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Antioxidant Activities and Phytochemicals of *Tagetes Erecta* Flowers As Affected by Drying Methods

Khanzadi Fatima Khattak[1,2*]

[1]Abdul Wali Khan University, Mardan, Pakistan
[2] Nuclear Institute for Food and Agriculture, P.O. Box 446, Peshawar, Pakistan

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ABSTRACT

The flowers extracts of Tagetes erecta Linn. Have extensive usages in industries like food, beverages, cosmetics, feed, fragrances and dyeing. In this study, the influence of four different drying techniques namely spray drying, shade drying, oven drying and sun drying on the levels of selected phytochemicals and scavenging activity of the flowers was studied. The data showed significant effect of drying treatments on the concentrations of the phytochemicals. Ascorbic acid, total phenolic and total flavonoid contents ranged from 57.3 to 91.0 mg/100 g, 487.0 to 634.0 mg/100 g and 137.0 to 161.3 mg/g, respectively. Highest concentration of ascorbic acid was found for shade dried sample, while lowest for sun dried sample. Similarly, the shade dried sample showed the highest phenolic content. The shade, oven and spray dried samples showed statistically similar values for flavonoid contents, whereas lowest value was observed for sun dried sample. The spray dried sample showed the highest values for both DPPH and hydrogen peroxide scavenging activities.

KEYWORDS: *Tagetes erecta;* Drying Methods; Scavenging Activities; Phytochemicals

1 INTRODUCTION

Tagetes species belong to family Asteraceae and are of high demand in dyeing, fragrances, cosmetics, beverages, food, feed and pharmaceutical industries. These plants have been widely used as folk medicine for treating various ailments and exploited as natural insecticides, insect repellents, herbicides, bactericides and fungicides (Batish *et al.*, 2007; Faizi *et al.*, 2008; Vasudevan *et al.*, 1997). The flower pigments are commercially consumed as natural colouring agents in food, feed and beverage industries (Vasudevan *et al.*, 1997).

Tagetes erecta Linn. is very popular in Pakistan as an ornamental plant. It is locally known as Genda Phul. The English name of the plant is African marigold. Different parts of this plant are used in folk medicines to cure several diseases. The whole herb is used for medicinal properties such as anthelmintic, astringent, aromatic, diuretic, sedative and antiseptic. Leaves are used to treat kidney troubles, muscular pain, ulcers, piles and applied externally to boils, wounds and carbuncles (Gopi *et al.*, 2012; Kumar *et al.*, 2006). The flowers also have numerous uses. These are used in salads, currys, tea, condiments and as flavoring and coloring agents (Kaisoon *et al.*, 2011). Medicinally, the flowers are useful for the treatment of fevers, epileptic fits, sore throats, indigestion, coughs, dysentery, scabies, liver complaints and eye diseases (Kirtikar and Basu, 1987; Ghani, 1998). Externally, the flowers are utilized to treat carbuncles, eczema, sore eyes, earaches and rheumatism. The flowers produce aromatic oil that is largely used in superior quality perfumes (Jothi, 2008; Prasad *et al.*, 2010). The flower pigments are extensively used in food industry, textile colouration and to colour egg yolks (Jothi, 2008; Vankar *et al.*, 2009).

Numerous scientific investigations showed that the plant have nematocidal, antifungal, insecticidal, herbicidal, antimalarial, antioxidant, antimutagenic, anthelmintic, antinociceptive, hepatoprotective and anti-inflammatory activities (Giri *et al.*, 2011; Gopi *et al*., 2012; Gupta and Vasudeva, 2010; Li-Wei *et al*., 2012; Shinde *et al*., 2009). Phytochemical analysis of different parts of the plant have resulted in the findings of various chemical constituents such as phenolics, alkaloids, thiophenes, steroids, terpenoids, flavonoids and carotenoids (Faizi and Naz, 2004; Li-Wei *et al*., 2012). The plant has been shown to contain lutein, quercetagetin, syringic acid, quercetin, thienyl gallate, ethyl gallate and methyl 3,5-dihydroxy-4-methoxy-benzoate (Ghani, 1998; Jothi, 2008).The petals extracts prepared by using different solvents lead to the isolation of dodecanoic acid, myristic acid, palmitic acid, stearic acid, octaeicosane-8-one and triacontane-1-ol (Prasad *et al*., 2010).

