Research Article

HPTLC Finger Print Profile and *in vitro* Antioxidant Activity of *Gomphrena globosa* L. Flowers

V. Arthi, G. Prasanna*
P.G and Research Department of Biochemistry, Women's College, Sundarakkottai, Mannargudi, Thiruvarur Dist, Tamil Nadu, India.
***Corresponding author's E-mail:** prasannakeertana@yahoo.in

Accepted on: 15-05-2016; **Finalized on:** 30-06-2016.

ABSTRACT

The present investigation was aimed to evaluate the phytochemicals, HPTLC finger print profile and *in vitro* antioxidant activity of ethanol extract of *Gomphrena globosa L.* flowers. Qualitative phytochemical analysis confirmed the presence of carbohydrates, tannins, saponins, flavonoids, quinones, glycosides, terpenoids, phenols, coumarins, steroids and phytosteroids and absence of alkaloids, phlobatannins and anthraquinones. HPTLC study confirms the presence of the flavonoid quercetin by comparing the total peak area of quercetin band in standard solution with that of the sample solutions. The *in vitro* antioxidant property was also carried out in three different concentrations (100, 200 and 300 µg/ml) of flowers extract using total antioxidant capacity, reducing power assay, and DPPH radical scavenging activity. Among the different concentrations, 300 µg/ml of plant extract showed highest antioxidant activity than others in all the models. All the results were compared with standard ascorbic acid. In conclusion, the methanolic extract of *Gomphrena globosa* L. flowers possesses high antioxidant activity which may be due to the presence of quercetin.

Keywords: Antioxidant, *Gomphrena globosa,* HPTLC finger printing, phytochemicals, quercetin.

INTRODUCTION

Oxidative stress is a harmful condition that occurs when there is an excess of ROS and a decrease in antioxidant levels, this may caused tissue damage by physical, chemical, psychological factors that lead to tissue injury in human and causes different diseases[1]. Living creatures have evolved a highly complicated defense system and body act against free radical-induced oxidative stress involve by different defense mechanisms, physical defenses and antioxidant defenses[2].

Antioxidant means "Against oxidation", antioxidants work to protect lipids from peroxidation by free radicals. Antioxidants are effective because, they are willing to give up their own electrons to free radicals[3]. Free radical is any species capable of independent existence that contains one or more unpaired electrons which reacts with other molecule by taking or giving electrons and involved in many pathological conditions[4]. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[5]. Antioxidants acts as a defense mechanism that protects against oxidative damage and include compounds to remove or repair damaged molecules.

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. Numerous other antioxidant phytonutrients present in a wide variety of plant foods. The main characteristic of an antioxidant is its ability to trap free radicals[6]. Therefore, investigations of antioxidants are focused on naturally occurring substances, especially plant phytochemicals[7]. Most sources of natural antioxidants originate from plant materials[8].

Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, tannins, coumarins, lignans and lignins, etc; these compounds have multiple biological effects including antioxidant activity[9]. Flavonoids are a collective term of polyphenolic compounds and ubiquitously exist in all parts of plants[10]. The antioxidant abilities of flavonoids are based upon the characteristics of their molecular structure. Hydroxylation (related to the position and numbers of hydroxyl groups) of 7 the B ring is particularly important for such activity. Quercetin, a flavonoid plays a potent role in antioxidation because it has all the right structural features for scavenging of free radicals[11,10]. Hence the present study has been designed to study the HPTLC finger print profile and *in vitro* antioxidant activity of *Gomphrena globosa* L. flowers.

Gomphrena globosa (Globe amaranth) is belongs to the family of Amaranthaceae. It is an annual budding plant that grows 1-2 ft (0.3-0.6- m) tall with a spread of about 1 ft (0.3 m). The leaves are opposite, oblong, 4-6 in (10-15 cm) long, clover like flower heads, 1.5 in (3.8 cm) long. The individual flowers within the flower heads are inconspicuous, but the stiff, papery bracts that form the bulk of the structure are colorful and showy and come in shades of white, pink and purple. Fruit a capsule, oblong-ovoid, compressed, 2.0 by 2.4 mm. Seeds compressed-ovoid, brown, shining, almost smooth.

Phytochemical screening of plant extract revealed the presence of saponins, alkaloids, reducing sugars and

coumarins. (Daniel Dias Arcanjo) Phytochemical evaluation of aerial parts reported that one new triterpenoid saponin (gomphrenoside) and one new hopane derivative (hopan-7ß-ol) along with known compounds, ß-sitosterol-ß-D-glucoside and 1-triacontanol[12]. Flower yields ß-cyanins, gomphrenin I, II, and III. Leaves yield methylene dioxy flavonol-gomphrenol.

