

## Antioxidant Activities of Methanolic Extract of *Tagetes erecta* Flower Growing in Bangladesh

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**Abstract:** The present study was designed to investigate the antioxidant activities of the methanolic extract of *Tagetes erecta* (TRF) flower. Total phenolic content, total antioxidant activity, scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical were used to evaluate antioxidant potential of TRF. In DPPH method, TRF showed moderate antioxidant potentiality in a dose dependent manner with the  $IC_{50}$  value of 117.47 $\mu$ g/ml. The phenolic content of methanol extract of TRF was 549.75 $\pm$ 0.006mg of GAE / gm of dried extract. Total antioxidant capacity of TRF was found to be 183.62 $\pm$ 0.001mg/gm equivalent of ascorbic acid. Altogether, these results suggest that the TRF has potential antioxidant activity.

**Key words:** Antioxidant • DPPH • Total Phenolic Content • Total Antioxidant Activity • *Tagetes erecta*

### INTRODUCTION

Nature has been a resource of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Free radicals are the main reason in lipid peroxidation, highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources [1]. Free radical oxidative stress caused a wide variety of clinical disorders [2]. A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress [3]. Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements [4].

Marigold (*Tagetes erecta* L.), an ornamental plant, belongs to the Asteraceae family and is commonly known as 'genda' in Bangladesh. Numerous traditional uses of this plant have been reported. The whole plant has been used to treat bronchitis, rheumatic pain, cold and respiratory diseases and as a stimulant and muscle relaxer [5]. The flowers have been used to treat fevers, epileptic fits, scabies, liver complaints and eye diseases and have been demonstrated their astringent, carminative and stomachic effects [6]. Experimentally, *Tagetes erecta* are shown to possess antibacterial, Rhama and Madhavan

[7], analgesic [8], antioxidant [8], larvicidal activity [9], nematicidal [10], insecticidal [11], hepatoprotective [12] and molluscicidal activity [13].

The present study was therefore aimed to analyze the antioxidant activity of methanolic extracts of *T. erecta* flowers, cultivated in Bangladesh.

### MATERIAL AND METHODS

**Plant Materials:** The samples were collected from the local market of Dhaka, Bangladesh and sample was taxonomically identified by the National Herbarium of Bangladesh.

**Preparation of Plant Extract:** The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in a tight container. The dried powder material (1.2 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract.

#### Antioxidant Activity Test

**Determination of Total Phenolics:** Total phenols were determined by determined Folin-Ciocalteu reagent [14]. A dilute extract of each plant extract (0.5ml) or gallic acid (standard) was mixed with Folin-Ciocalteu reagent

(2.5ml, 1: 10 diluted with distilled water) and aqueous Sodium carbonate (2.5 ml). The mixture were allowed to stand for 20 min and the total phenols were determined by spectrophotometer at 760 nm. The standard curve was prepared using 6.25, 12.5, 25, 50, 100, 200 µgm/ ml solution of gallic acid. Total phenol values are expressed in terms of gallic acid equivalent (mg/ g) which is a common standard.

**Determination of Total Antioxidant Capacity:** Total antioxidant capacity of the extracts of *Tagetes erecta* flower was determined by the method of Prieto *et al.* [15]. An aliquot of 0.3 ml of sample solution was combined in a test tube with 3ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The test tube were incubated at 95 ° C for 90 minutes. After the sample had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank.

**Dpph (1, 1-diphenyl-2-picrylhydrazyl) Free Radical:** The antioxidant potential of the extracts were determined on the basis of its scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The DPPH assay was carried out as per the procedure outlined by Fresin [16]. 0.1 ml of different fraction of extract, at various concentration was added to 3 ml of a 0.004% methanol solution of DPPH and was allowed to stand for 30 min for the reaction occur. Thirty minutes later, the absorbance was measured at 517 nm using spectrophotometer. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation: % inhibition =  $[(A_B - A_S) / A_B] \times 100$

Where,  $A_B$  is the absorbance of the control reaction (Containing all reagents except the test compound and  $A_S$  is the absorbance of the test compound.

Ascorbic acid was used as positive control. The tests were carried out in triplicate. The extract concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph of inhibition percentage plotted against extract concentration.

**RESULTS**

**Total Phenolics Compound:** Phenolic content of the sample was calculated on the basis of the standard curve for gallic acid as shown in Table 1.1 and in Fig.1.1. The results were expressed as mg of gallic acid equivalent (GAE)/gm of dried extractives. The phenolic content of methanol extract of TRF was 549.75±.006 mg of GAE / gm of dried extract.