*** Corresponding Author:** Dr. Khanzadi Fatima Khattak, Abdul Wali Khan University, Mardan, Pakistan. & Nuclear Institute for Food and Agriculture, P.O. Box 446, Peshawar, Pakistan. khattakkf@yahoo.com

Recent worldwide penchant for the use of natural compounds has extremely augmented the importance of plant materials for usage in industries like pharmaceutical, nutraceutical, food, cosmetic and pesticides etc. Correspondingly, there is an increasing interest in the assurance of quality, safety and efficacy of raw materials and plant based products (WHO, 2000, 2003). Plant materials are now abundantly available in raw, semi-processed and product forms all over the world markets. Though, plant extract powders and dried materials (barks, roots, stems, seeds, flowers and leaves) show a number of advantages over the conventional fluids and unprocessed forms because they have higher amounts of active compounds which increase the therapeutic efficacy and product value (Calixto, 2000; Rocha *et al*., 2011: Runha *et al*.,2001). Raw plant materials are also difficult to transport because of their bulkiness and high transportation charges. Drying reduces the moisture contents and therefore virtuous for reducing storage and transportation costs (Calixto, 2000). It also decreases the chances of microbial contamination (Hassanain, 2011). Drying can affect both the quantity and quality of the active constituents, therefore the selection of the appropriate drying method for plant materials and their extracts remains a serious criterion (Rocha *et al*., 2011).

The present study was intended to assess the effect of different methods of drying (sun, shade, oven and spray drying) on the phenolic, flavonoid and ascorbic acid contents as well as scavenging activities of *Tagetes erecta* flowers.

2. MATERIALS AND METHODS

Collection of plant samples: *Tagetes erecta* flowers were collected from NIFA Tarnab, Peshawar, in the month of December and immediately transferred to the laboratory. The plant material was authenticated by PCSIR Labs., Peshawar, Pakistan.

Sample preparation: The flowers were cleaned from foreign particles, cut into small pieces and divided into three equal parts. One part was shade dried with natural air circulation. The average room temperature was 19 °C. The second portion was sun dried in a clean place. The third part was dried in a laboratory oven at 45 °C. Afterward, the dried plant materials were ground in a grinder (Retch Muhle-Germany) and passed through the sieves of mesh size 30. The powdered samples were packed in clear polyethylene pouches and sealed with electric Sealer PFS 300, Japan.

For spray drying, about 500 g of fresh flowers of *Tagetes erecta* were properly washed with tap water and then rinsed with distilled water. The flowers were mixed with 1.5 litre of distilled water and homogenized in a blender. Later, the solution was filtered through Whatman No. 1 filter paper. The filtrate was concentrated to 50 % in a rotary evaporator at 45 °C. The sample was then dried using a spray-drier model Pamico Lab. 01/08 (Pamico Technologies, Faisalabad, Pakistan) containing a co-current nozzle and cyclone with powder collecting bottle. The dryer has the capacity of drying up to 10 litres per hour. The operational conditions employed during spray-drying are shown as follows:

Inlet temperature: 175 ± 10 °C
Outlet temperature: 65 ± 5 °C
Atomization pressure: 1.5 bars

Preparation of extracts: The dried flower samples (50 g, each) were extracted separately using distilled water. The mixtures were kept in an orbital shaker at room temperature for 8 hours. The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted thrice with fresh distilled water and finally the extracts were combined. The pooled extracts were concentrated under reduced pressure at 45 °C using a rotary evaporator and stored at 4 °C until further processing.