Medicinal Uses

Decoction of leaves is used for bronchial asthma, acute and chronic bronchitis, whooping cough. The plant is used for diabetes[13] and for hypertension[14], jaundice and other kidney problems and as heart tonic. Infusion of flower is used in treating oliguria and empacho and expectorant for animals[15]. Used the moist petal of this plant under the eyes to lighten the skin and firm up dark bags under the eyes. It is used for stress periods, because *Gomphrena* allows a relaxing of the nerves and muscles, to be serene and full of energy. It decreases the mental concern and fights against tiredness and aggressiveness. It favors sleep. It allows memory stimulation, to increases concentration and lucidity. It has a beneficial action on digestion. Its properties of anti-stress and anti-oxidation promotes against skin aging.

MATERIALS AND METHODS

Collection of Plant Materials

The plant materials used in the present study are the flowers of *Gomphrena globosa*. The flowers were collected from in and around Kumbakonam, Thanjavur district, Tamil Nadu, India.

The collected samples were carefully kept in polythene bags. These plant samples were authenticated and a voucher specimen was deposited in the Department of Biochemistry, S.T.E.T. Women's College, Mannargudi, Thiruvarur District, Tamil Nadu. The flowers were dried in shade and stored in air tight containers until further studies.

Preparation of Extracts

100g of flower powder was weighed and macerated in 750 ml methanol. They were kept at the room temperature for 72 hours. The mixture was stirred every 24 hours using a sterile glass rod. Then it was filtered through the Whatmann filter paper. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure. The dried residue of methanol extracts was used for further work.

Preliminary Phytochemical Screening

The flower extract was subjected to preliminary phytochemical investigations to determine the different phytoconstituents using different standard methods[16].

HPTLC Finger Print Analysis

About 1mg of the extract of the *Gomphrena globosa* flowers was taken and dissolved in respective solvent and the volume was made up to 1ml in a standard flask (1000 µg/ml). This solution was used as test solution for HPTLC analysis. Silica gel 60 F254 and HPTLC aluminum sheets were used as adsorbent (stationary phase). 5 and 10 µL of the above test solution and 2 µL of a standard solution (quercetin) were loaded on HPTLC aluminum sheets as different tracks in the form of 6 mm wide bands by using a CAMAG semi-automatic Linom at 5 spotters at a distance of 12mm. Nitrogen gas was also supplied for simultaneous drying of bands. The samples-loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor) with mobile phase toluene: Ethyl acetate: Formic acid (5:4:1).

The developed plates were then dried and scanned using a TLC scanner 3 with Wincats software under 364 nm (Wager and Bladt, 1996). All plates were visualized directly after drying and a fingerprint profile was photo documented using a CAMAG Reporter- 3 under 254 nm and 366 nm in UV and visible light. The peak table, peak display and peak densitogram were recorded[17,18].

In vitro Antioxidant Activity

The antioxidant activity of plant extract was determined by different *in vitro* models such as total antioxidant activity[19], Reducing power assay[20], and DPPH radical scavenging activity[21].

Different concentration (100, 200 and 300 µg/ml of plant extract were used for antioxidant activity. Ascorbic acid was used as standard. All the experiments were performed thrice and the results were averaged.

RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical Analysis of *Gompherena globosa* Flowers

S. No.	Phytochemical	Result
1	Carbohydrates	+
2	Tannins	+
3	Saponins	+
4	Flavonoids	+
5	Alkaloids	-
6	Quinones	+
7	Glycosides	+
8	Cardiac glycosides	+
9	Terpenoids	+
10	Triterpenoids	+
11	Phenols	+
12	Coumarins	+
13	Steroids and phytosteroids	+
14	Phlobatannins	-
15	Anthraquinones	-

+ indicates presence; - indicates absence

In the present study, phytochemical screening, HPTLC finger printing profile and *in vitro* antioxidant activity were carried out in methanolic flowers extract of *Gomphrena globosa*. Results were recorded, tabulated and discussed in this chapter.

