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# *IN-VITRO* ANTIOXIDANT ACTIVITY STUDIES ON THE FLOWERS OF *TAGETES ERECTA L.* (COMPOSITAE)

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## ABSTRACT

In the present study, *in Vitro* Antioxidant studies were performed on the ethanolic extract of *Tagetes erecta* flowers. During the study preliminary phytochemical analysis were carried out on ethanolic extract of flowers of *Tagetes erecta* and found the presence of Alkaloids, Flavonoids, Proteins, Steroids and tannins. For the study of *in Vitro* antioxidant activity three different assays like DPPH, reducing power and super oxide radical scavenging activity at different concentrations were used. In all the three assay, *Tagetes erecta* showed better reducing power than the standard (i.e. ascorbic acid), and super oxide anion scavenging activity and DPPH antioxidant activity showed less than standard. However, ethanolic extract of *Tagetes erecta* demonstrated antioxidant property in all the *in Vitro* models.

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## KEYWORDS

*In vitro* antioxidant activity, DPPH, Reducing power, super oxide scavenging activity, *Tagetes erecta.*

## INTRODUCTION

If free radicals are at reasonable levels, the human body produces enzymes to combat them and useful immune system and antibacterial cell activity. Excess free radicals attack DNA, a cell's genetic material, blood vessels. They are also implicated in arthritis, strokes, cataracts and degenerative health problems such as diabetes, Alzheimer's disease, retinal degeneration, ischemic dementia and aging. Fried foods, cigarette smoke, air and water pollution as well as toxins also create free radicals. When these free radicals are added to metabolic free radicals, they may lead to over exposure, which causes oxidative stress, a condition in which the body's natural defenses are overrun. Antioxidants thereby protect against oxidative damage of cells. They are found naturally in wide variety of foods and plants including many vegetables and fruits. In previous report, screened the ethanolic (50% v/v) extract of the leaves of *Tagetes erecta* for wound-healing activity on adult albino rats[1], volatile oil of flowers of *Tagetes erecta* was tested against bacteria and fungi and found 100% inhibitory effect against gram-negative bacteria and 95% inhibitory effect against fungi[2], Carotenoids are cheap source of flowers of *Tagetes erecta* extract, lutein constituents 85-95% of Carotenoid. Lutein is found as esterified to palmitic or stearic acid[3], the extracts of flowers of *Tagetes erecta* posses' significant insecticidal activity against *S oryzae*[4], in the study preliminary qualitative phytochemical analysis were undertaken and in vitro antioxidant assay were performed for further conformation of antioxidant activity.

## MATERIALS AND METHODS

***Collection of Plant material:***
The flowers of Tagetes erecta were obtained from local market, and it was authenticated by Dr. Noeline.J.Pinto. H.O.D. Dept. of Botany, St Agnes College, Mangalore-2.

***Preparation of extract:***
After collection flowers were cleaned from dust and other materials, and then it was dried under the shade for 15 days. The dried flowers were pulverized in an electric grinder. The powdered material was soaked in 90% ethanol for four days. Stirring of the mixture was done twice daily. After the fourth day, the mixture was filtered and the marc was pressed. This process was repeated 3 times. All the alcoholic fractions were combined and the ethanol was subjected for evaporation. The syrupy consistency material obtained was heated on the water bath until dry extract was obtained. Thus obtained ethanolic extract of flowers of *Tagetes erecta* were labelled and stored in the desiccator for further usage[5].

***Qualitative phytochemical analysis:***
The ethanolic extract of flowers of *Tagetes erecta* were subjected to qualitative examination for different phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Proteins, Saponins, Terpenoids and Steroids by using standard methods[6].

***Evaluation of in vitro antioxidant activity:***
The in vitro scavenging activities of the ethanol extracts of flowers of *Tagetes erecta* against different free radicals were performed. The results of the in vitro antioxidant scavenging

activities were expressed in terms of $IC_{50}$, which is the concentration of sample required to cause 50% inhibition of free radicals.

***General chemicals and instruments:***

All chemicals and solvents used in the study were of analytical grade. 2, 2-diphenyl-1-picryl hydrazyl (DPPH),methano, ethanol, trichloro acetic acid (TCA) ,Ascorbic acid and gallic acid are purchased from Himedia, India., monobasic and dibasic sodium phosphate, potassium ferri cyanide, ferric chloride, sodium carbonate, sulphuric acid, sodium phosphate, nitro blue tetrazolium, EDTA, Hydroxylamine hydrochloride is procured from Sd Fine chem. Ltd, India. UV-Vis Spectrophotometer, centrifuge, weighing balance and pH meter were the instruments used for the study.