Estimation of ascorbic acid: Ascorbic acid was measured by titration method (AOAC, 1984). Four grams of the dried samples of *Tagetes erecta* flowers were extracted in 3% metaphosphoric acid (50 ml), filtered and the extracts were titrated with dye solution (2,6-dichlorophenol indophenol). The end point is the appearance of light pink colour, which persists for a few minutes. Ascorbic acid content was then calculated based on the following formula:

Ascorbic acid (mg/100 g) = (titre × dye factor × volume made up × 100)/(aliquot of extract × volume of sample).

Estimation of phenolic content: The total phenolic contents of plant samples were estimated by the Folin-Ciocalteau colorimetric method (Khattak, 2012). The filtered extracts of different concentrations were taken in 10 ml glass tubes and total volumes were made to 2 ml with distilled water. 4 ml of 2% aqueous sodium carbonate solution was added to these and mixed thoroughly. Then 500 µl of Folin-Ciocalteau reagent was added to the mixtures. The mixtures were allowed to stand for 1 hour with intermittent shaking

and the absorbance of the green-blue complexes were measured at 750 nm in a spectrophotometer against blank. The standard curve was prepared using known concentrations of gallic acid. The total phenol content in the test samples were calculated from the standard curve and the results were expressed as milligram of gallic acid equivalents per 100 grams (mg/100 g) of the dry extract.

Estimation of total flavonoids: Flavonoids contents were determined by following the method of Aiyegroro and Okoh (2010). One ml of the different extracts of the plant were mixed with 3ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The mixtures were kept at room temperature for 30 minutes. The absorbance of the mixtures was measured at 420 nm with UV visible spectrophotometer. Quercetin was used as standard (0–1 mg/ml). Flavonoid contents were determined from the standard curve and expressed as quercetin equivalent (mg/g).

Determination of DPPH radical scavenging activity: The antioxidant activity of the samples was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Khattak, 2012). Various concentrations of the dried extracts were added to 2 ml of DPPH in methanol solution (100 µM) in a test tube and shaken vigorously. After incubation at 37 °C for 50 minutes in the dark, the absorbance of each solution was estimated at 517 nm. The percentage of free radical scavenging effect was calculated as follows:

$$\text{Scavenging effect (\%)} = [(A_{517}\text{control} - A_{517}\text{test sample})/A_{517}\text{control}] \text{ X } 100$$

Where A_{517}control = Absorbance of the control at 517 nm

A_{517}test sample = Absorbance of the test sample at 517 nm.

The results obtained from the radical scavenging experiments were expressed as EC_{50} values. EC_{50} value is the extract concentration at which DPPH radicals were reduced by 50% and calculated from the linear regression analysis.

Hydrogen peroxide assay: The ability of plant extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide is determined by using a spectrophotometer. Extracts (20–100 µg/ml) was added to hydrogen peroxide and mixed. After 10 minutes the absorbance was determined at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Alpha tocopherol was used as a positive control. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\text{\% Scavenged } (H_2O_2) = (A_0 - A_1 / A_0) \text{ X } 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test sample.

Data Analysis: All determinations were obtained from triplicate measurements and results were expressed as means ± standard deviations. The data were analyzed using one-way ANOVA and least significant difference tests for the mean differences. Statistical significance was declared at $p < 0.05$.

3. RESULTS AND DISCUSSION

The effect of four drying treatments namely, sun drying, shade drying, oven drying and spray drying was checked on the concentrations of flavonoids, ascorbic acid and total phenolics as well as scavenging activity using DPPH radical and hydrogen peroxide of *Tagetes erecta* flowers.