Table 1.1: Determination of total phenolic content of methanol extract of TRF

Sample (TRF)	GAE/gm of dried Sample Mean ±STD
Total phenolic content	549.75±.006

Table 1.2: Determination of total antioxidant capacity of methanol extract of Kathal

Sample	GAE/gm of dried Sample Mean ±STD
Total antioxidant capacity	183.62±0.001

Table 1.3:  $IC_{50}$  value of methanolic extract of TRF and standard ascorbic acid

Sample	$IC_{50}$ value(µg/ml)
Ascorbic acid	9.02
TRF	117.47

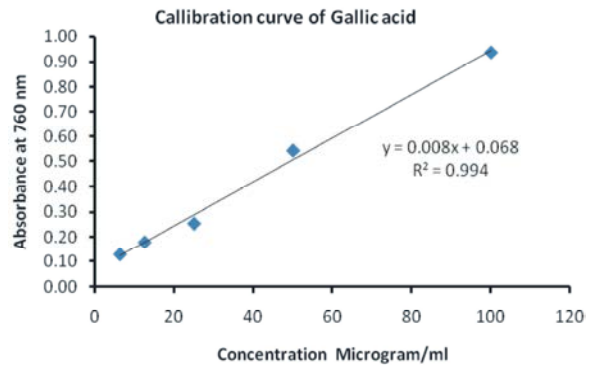


Fig 1.1: Standard curve of Gallic acid for the determination of total Phenolics

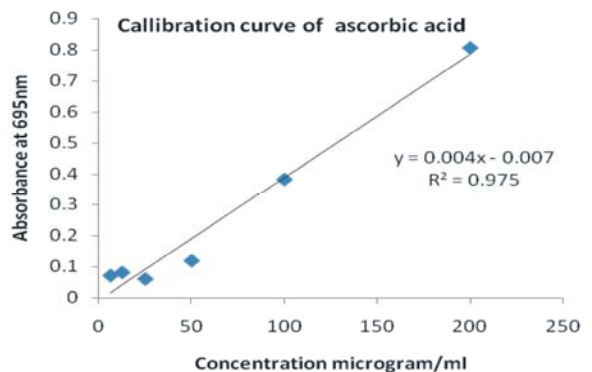


Fig 1.2: Standard curve of Ascorbic acid for the determination of total antioxidant activity

**Determination of Total Antioxidant Activity:** Total antioxidant activity of the sample was calculated on the basis of the standard curve for ascorbic acid as shown in Fig.1.2. Total antioxidant capacity of TRF is expressed as the number of equivalents of ascorbic acid. Total antioxidant capacity of ATS was found to be 183.62±0.001mg/gm equivalent of ascorbic acid (Table 1.2).

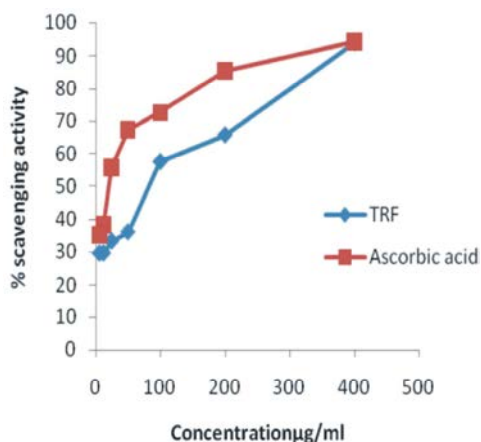


Fig 1.3: DPPH radical scavenging activity of methanolic fractions of TRF and Ascorbic acid by DPPH method

**DPPH Radical Scavenging Activity:** The percentage% scavenging of DPPH radical was found to be concentration dependent with the  $IC_{50}$  value of 117.47  $\mu\text{g/ml}$ , while  $IC_{50}$  value of standard ascorbic acid was found to be 9.02  $\mu\text{g/ml}$  (Table 1.3). The  $IC_{50}$  value was obtained from fig 1.3.

## DISCUSSION

To determine the efficacy of natural antioxidants either as pure compounds or as plant extract, a great number of in vitro methods have been developed in which antioxidant compounds act by several mechanisms. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the compounds having antioxidant property and is successfully used to quantify vitamin E in seeds [15].

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [17] and is usually used as a substrate to evaluate the antioxidant activity of a compound [18]. Based on the data obtained from this study, DPPH radical scavenging activity of *Tagetes erecta* flower extract was ( $IC_{50}$  117.47 $\mu\text{g/ml}$ ) was where  $IC_{50}$  value of the standard (9.02 $\mu\text{g/ml}$ ). It was revealed that extract of *Tagetes erecta* flower did show the proton donating ability and could serve as free radical inhibitor or scavenger. In fact, the radical scavenging capability of phenolic compounds are due to their hydrogen donating ability/number of hydroxyl groups present, which in turn is closely related both to the chemical structure and spatial conformation, that can modify the reactivity of the molecules [19].

## CONCLUSION

Based on the results of the present study, we conclude that the methanolic extract of *Tagetes erecta* possesses remarkable antioxidant activity. However, further studies are indispensable to examine underlying mechanisms of antioxidant effects and to isolate the active compounds responsible for these activities.

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