***DPPH (2, 2 diphenyl1picryl hydrazyl) radical scavenging activity[7]:***

DPPH free radical scavenging assay was measured using the method of Molyneux P *et* 2004. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentration (1-16 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid (1%) at various concentrations (1 to16 µg/ml) was used as standard. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\textbf{DPPH Scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction and $A_{test}$ is the absorbance in the presence of the sample of the extracts. Results are expressed as $IC_{50}$, which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%.

***Reducing power assay[8]:***

The reducing power of 90% ethanolic extract of flowers of *Tagetes erecta* was determined by the method of Oyaizu 1986. Various concentrations of the extracts (1to16 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (1%) (2.5 ml).The mixture was incubated at $50^0$ C for 20 min. Aliquots of trichloroacetic acid (10%) (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.1%) (0.5ml). the absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid (1%) at various concentrations (1 to16 µg/ml) was used as standard.

Increased absorbance of the reaction mixture indicates increase in reducing power.

$$\% \text{ increase in Reducing Power} = \left[ \frac{A_{test}}{A_{blank}} - 1 \right] X\,100$$

$A_{test}$ is absorbance of test solution; $A_{blank}$ is absorbance of blank.
The antioxidant activity of the flower extract was expressed as $EC_{50}$ and compared with standard.

***Scavenging of Superoxide Radical by Alkaline DMSO Method[9]:***

Superoxide radical scavenging assay was measured using the method of Yasushira K *et al.,* 1978. To different aliquots of (100 to 800 µg/ml) sample added (50mM) sodium carbonate (1 ml), NBT (0.4 ml) and (2µM) EDTA (0.2 ml). The zero minute reading was taken at 560 nm. To the above added hydroxylamine hydrochloride (0.4 ml), incubated at $25^0$C for 15 minute. The final reading is taken at 560 nm again. The difference in the initial and final reading is taken for each sample. Gallic acid (1%) at various concentrations (100 to 800 µg/ml) was used as a control. Decreased absorbance of the reaction mixture indicates increased super oxide anion scavenging activity.
The % inhibition of superoxide anion generation was calculated using the following formula:

$$\text{SOD Scavenged (\%)} = \frac{A_{initial} - A_{test}}{A_{initial}} \times 100$$

Where $A_{initial}$ is the absorbance of the initial reaction and $A_{ttests}$ is the absorbance in the presence of the sample of the extracts

## RESULTS

The preliminary phytochemical analysis of ethanolic extract of flowers of *Tagetes erecta* indicated the presence of Alkaloids, Flavonoids, Proteins, Steroids and tannins (table no 1).

***Qualitative phytochemical analysis:***

**Table 1**
***Results of Qualitative Phytochemical analysis of ethanolic extract of flowers of Tagete erecta:***

| Tests | Ethanolic extract of flowers of *Tagetes erecta* |
|---|---|
| Alkaloids | Negative |
| Carbohydrates | Negative |
| Flavonoids | Positive |
| Steroids | Positive |
| Triterpenoids | Positive |
| Proteins | Positive |
| Saponins | Negative |
| Tannins | Positive |

***In vitro antioxidant activity studies:***
***Reducing power assay:***

The reducing power of ethanolic extract of flowers of *Tagetes erecta* increased with the increasing amount of sample. All the

concentration of ethanolic extract of *Tagetes erecta* flowers showed significant activities when compared to standard ascorbic acid. The $EC_{50}$ values were found to be 3.9 μg/ml and 9.5 μg/ml of ethanolic extract of flowers of *Tagetes erecta* and ascorbic acid respectively **(figure no 1).**

**Figure 1**
***Reducing power assay***

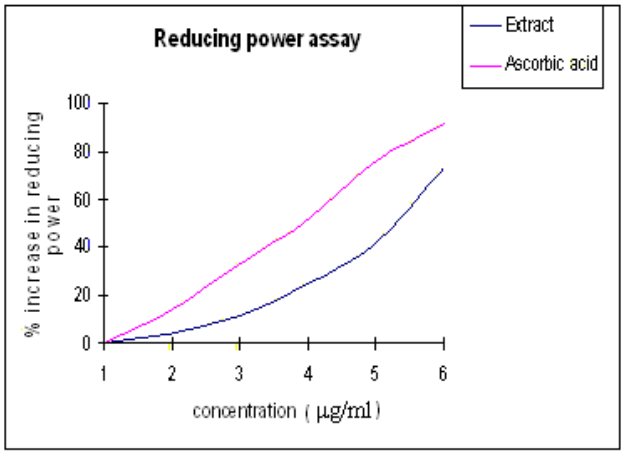



**Figure 1**

***DPPH Radical scavenging activity:***

Figure 2 illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of the soluble constituents in the ethanolic extract of flowers of *Tagetes erecta* and the standard ascorbic acid, as a reference compound, presented the highest activity at all concentrations. The $IC_{50}$ values were found to be 3.4 μg/ml and 7 μg/ml for ethanolic extract of flowers of *Tagetes erecta* and ascorbic acid respectively.