Vitamin C takes part in numerous biological functions such as collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosoamine formation, boosting of immune system and scavenging of free radicals, and thus plays a vital role in the maintenance of healthy skin, gums and blood vessels (Lee and Kader, 2000). The ascorbic acid contents of the flowers of *Tagetes erecta* were determined on dry weight basis and results are presented in Figure 1. The content of the tested samples ranged from 57.3 to 91.0 mg/100 g. The results showed that drying treatments significantly affected ($p< 0.05$) the contents of ascorbic acid in *Tagetes erecta* flowers. The shade dried sample showed the highest value (91.0 mg/100 g), followed by spray dried (90.0 mg/100 g) and oven dried (84.3 mg/100 g). The sun dried (57.3 mg/100 g) sample showed the minimum concentration of ascorbic acid. The reason might be the comparatively high rate of oxidation in the presence of direct sunlight. No information is available on the effect of drying conditions on vitamin C content of the plant. In comparison to our findings, a study conducted by Mishra *et al*. (2009) on different dehydration techniques (freeze drying, sun drying, spray drying, hot air drying and vacuum drying) showed thelowest concentration of ascorbic acid in the sun dried powder of Amla (*Emblica officinalis*).Similarly, a scientific investigation carried out by Sohail *et al*. (2011) on the physicochemical and microbiological properties of sun dried tomatoes revealed that the nutrient which is highly affected by sun drying was vitamin C. In fresh tomatoes, the ascorbic acid

content was 32.5 mg/100 g which was reduced to 24.6 mg/100 g after sun drying. Joshi and Mehta (2010) reported that after dehydration the maximum amount of vitamin C was noted in shade dried sample, as in this technique the leaves were not exposed to direct heat and air.

Plant derived phenolic compounds have received considerable attention due to their antioxidant activities and free radical scavenging abilities, which have beneficial implications in human health and usages in food and cosmetic industries (Oksana *et al*., 2012).The total phenolic content of the flower extracts were determined by Folin-Ciocalteau colorimetric method and the results are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of the extracts (Figure 2). The phenolic contents ranged from 487.0 to 634.0 mg/100 g. Previously, a study conducted by Kaisoon *et al*. (2012) on selected edible flowers showed 1107.5 mg/100 g of phenolic compounds in the dry weight of the extracts of *Tagetes erecta*. Gong et al. (2012) worked on the optimization of extraction parameters of bioactive components in the petals of *Tagetes erecta* and reported 70.01 mg GAE/g of total phenolics. It was observed that drying methods significantly ($p < 0.05$) affected the total phenolics in the aqueous extracts of the flowers. The shade dried powder had the highest phenolic content, followed by sun dried powder while spray dried sample exhibited the lowest concentration of phenolics. Eearlier research studies showed diverse results. Comparable findings were reported by Ramamoorthy and Bono (2007). Their investigations revealed lowest phenolic content in spray dried while highest in vacuum dried samples of the fruit extracts of *Morinda citrifolia*. Hossain *et al*. (2010) checked the effect of effect of drying methods (shade, freeze and oven drying) on the total phenolic content of rosemary, oregano, marjoram, sage, basil and thyme. They reported that the shade dried samples had significantly higher phenolics contents than freeze- and oven-dried samples throughout the storage period of 60 days. Chaovanalikit *et al*. (2012) revealed that spray drying can preserve total phenolic in mangosteen powder better than vacuum drying. Novakovic *et al*. (2011) investigated the influence of different drying treatments (convective, osmotic and freeze) on the antioxidant activity and phenolic content of raspberry fruits. The convective and osmotic drying showed a significant decrease in phenolic content of the fruit samples, when compared with freeze drying treatment. Hung and Duy (2012) compared the effect of freeze and oven drying on the phytochemicals of carrot, taro, tomato, red beetroot and eggplant, grown in Vietnam. Their results indicated that rise of temperature in heat-drying method significantly reduced the total phenolics.