Plants are the source of energy for the animal kingdom. In addition, Plants can synthesize a large variety of chemical substances that are of physiological importance[22]. Medicinal, herbal and aromatic plants constitute a large segment of the flora, which provide raw materials for use by pharmaceutical, cosmetic, fragrance and flavor industries. They have been used in the country for a long time for their medicinal properties[23]. In the present study, phytoconstituents of methanolic extract of *Gomphrena globosa* were analysed qualitatively and the result revealed the presence of carbohydrates, tannins, saponins, flavonoids, quinones, glycosides, terpenoids, phenols, coumarins, steroids and phytosteroids and absence of alkaloids, phlobatannins and anthraquinones (Table 1). It was proposed that the antioxidant activity of plant extract could possibly be related to flavonoids[24].

HPTLC Finger Print Analysis

Chromatographic fingerprint analysis has shown to be a rational and feasible approach for the quality assessment and species authentication of traditional medicine. It utilizes chromatographic techniques to construct specific patterns of recognition for medicinal plants. The developed fingerprint pattern of components can then be used to determine not only the absence or presence of markers of interest but the ratio of all detectable analysts as well. HPTLC, High Performance Thin Layer Chromatography, is the most recent evolution of planar chromatography and has been specifically tailored for analysis of natural products[25,26].

Although high performance thin layer chromatography (HPTLC) has a few limitations, such as the limited developing distance and lower plate efficiency by comparison with HPLC and GC, it is still an effective tool for quality evaluation of medicinal plants due to its simplicity, low cost, and requirement, and it has been successfully utilized to develop the chromatographic fingerprint for medicinal plants.

In the present study, HPTLC analysis of *Gomphrena globosa* (5µl and 10µl) was carried out along with the standard flavonoid quercetin (1µl) and toluene: ethyl acetate: formic acid (5:4:1) as the mobile phase. Silica gel F_{254} was a stationary phase. The identity of the bands of quercetin in the methanol extract was confirmed by comparing the UV-Vis absorption spectra at 254 nm with that of standard using a CAMAG REPROST scanner. The band range of Rf value was from 0.55 to 0.62 corresponding to quercetin in test solution has been identified in plant extract. The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the reference standard solution and by comparing retention factors of quercetin from sample and standard solution. Good correlation was obtained between the standard and the sample of quercetin (Table 2, 3 and 4; Figure 1, 2, 3 & 4).

In previous study, HPLC was performed for quantitative analysis of methanol extracts of *G. globosa* for phenols and flavonoids following the method described by Deshmukh and Prabhu, 2011[27]. Quantification was performed on the basis of calibration curve for standards of phenols and flavonoids (i.e. gallic acid and quercetin respectively). The percentage recovery of gallic acid was 0.093% (w/w) for *G.globosa*. However, for quercetin the percentage recovery is 0.363 % (w/w) for *G.globosa* which supports our study that the flavonoid quercetin is present in the flowers of *Gomphrena globosa.* Previous studies reported that flavonoids were the predominant polyphenols in flower sample extracts, which are in agreement with the other reports[28-31] which suggesting the occurrence of flavonoids, iso flavonoids and flavones-C glycosides in the family Amaranthaceae.

