FREE RADICAL SCEVENGING ACTIVITY OF *Tagetes erecta* FLOWER EXTRACT – AN *IN VITRO* STUDY

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ABSTRACT

Free radicals are reactive molecules involved in many physiological processes and have been associated with many diseases, such as cancer, arthritis and liver injury. As a result, there is need to explore substances with free radical scavenging and or antioxidant activity. The present study was designed to evaluate the free radical scavenging activity of Tagetes erecta flower through various model including DPPH, total antioxidant assay, reducing power assay, superoxide and iron chelating activity. The antioxidant activity is directly proportional to the concentration. In the present study analysis of free radical scavenging activity of Tagetes erecta flower can be the potent source of natural antioxidant. The flower extract is a promising candidate for use as natural products based antioxidant for health preservation and diseases prevention.

Keywords: *Free radical, Antioxidant, DPPH, Total antioxidant assay, Reducing power assay, Superoxide and iron chelating activity*

INTRODUCTION

The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immunosuppression and neurodegeneration (Harman, 1998). An imbalance between ROS and the inherent antioxidant capacity of the body, directed the use of dietary and /or medicinal supplements particularly during the disease attack. Studies on herbal plants, vegetables, and fruits have indicated the presence of antioxidants such as phenolics, flavonoids, tannins, and proanthocyanidins. The antioxidant contents of medicinal plants may contribute to the protection they offer from disease. The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gulcin, 2012). Liver diseases remain a serious health problem. It is well known that free radicals cause cell damage through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injury. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities (Osawa *et al*., 1990). The use of medicinal plants with high level of

antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages (Govind, 2011). The search for novel natural antioxidants of plant origin has ever since increased. It is not known which constituents of plant are associated in reducing the risk of chronic diseases, but antioxidants appear to play a major role in the protective effect of plant medicine. The present study was designed to investigate the antioxidant activity of *Tagetes erecta* flower through various model including DPPH, total antioxidant assay, reducing power assay, superoxide, and iron chelating activity

MATERIALS AND METHODS

Collection of Plant materials

During the month of February, *Tagetes erecta* flowers were collected from various gardens in Keelavandanviduthy Village, Pudukkottai district, Tamil Nadu, India.

Authentication of plants

The plant was identified and carefully examined with the help of region floras. Specimens were further confirmed with reference to herbarium sheets available in the Rapinat Herbarium, St, Joseh's College, Tiruchirappalli, Tamil Nadu, and India.

Preparation of extracts

The powdered rhizome and flowers material (20 g) was soaked in 50 ml of 70% Methanol for 12 hours and then filtered through a Whatmann filter paper along with 2 g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1 ml. The extract contains both polar and non-polar phytocomponents. Doses 20, 40, 60 and 80 were selected for antioxidant actvity

In vitro antioxidant activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992). The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*., (1999). The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (1986). The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al*., (1994).

RESULTS

In Vitro Antioxidant Activity of *Tagetes erecta* flower DPPH Assay

DPPH radical scavenging activity of plant extract of *Tagetes erecta* flower and standard as ascorbic acid are presented in Fig 1. The half inhibition concentration (IC_{50}) of *Tagetes erecta* flower and ascorbic acid were 59.72 and 34.91µg ml^{-1} respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH activity (Table 1). The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

Table.1: DPPH radical scavenging activity of *Tagetes erecta* flower (Marigold)

Concentrations (µg/ml)	*Tagetes erecta* flower (Marigold)	Standard (Ascorbic acid)
20	17.27±1.20	25.6±1.79
40	36.36±2.54	95.45±6.68
60	45.45±3.18	63.61±4.45
80	69.09±4.83	99.34±6.95
IC_{50} (µg/ml)	59.72	34.91