Flavonoids are low molecular weight compounds that have a wide range of biological activities including anti-inflammatory, antibacterial, antiviral, antioxidant, antiallergic, cytotoxic, antitumour and vasodilatory etc. (Sandhar *et al*., 2011). The flavonoid contents of the powdered samples of *Tagetes erecta* were determined and presented in Figure 3. The levels of flavonoids ranged between 137.0 to 161.0 mg/g. Previously, Kaisoon *et al*. (2011) discovered that the flowers of *Tagetes erecta* had 68.9 mg/g of flavonoid content. Comparable results were described by Gong *et al*. (2012). They revealed that fermented petals of defatted *Tagetes erecta* had 109.38 mg RE/g of total flavonoid content. The present study showed that the flavonoid contents observed for shade dried (161.3 mg/g), oven dried (155.7 mg/g) and spray dried (158.0 mg/g) are statistically same ($p > 0.05$). While, significant decrease ($p < 0.05$) in flavonoid content was observed for sun dried sample (137.0 mg/g). Earlier, Ramamoorthy and Bono (2007) reported highest flavonoid content in the ethyl acetate extract of spray dried samples of *Morinda citrifolia* fruit. A scientific investigation conducted by Hung and Duy (2012) showed significantly reduced flavonoid contents in carrot, taro, tomato, red beetroot and eggplant after heat-drying method. Novakovic *et al*. (2011) reported that convective and osmotic drying resulted in significant decreases in the concentration levels of flavonoids in raspberry fruits when compared with freeze drying.

The free radical scavenging activity of the extracts of the plant was analyzed by using DPPH radical. The results obtained from these experiments are expressed as EC_{50} values (µg/ml) and presented in Figure 4. A low EC_{50} value is the sign of high DPPH scavenging activity. The EC_{50} value of *Tageteserecta* samples ranged between 13.9 to 25.3 µg/ml. Previously, Chivde *et al*. (2011) reported that the IC_{50} values was 3.4 µg/ml for the ethanolic extract of flowers of *Tagetes erecta*. Whereas, Gutierrez*et al*. (2011) worked on the oil content of *Tagetes erecta* and reported Lower DPPH scavenging activity as EC_{50} value was 71.5 µg/ml. The current data showed a strong correlation ($p < 0.05$) of drying treatments with DPPH scavenging activity. Highest DPPH scavenging activity was recorded for spray dried flowers, while lowest for sun dried samples. Exposure to direct sunlight may lead to initiation of accelerated oxidation. The results of the present study came in consistence with the findings of Ramamoorthy and Bono (2007). They noted the highest DPPH scavenging activity in spray dried extracts of *Morinda citrifolia* fruit. Likewise, Hossain *et al*. (2010) reported significantly higher antioxidant activity for shade-dried samples of rosemary, oregano, marjoram, sage, basil and thyme, when compared with freeze-dried and oven-dried samples.

Conversely, Annamalai *et al*. (2011) reported higher DPPH scavenging activity for microwave dried sample of *Cardiospermum halicacabum* as compared to that of the shade and sun dried samples. Hung and Duy (2012) compared the effect of freeze drying and oven drying on DPPH scavenging activity of carrot, taro, tomato, red beetroot and eggplant. Their results indicated significant losses in the antioxidant capacity of oven dried samples.

The Figure 5. showed the effect of sun, shade, oven and spray drying treatments on the hydrogen peroxide scavenging assay of the flowers of *Tagetes erecta*. It was clear from the data that drying treatment has significantly ($p < 0.05$) affected the hydrogen peroxide scavenging activity of *Tagetes erecta* flowers extracts. The exhibited EC_{50} values were 94.9, 96.8, 85.4 and 79.9 µg/ml for sun dried, shade dried, oven dried, and spray dried samples, respectively. There is no information available in the literature on the effect of drying methods on the scavenging activity of the plant. However, earlier Novakovic *et al*. (2011) investigated the influence of different drying treatments (convective, osmotic and freeze) on the antioxidant activity of raspberry fruits and reported that the convective and osmotic drying showed significant reductions in hydrogen peroxide scavenging activity, when compared with freeze drying.