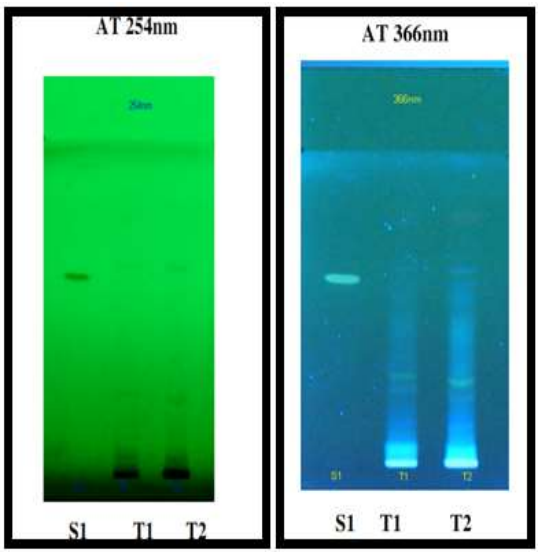

Figure 1: Photo Documentation under UV

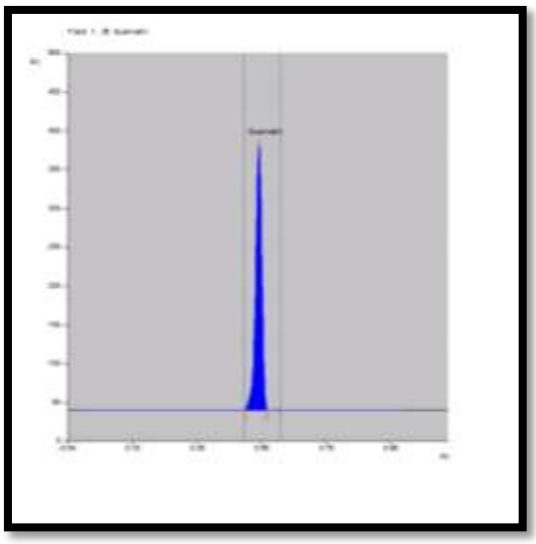

Figure 2: HPTLC Chromatogram for Standard

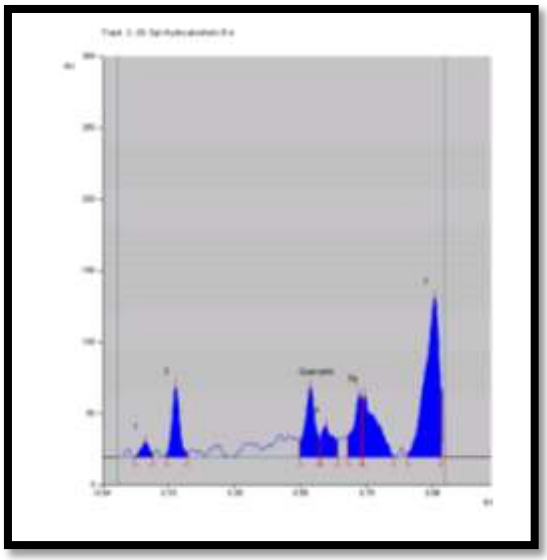

Figure 3: HPTLC Chromatogram for Sample (5µl)

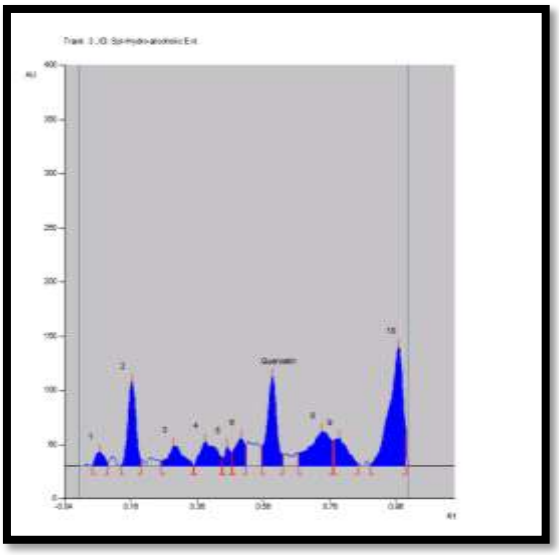

Figure 4: HPTLC Chromatogram for Sample (10µl)

Table 2: HPTLC Finger Print Profile for Standard (1µl)

Peak	Start Rf	Start height	Max Rf	Max height	Height %	End Rf	End height	Area	Area %	Assigned substance
1	0.50	7.3	0.55	344.1	100.00	0.58	0.1	5472.1	100.00	Quercetin

Table 3: HPTLC Finger Print Profile for Sample (5µl)

Peak	Start Rf	Start height	Max Rf	Max height	Height %	End Rf	End height	Area	Area %	Assigned substance
1	0.05	0.4	0.09	10.5	3.18	0.11	2.2	202.7	2.33	Unknown*
2	0.15	1.5	0.18	49.5	14.97	0.21	2.8	851.9	9.79	Unknown*
3	**0.55**	**10.6**	**0.59**	**49.0**	**14.83**	**0.62**	**12.8**	**1128.7**	**12.97**	**Quercetin**
4	0.62	12.9	0.63	22.3	6.75	0.67	11.3	597.2	6.86	Unknown*
5	0.70	14.2	0.74	44.2	13.37	0.74	38.2	849.7	9.78	Unknown*
6	0.74	38.3	0.75	43.2	13.08	0.84	0.4	1424.5	16.37	Unknown*
7	0.88	2.6	0.96	111.8	33.83	0.99	46.0	3648.5	41.92	Unknown*

Table 4: HPTLC Finger Print Profile for Sample (10µl)

