:144-158.