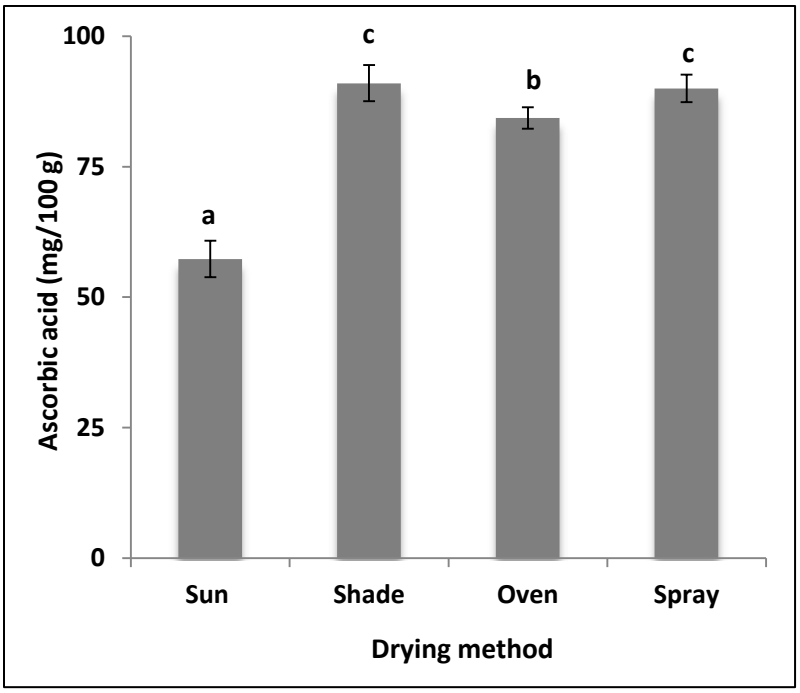

Figure 1. Effect of drying methods on the ascorbic acid content of *Tagetes erecta* flower. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

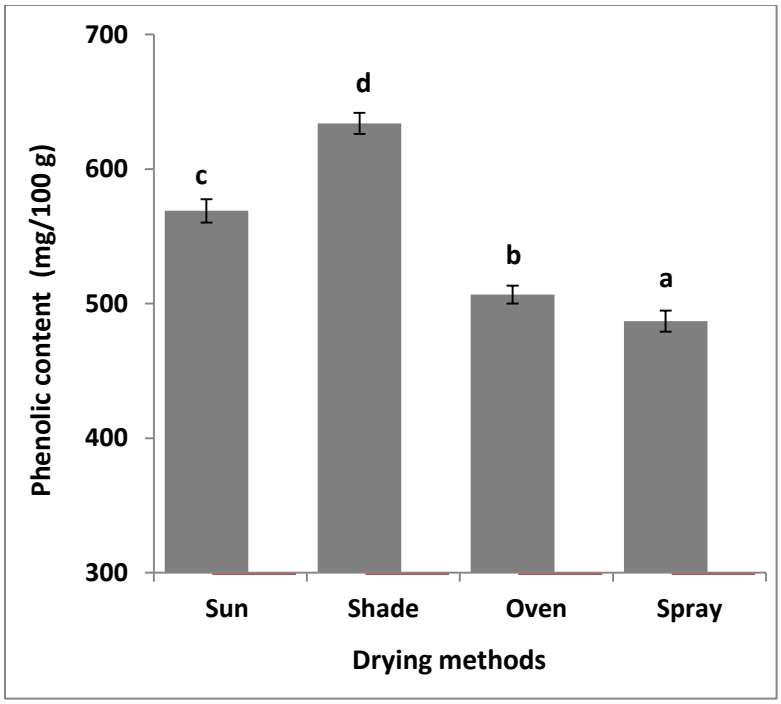

Figure 2. Effect of drying methods on the phenolic content yields of *Tagetes erecta* flower. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