Peak	Start Rf	Start height	Max Rf	Max height	Height %	End Rf	End height	Area	Area %	Assigned substance
1	0,04	0.3	0.06	12.7	3.00	0.09	2.6	258.6	2.26	Unknown*
2	0.13	0.3	0.16	77.8	18.39	0.19	5.1	1421.5	12.41	Unknown*
3	0.25	3.8	0.29	18.2	4.31	0.35	0.1	624.2	5.45	Unknown*
4	0.35	0.2	0.38	22.2	5.25	0.43	7.9	818.4	7.14	Unknown*
5	0.44	7.3	0.45	17.6	4.17	0.46	12.4	253.4	2.21	Unknown*
6	0.46	12.7	0.49	25.2	5.97	0.50	18.9	571.3	4.99	Unknown*
7	**0.55**	**17.4**	**0.58**	**82.1**	**19.42**	**0.62**	**10.1**	**1771.7**	**15.46**	**Quercetin**
8	0.66	11.6	0.73	31.5	7.44	0.77	23.1	1551.7	13.54	Unknown*
9	0.77	22.6	0.79	25.6	6.06	0.84	2.1	795.5	6.94	Unknown*
10	0.88	2.1	0.97	110.0	26.00	0.99	36.2	3391.7	29.60	Unknown*

Table 5: Total Antioxidant Activity of *Gomphrena globosa* Flowers

S. No	Concentration (µg/ml)	Antioxidant Activity in %	
		Plant extract	Standard drug
1.	100	30.6±0.8	45.68±3.18
2.	200	35.9±0.3	51.69±2.14
3.	300	40.7±1.2	54.53±0.57
IC_{50} value		200 µg /ml	200 µg /ml

Table 6: Reducing Power Assay of *Gomphrena globosa* Flowers

S. No	Concentration (µg/ml)	Reducing Power in %	
		Plant extract	Standard drug
1.	100	23.07±0.06	38.68±1.15
2.	200	41.17±1.10	47.22±1.02
3.	300	58.81±0.90	62.80±2.02
IC_{50} value		200 µg/ml	230 µg/ml

Table 7: DPPH Scavenging Activity of *Gomphrena globosa* Flowers

S. No	Concentration (µg/ml)	DPPH scavenging activity in %	
		Plant extract	Standard drug
1.	100	45.7±2.3	53.06±0.12
2.	200	60.3±0.70	69.36±0.08
3.	300	70.3±0.03	79.30±0.10
IC_{50} value		140 µg /ml	190 µg /ml

In vitro Antioxidant Activity

The oxidative stress leads to numerous pathophysiological conditions including cancer, cardiovascular diseases, neural disorders, alzheimer's disease, parkinson's disease, insulin dependent diabetes, fatty liver associated with chronic alcoholism and aging[32-35]. For the last few decades, an immense interest has been developed in plants with medicinal properties, with prime focus on therapeutic significance and research is carried out to identify and develop the plants with antioxidant properties to combat the oxidative stress. In the present study, *in vitro* antioxidant activity of methanolic extract of *Gomphrena globosa* flowers were evaluated by different models, including total antioxidant capacity, reducing power assay and DPPH radical scavenging activity. Standard ascorbic acid was also analyzed to compare the efficacy of plant extract on antioxidant activity.

Total Antioxidant Activity

Table 5 represents the total antioxidant activity of three different concentrations of (100, 200 and 300 µg /ml**)** methanolic extract of *Gomphrena globosa* flowers**.** Percentage of antioxidant activity of plant extracts were 30.6, 35.9 and 40.7 for 100, 200 and 300 µg /ml respectively. For the same concentration of standard ascorbic acid, percentage of activity was 45.68, 51.69 and 54.53. IC_{50} value for both plant extract and standard drug were 200 µg/ml. Total antioxidant capacity by Phospho molybdenum method assay has been routinely used and it is based on the reduction of Mo (VI) to Mo (V) by the sample analyst and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphor molybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid[36]. Finding of our study showed that plant extract exhibited antioxidant activity which was less than that of standard.

Reducing Power Assay

The reducing ability of the extract served as a significant indicator of the potential antioxidant activity. For reducing power assay, plant extract and standard ascorbic acid were used at dose range of 100-300µg/ml (Table 6). The percentage of reducing power of extract increased from 100 µg/ml (23.07%) followed by 200 µg/ml (41.17%) and 300µg/ml (58.81±0.90) and for standard ascorbic acid, the percentage were 38.68, 47.22 and 62.80 for 100, 200 and 300 µg/ml respectively. IC_{50} value of plant extract was 200 µg/ml and standard drug was 230 µg/ml.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The reducing ability is generally associated with the presence of reductones, which breaks the free radical chain by donating a hydrogen atom[37]. Reducing power activity is often used to evaluate the ability of natural antioxidant to donate electron[38,39]. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts[40,41,38]. In the present study, the reducing power of flower extract of *Gomphrena globosa* increased consistently with the increase in the volume of extract from 100µg to 300µg and the extract exhibited a moderate reducing power when compared to that of standard ascorbic acid.