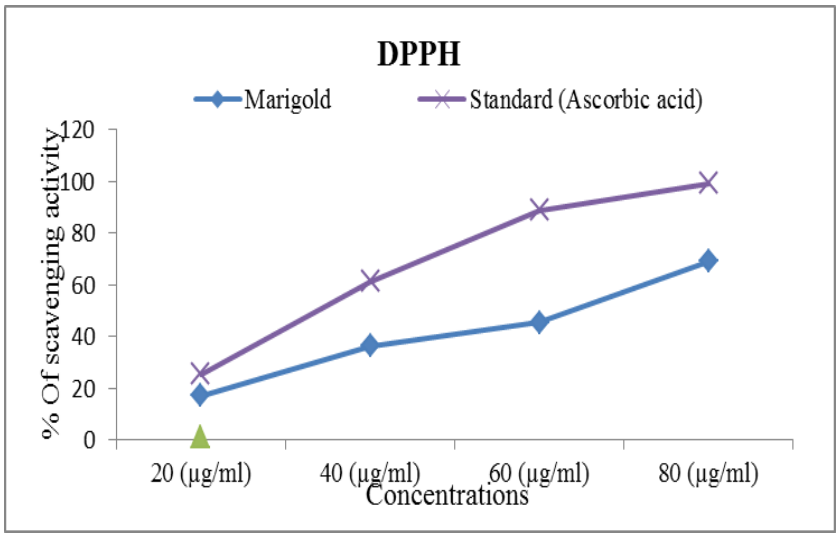

Fig.1: DPPH radical scavenging activity of *Tagetes erecta* flower (Marigold)

Total antioxidant activity

The yield of the ethanol extract of the plant extract and its total antioxidant capacity are given in Fig. 2. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract (Table 2). The half inhibition concentration (IC_{50}) of *Tagetes erecta* flower and ascorbic acid were 60.14 and 42.41 µg ml^{-1} respectively.

Table.2: Total antioxidant activity of *Tagetes erecta* flower (Marigold)

Concentrations (µg/ml)	*Tagetes erecta* flower (Marigold)	Standard (Ascorbic acid)
20	18.75±1.31	22.35±1.56
40	35.62±2.49	74.37±5.20
60	53.12±3.71	73.75±5.16
80	62.5±4.37	86.35±6.04
IC_{50} (µg/ml)	60.14	42.41

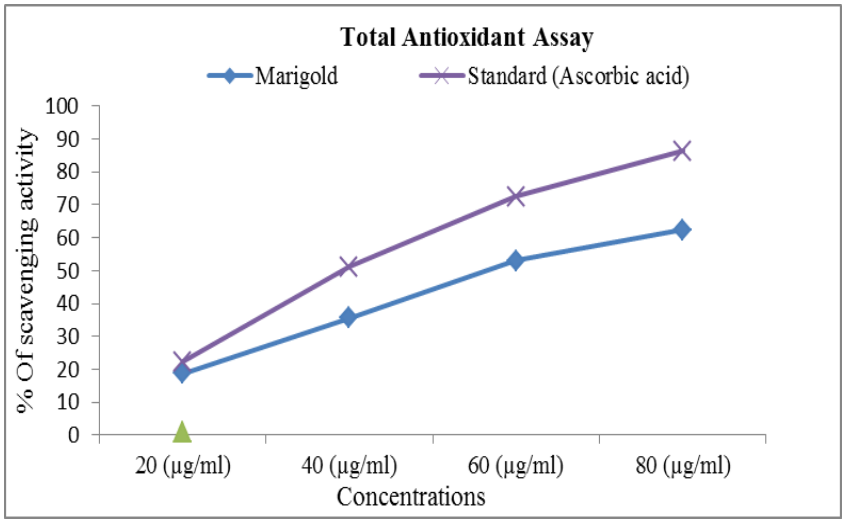

Fig.2: Total antioxidant activity of *Tagetes erecta* flower (Marigold)

Superoxide scavenging activity

The superoxide anion radical scavenging activities of the extract from *Tagetes erecta* flower assayed by the PMS-NADH system was shown in Fig 3. The superoxide scavenging activity of *Tagetes erecta* flower was increased markedly with the increase of concentrations (Table 3). The half inhibition concentration (IC_{50}) of *Tagetes erecta* flower and ascorbic acid were 59.72 and 34.91µg ml^{-1} respectively.