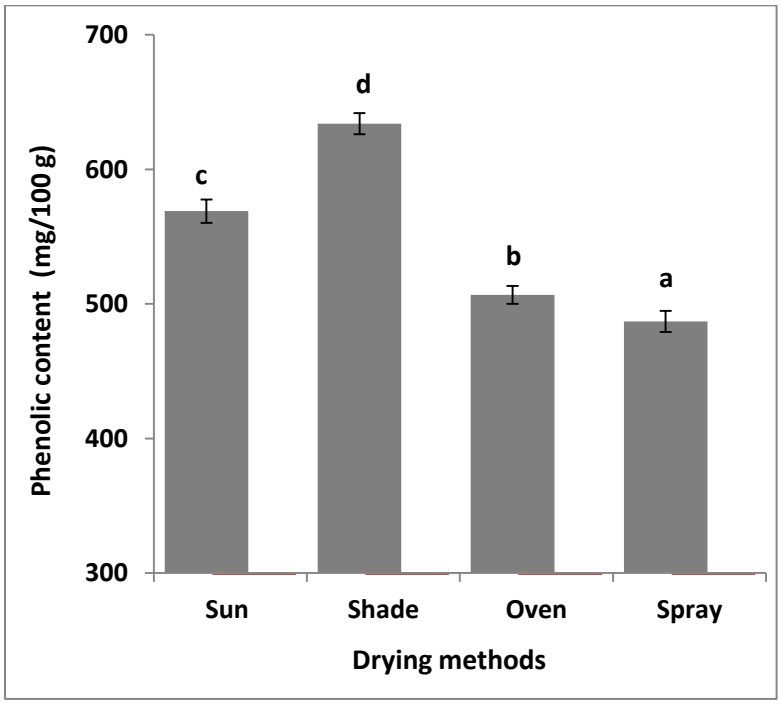

Figure 2. Effect of drying methods on the phenolic content yields of *Tagetes erecta* flower. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

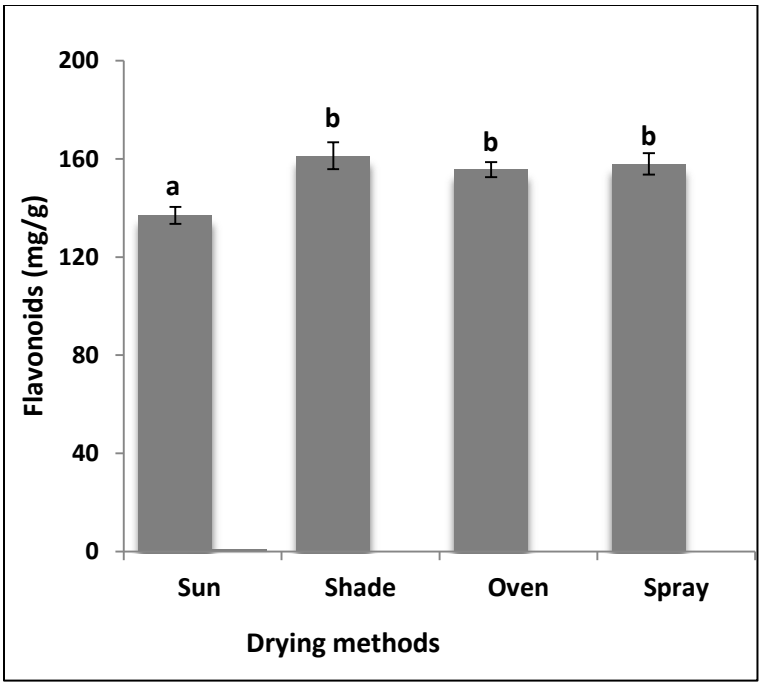

Figure 3. Effect of drying methods on the flavonoid content yields of *Tagetes erecta* flower. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$)