DPPH Scavenging Activity

Table 7 shows the result of DPPH radical scavenging activity at different concentration (100, 200 and 300 µg/ml**)** of methanol extract of *Gomphrena globosa* flowers. Among the three concentrations, highest % of inhibition (70.3%) was found in 300 µg. Moderate activity was found in 200 µg **(**60.3%**)** and least activity was observed in 100 µg **(**45.7%**)**. Similarly, radical scavenging activity of ascorbic acid were also observed for 100 µg/ml (53.06%), 200 µg/ml (69.36%) and 300 µg/ml (79.30%). From the results, dose dependent response was observed, that is the concentration of extract increased, the scavenging activity was also increased. IC_{50} value of plant extract was 140 µg/ml and standard drug was 190 µg/ml.

Good stability, credible sensitivity, simplicity and feasibility are the advantages of DPPH assay[42-44]. This assay is often used to evaluate the ability of antioxidants to scavenge the free radicals from the supplied samples, whereby the free radicals because biological damage through oxidative stress and such processes leads to many disorders like neurodegenerative disorders, cancer and AIDS[45]. Therefore, DPPH assay is an effective method to measure their scavenging power.

The principle of the DPPH is based on the color changes from purple (DPPH solution) to yellow[46]. The color changes can be measured quantitatively at the absorbance 517nm. In the present study, the methanol flower extract exhibited a dose dependent higher radical quenching activity. The 100 µg/ml concentration showed moderate radical scavenging activity (45.70%) than ascorbic acid (53.06%) and thereafter the pattern continued till 300 µg/ml.

The results of this study infer that the plant extract exhibit strong antioxidant properties in a dose dependent manner in all the models which is consistent with earlier observations[47,48].

The plant flavonoids are classified as the most abundant secondary metabolites, with high antioxidant properties, which could contribute to reduce oxidative stress[49-51]. Previous study have reported that extracts prepared from whole plant of *G.globosa* possess antioxidant and cytotoxic potential[52]. Sakia and Upadhayaya, (2011) also suggested that the leaves of *G. globosa* have phenols and flavonoids and exhibit antioxidant activities.

CONCLUSION

The result of the present study showed that the flowers extract of *Gomphrena globosa* exhibited the greatest antioxidant activity.

The presence of quercetin enhances the effective antioxidant potential of the plant. The result supports the *Gomphrena globosa* can be used as a source of safe and natural antioxidant compounds for treating chronic diseases.

Acknowledgement: Authors are thankful to the Managing Trustee and the Principal of S.T.E.T. Women's College, Mannargudi, Thiruvarur, Tamil Nadu for their moral support, encouragement, guidance and providing infrastructure facility to carry out this work successfully.

REFERENCES

1. Tian Y, Jiang B, An L, Bao Y, Neuroprotective effect of catalpol against MPP+-induced oxidative stress in mesen cephalic neurons, Eur. J. Pharmacol, 568, 2007, 142-48.
2. Valko M, Morris H and Cronin MTD, Metals, toxicity and oxidative stress, Current Medicinal Chemistry, 12, 2005, 1161-1208.
3. Kaczmarski M, Wojicicki J, Samochowiee L, Dutkiewicz T, and Sych Z, The influence of exogenous antioxidants and physic al exercise on some parameters associated with production and removal of free radicals, Pharmazie, 54, 1999, 303-306.
4. Madhavi DL, Deshpande SS, Sulunkhe DK, Food antioxidant: technological, toxicological and health perspectives, New York, Marcel Dekker. 1(3), 1996, 51-54.
5. Stanner SA, Hughes J, Kelly C.N, Buttriss Review of the epidemiological evidence for the antioxidant hypothesis, public Health. Nutrition, 7, 2000, 401-422.
6. Rumit Shah A, Heena Kathad D, Rajal sheth S, Naveen Sheth B. *In vitro* Antioxidant Activity of Roots of *Tephrosia purpurea* Linn, International journal pharmacy sci. 2, 2010, 30-33.
7. Gokhan zengin A, Abdurrahman S, Akumsek, Gokalp Ozmen Guler, Yavuz Selim Cakmak, Evren Yildiztugay, Antioxidant capacity and fatty acid profile of *Centaurea kotschyi* (Boiss. & Heldr.) Hayek var. persica (Boiss.) Wagenitz from Turkey, Grasasy aceites, 62(1), 2011, 90-95.
8. Elizabeth M, Williamson, Text book of major herbs of Ayurveda, 1st edn, 7, 2002.
9. Sharma SK, Singh Lalit, Singh Suruchi, A review on medicinal plants having antioxidant potential, Indian. J of Research in Pharmacy and Biotechnology, 3, 2013, 404-409.
10. Nijveldt RJE, Van Nood DEC, Van Hoorn PG, Boelens K, Van Norren, and Van Leeuwen PAM, Flavonoids: a review of probable mechanisms of action and potential applications, American journal of clinical nutrition, 74(4), 2001, 418-425.
11. Namiki M, Antioxidants/antimutagens in food. Critical reviews in food science and nutrition, 29(4), 1990, 273-300.