***Superoxide Radical Scavenging activity:***

The superoxide scavenging activity has been investigated using nitroblue tetrazolium (NBT). From the ethanolic extract of flowers of *Tagetes erecta* showed greater percentage of inhibition with increase in concentrations. The $IC_{50}$ values were found to be 340 μg/ml and 735 μg/ml for ethanolic extract of flowers of *Tagetes erecta* and gallic acid respectively **(figure no 3).**

**Figure 2**
***DPPH radical scavenging activity***

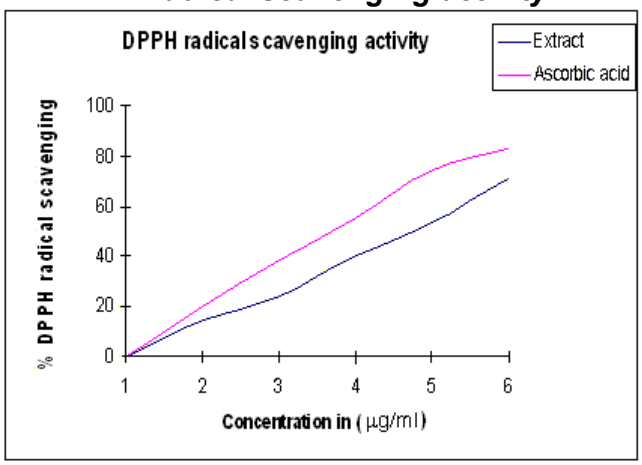
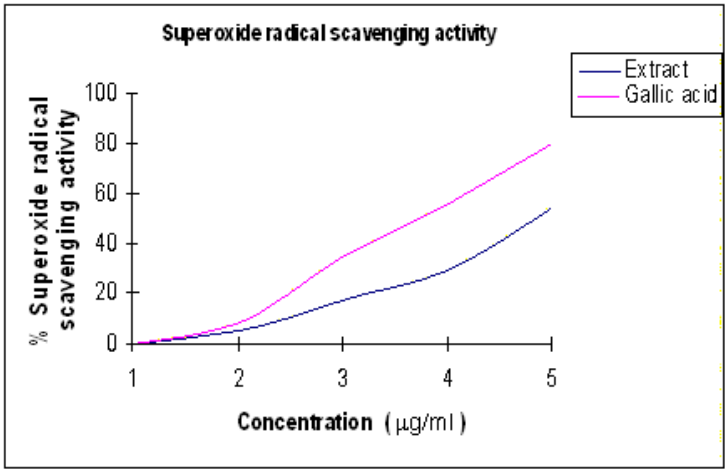


**Figure 3**
***Superoxide radical scavenging activity***

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## DISCUSSION

***Qualitative phytochemical analysis:***
The present investigation revealed that, the preliminary phytochemical analysis of the flowers of *Tagetes erecta* extracts are bestowed with the presence of several bioactive compounds *viz.* proteins, tannins, steroids, flavonoids and terpenoids in *Tagetes erecta* flower extracts which therefore encourages antioxidant studies.

***Reducing power assay:***
The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant. The reducing power of ethanolic extract of *Tagetes erecta* flowers increased with the increasing amount of sample. All the concentration of ethanolic extract of *Tagetes erecta flower* showed significant activities when compared to standard ascorbic acid. The $EC_{50}$ values were found to be 3.9 µg/ml and 9.5 µg/ml of ethanolic extract of flowers of *Tagetes erecta* and ascorbic acid respectively.

***DPPH radical scavenging activity:***
The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.figure 2 illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of the soluble constituents in the ethanolic extract of flowers of *Tagetes erecta* and the standard ascorbic acid, as a reference compound, presented the highest activity at all concentrations. The $IC_{50}$ values were found to be 3.4 µg/ml and 7 µg/ml for ethanolic extract of flowers of *Tagetes erecta* and ascorbic acid respectively.

***Superoxide radical scavenging activity:***
The superoxide scavenging activity of the medicinal herb has been investigated using nitroblue tetrazolium (NBT). From our studies we found that, the ethanolic extract of flowers of *Tagetes erecta* showed greater percentage of inhibition with increase in concentrations. The $IC_{50}$ values were found to be 340 µg/ml and 735 µg/ml for ethanolic extract of flowers of *Tagetes erecta* and gallic acid respectively.

## CONCLUSION

Substantial evidence has been generated from the three *in Vitro* antioxidant assay, the ethanolic extracts of flowers of *Tagetes erecta* exhibited strong anti-oxidant property. However, *Tagetes erecta* showed better reducing power than the standerd ie ascorbic acid. In other models ie superoxide scavenging activity and DPPH antioxidant activity less than

that of standerd. Further work may undertaken to isolate, characterize and screen the active principles from the flowers of *Tagetes erecta* that possess antioxidant properties.

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