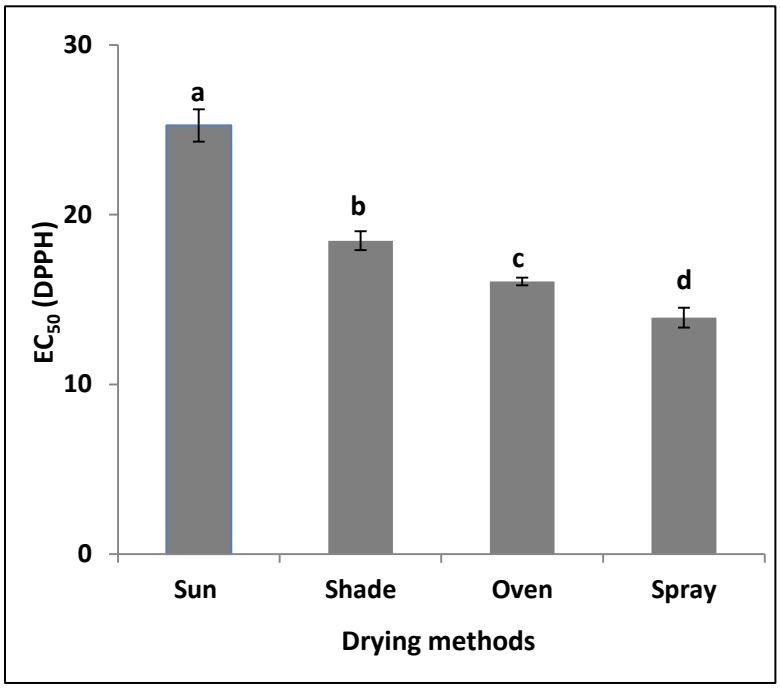

Figure 4. Effect of drying methods on the EC_{50} value (µg/ml) of the DPPH scavenging activity of *Tagetes erecta* flower. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

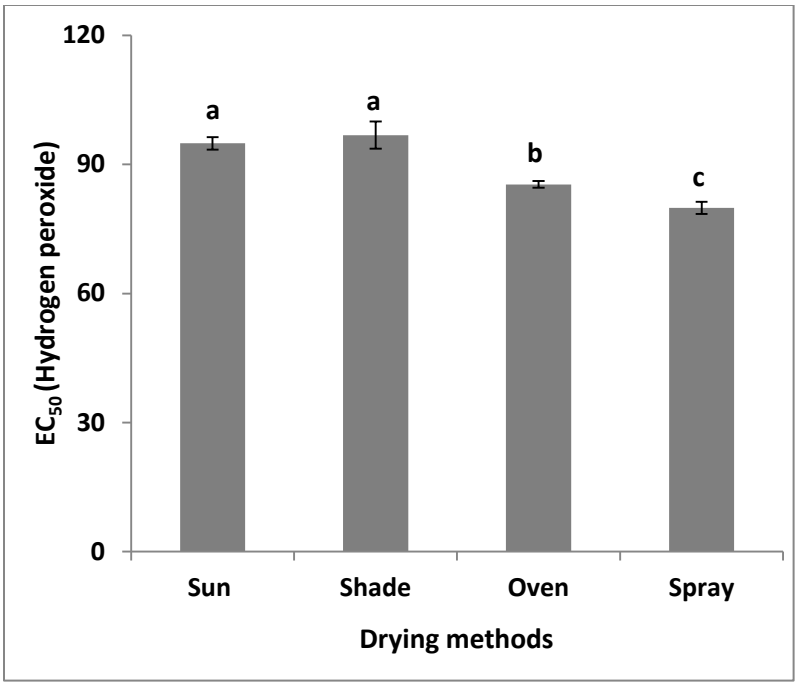

Figure 5. Effect of drying methods on the EC_{50} value (µg/ml) of the hydrogen peroxide scavenging activity of *Tagetes erecta* flower. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

4. CONCLUSION

With the increased consumer interest in plant based value-added products, there is a growing scientific emphasis in processing technologies and consequent evaluation of the influence of these techniques on the chemical constituents, biological activities and physical characteristics of the plant materials. Processing and value addition activities are imperative for getting concentrated and quality products.

The present study showed that spray drying is the best technique to have maximum scavenging activity and better retention of beneficial phytochemicals in the dried extracts of *Tagetes erecta*, followed by shade drying.

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