12. Biswanath Dinda, BiplabGhosh, Shiho Arima, Nariko Sato, and Yoshihiro Harigaya, Phytochemical investigation of *Gomphrena globosa* aerial parts, Indian Journal of Chemistry, 43, 2004, 2223-2227.
13. Arcanjo DDR, De Albuquerque ACM, Neto BN, Silva NCBS, and Moita MM, Phytochemical screening and evaluation of cytotoxic, antimicrobial and cardiovascular effects of *Gomphrena globosa* L ethanolic extraction, J. Med. Plants Res, 5, 2006-2010.
14. Yusuf AA, Iwuafor ENO, Abaidoo RC, Olufajo OO and Sanginga N, Grain legume rotation benefits to maize in the northern Guinea savanna of Nigeria: Fixed-nitrogen vs. other rotation effects, Nutr. Cycl. Agroecosyst, 84, 2009, 129-139.
15. Asolkar LV, Kakkar KK, Chakre OJ, Glossary of Indian Medicinal Plants with Active Principles New Delhi, Publication and Informat ion Directorate, 1992, 72-73.
16. Harborne JB, Phytochemical methods; 3rd edition, London: Chapman and Hall; 1998.
17. Srivastava M, High-Performance Thin-Layer Chromatography (Hptlc). Springer, Heidelberg Dordrecht London, New York, 2011.
18. Sethi PD, High Performance Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Formulations, CBS Publishers and Distributers, New Delhi, 1996, 10-60.
19. Shirwaikar A, Rajendran K, Kumar CD, *In vitro* antioxidant studies of *Annona squamosa* Linn leaves, Indian Journal of Experimental Biology, 42, 2004, 803-807.
20. Oyaizu M, Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn J Nutr, 103, 1986, 413-419.
21. Gyamfi MA, Aniya Y, Antioxidant properties of thonningianin A, isolated from the African medicinal herb, *Thonningia sanguine*, Biochem Pharmacol, 63, 2002, 1725-1737.
22. Padmaja M, Sravanthi M, Hemalatha KP, Evaluation of antioxidant activity of two Indian medicinal plants, Journal of Phytology, 3(3), 2011, 86-91.
23. Kretovich UL, Principles of plant biochemistry permagon, Press oxford J. Food Sci. 54, 2005, 254-260.
24. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S, The comet assay with 8 mouse organs: Results with 39 currently used food additives, Mut Res-Gen Tox En, 519, 2002, 103-109.
25. Pharmacopéè Francaise, 7thedn, Wolter Kluver France, 1965.
26. Reich E, Schibli A, A High-performance thin-layer chromatography for the analysis of medicinal plants, Thieme Medical Publishers Inc, 2006.
27. Deshmukh H, Prabhu JP, Development of RP-HPLC method for Qualitative Analysis of Active Ingredient (Gallic acid) from Stem Bark of *Dendrophthoe falcate* Linn. International Journal of Pharmaceutical Sciences and Drug Research, 3, 2011, 146-149.
28. Khalil MY, Moustafa AA and Naguib NY, Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition, World Journal of Agricultural Sciences, 3(4), 2007, 451-457.
29. Salvador MJ, and Dias DA, Flavone C-glyco-sides from *Alternanthera maritime* (Mart.) St. Hil.(Amaranthaceae), Biochem. Syst. Ecol, 32, 2004, 107-110.
30. Salvador MJ, Zucchi OLAD, Candido RC, Ito IY, and Dias DA, *In vitro* antimicrobial activity of crude extracts and isolated constituents of *Alternanthera maritime* (Amaranthaceae). Pharm. Biol, 42, 2004, 138-148.
31. Salvador MJ, Ferreira EO, Mertens-Talcott SU, Castro WV, Butterweck V, Derendorf H, Dias DA, Isolation and HPLC quantitative analysis of antioxidant flavonoids from *Alternanthera tenella* Colla, Z. Naturforsch C, 61, 2006, 19–25.
32. Arteel GE, Oxidants and antioxidants in alcohol induced liver disease, Gastroenterology, 124, 2003, 778-790.
33. Kinnula VL, Crapo JD, Superoxide dismutases in malignant cells and human tumors, Free Rad. Biol. Med, 36, 2004, 718-744.
34. Guidi I, Galimberti D, Lonati S. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease, Neurobiol Aging, 27, 2006, 262-269.
35. Hyun DH, Hernandez JO, Mattson MP. The plasma membrane redox system in aging, Aging Res. Rev, 5, 2006, 209-220.
36. Halliwell H, Free radicals, antioxidants, and human disease: Curiosity, cause or consequence, Lancet, 344, 1994, 721-724.
37. Subhashini N, Thangathirupathi A and Lavanya N, Antioxidant activity of *Trigonella boenumgraecum* using various *In vitro* and *Ex vivo* models, I J Pharm and pharm Sciences, 3(2), 2011, 96-102.
38. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V, Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf Ex DC), Sage (*Savia triloba* L.) and Black tea (*Camellia sinensis*) extracts, J Agric Food Chem, 48(10), 2000, 5030-5034.
39. Dorman HJD, Kosar M, Kahlos K, Holm Y, and Hiltunen R, Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars, J. Agric. Food Chem, 51, 2003, 4563-4569.
40. Duh PD, Antioxidant activity of burdock (*Arctium lappa* Linn.): Its scavenging effect on free -radical and active oxygen, J. Am. Oil Chem. Soc, 75, 1998, 455-461.
41. Duh PD, Du PC, Yen GC, Action of methanolic extract of Mung beans hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage, Food Chem. Toxicol, 37, 1999, 1055-1061.
42. Jin J, Li Z, and Zhang F, Scavenging function of *Mulberry vinegar* extractives for 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Journal of Northwest Sci-Tech University of Agriculture and Forestry, 34(3), 2006, 135–137.
43. Ozcelik B, Lee JH, Min DB, Effects of light, oxygen, and pH on the absorbance of 2, 2-Diphenyl-1-picrylhydrazyl, J Food Sci, 68(2), 2003, 487–490.