Table.3: Superoxide radical scavenging activity *Tagetes erecta* flower (Marigold)

Concentrations (µg/ml)	*Tagetes erecta* flower (Marigold)	Standard (Ascorbic acid)
20	20.31±1.42	31.25±2.18
40	41.41±2.89	80.41±5.62
60	63.45±4.44	78.41±5.48
80	79.56±5.56	98.51±6.89
IC_{50} (µg/ml)	59.72	34.91

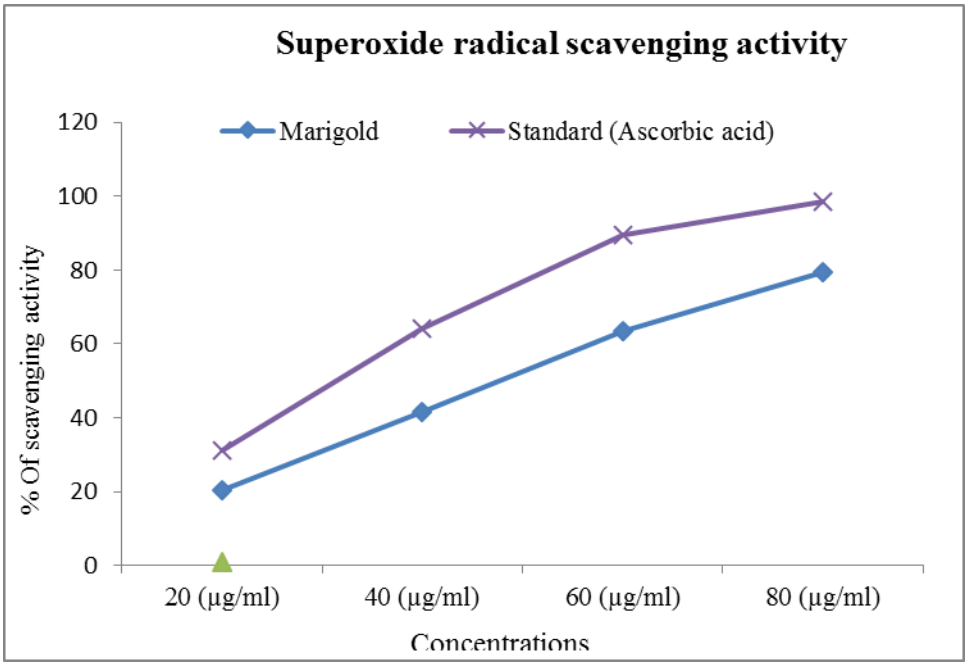

Fig.3: Superoxide radical scavenging activity *Tagetes erecta* flower (Marigold)

The ferrous iron chelating activity

The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of extract of *Tagetes erecta* flower indicating that have Chetlating activity with an $IC_{50}$of 45.64µg $ml^{-1}$and ascorbic acid was 30.96µg ml^{-1} respectively (Fig. 4, Table 4).

Table.4: Iron chelating activity of *Tagetes erecta* flower (Marigold)

Concentrations (µg/ml)	*Tagetes erecta* flower (Marigold)	Standard (Ascorbic acid)
20	19.23±1.34	35.23±2.46
40	38.46±2.69	92.3±6.46
60	76.92±5.38	80.76±5.65
80	86.46±6.05	98.65±6.90
IC_{50} (µg/ml)	45.64	30.96

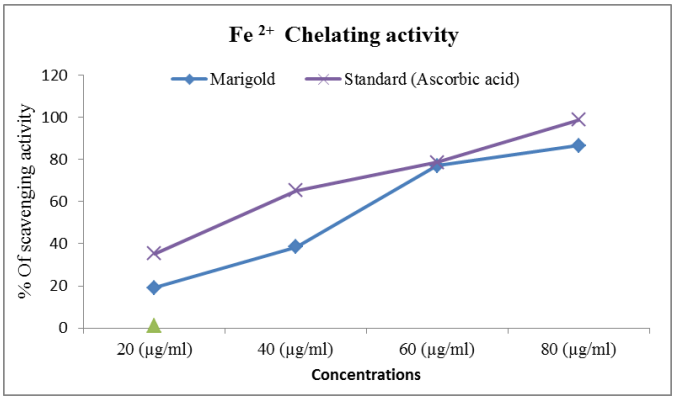

Fig.4: Iron chelating activity *Tagetes erecta* flower (Marigold)