44. Aparadh VT, Naik VV, Karadge BA, Antioxidative Properties (TPC, DPPH, FRAP, Metal Chelating Ability, Reducing Power And TAC) Within Some Cleome Species, Annali Di Botanica, 2, 2012, 49–56.

45. Rabe TSJ, Isolation of an antimicrobial sesqui terpenoid from *Warbugie salutaris*, J Ethnopharcol, 93, 2000, 171-174.

46. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, Selenium: Biochemical role as a component of glutathione peroxidase, Science, 179, 1973, 588-590.

47. Sakia LR and Upadhayaya S, Antioxidant activity, phenol and flavonoid content of some less known medicinal plants of Assam, International Journal of Pharma and Bio Sciences, 2(2), 2011, 383-38.

48. Rice –Evans C, Miller NJ, Methods in enzymology, 234, 1994, 279-293.

49. Robak J, Gryglewski RJ, Flavanoids are scavengers of superoxide anions, Biochemistry pharmacol, 37(5), 1988, 837-841.

50. Rice-Evans C, Miller N, Paganga G, Antioxidant properties of phenolic compounds, Trends Plant Science, 2, 1997, 152-159.

51. Nordberg J, Arner ESJ, Reactive oxygen species, antioxidants, and the mammalian thioredoxin system, Free Radic. Biol. Med, 31, 2001, 1287–1312.

52. Himuduzzaman and Azam ZMTA, Antimicrobial, Antioxidant and Cytotoxic Activities of *Gomphrena globosa* (L.), Bangladesh Pharmaceutical Journal, 15(2), 2012, 183-185.

Source of Support: Nil, **Conflict of Interest:** None.