Reducing power activity

Fig. 5 depicts the reductive effect of *Tagetes erecta* flower. Similar to the antioxidant activity, the reducing power of *Tagetes erecta* flower increased with increasing dosage (Table 5). All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Tagetes erecta* flower consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Table.5: Reducing power activity of *Tagetes erecta* flower (Marigold)

Concentrations (µg/ml)	*Tagetes erecta* flower (Marigold)	Standard (Ascorbic acid)
20	0.18±0.01	0.75±0.05
40	0.22±0.01	0.78±0.05
60	0.21±0.01	0.69±0.04
80	0.41±0.02	0.98±0.06

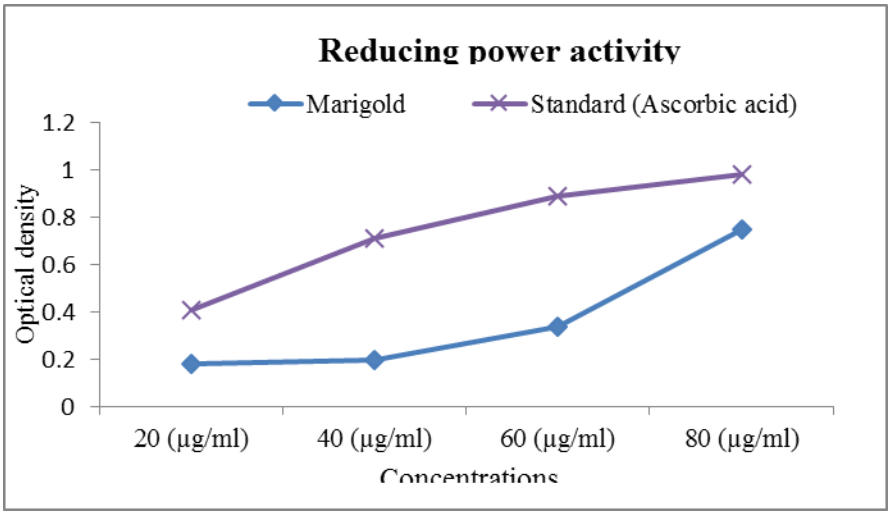

Fig.5:
Reducing power activity *Tagetes erecta flower (Marigold)*

DISCUSSION

In Vitro Antioxidant Activity *Tagetes erecta* flower *(*Marigold*)*

DPPH Assay

The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al.,* 2003). Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

Total antioxidant activity

Total antioxidant capacity of *Tagetes erecta* flower is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximum absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al.,* 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. Enas Mehjen Numan *et al.* (2016) reported the DPPH radical scavenging activity in *Tagetes erecta* flower.

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978). The superoxide anion radical scavenging activities of the extract from *Tagetes erecta* flower assayed by the PMS-NADH system. The superoxide scavenging activity of *Tagetes erecta* flower was increased markedly with the increase of concentrations. These results suggested that *Tagetes erecta* flower had notably superior superoxide radical scavenging effects.

The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of ethanol extract of *Tagetes erecta* flower, indicating that have chelating activity. Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals (Halliwell, 1991; Fridovich, 1995). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that forms bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion (Gordon, 1990). Thus, *Tagetes erecta* flower extract demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

Reducing power activity

For the measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Tagetes erecta* flower extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, and prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al*, 2000). Similar to the antioxidant activity, the reducing power of *Tagetes erecta* flower increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Tagetes erecta* flower consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Conclusion

The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. In the present study analysis of free radical scavenging activity of *Tagetes erecta* flower can be the potent source of natural antioxidant. The flower extract is a promising candidate for use as natural products based antioxidant for health preservation and diseases prevention.

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