ISSN: 0976-3031

*Available Online at http://www.recentscientific.com*

**CODEN: IJRSFP (USA)**

*International Journal of Recent Scientific Research*
*Vol. 9, Issue, 8(B), pp. 28355-28358, August, 2018*

**International Journal of Recent Scientific Research**

**DOI: 10.24327/IJRSR**

**Research Article**

# ESTIMATION OF FREE RADICAL SCAVENGING ACTIVITY OF COSMOS LEAVES EXTRACT

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**DOI: http://dx.doi.org/10.24327/ijrsr.2018.0908.2445**

**ARTICLE INFO**

***Article History:***

Received 13th May, 2018
Received in revised form 11th June, 2018
Accepted 8th July, 2018
Published online 28th August, 2018

***Key Words:***

Ethyl acetate, *Cosmos sulphureus*, Nitric Oxide Radical, Phenolic, Flavonoid, Antioxidants.

**ABSTRACT**

Cosmos leaf (*Cosmos sulphureus*) contains various secondary metabolites such as phenolic, flavonoid, alkaloid, and essential oil; hence it needs to be fractionated to find out the phenolic compound with the greatest potential as an antioxidant. This research study was aimed to know an antioxidant potential of ethyl acetate extract from Cosmos leaves. It was extracted by Soxhlet apparatus. The crude extract was fractionated with ethyl acetate. Ethyl acetate fraction was screened for phytochemical content including identification of phenolic and flavonoids. The antioxidant activity of ethyl acetate fractions were tested qualitatively with Nitric Oxide radical assay. Phytochemical screening test showed that ethyl acetate fraction from Cosmos leaves contain phenolic and flavonoids. Concentration of phenolic content was found to be 0.164mg/ml and flavonoid concentration was found to be 0.169mg/ml. The qualitative analysis of ethyl acetate fractions from Cosmos leaves showed an antioxidant activity. The IC50 value of ethyl acetate fractions and Standard Ascorbic acid were 129.21µg/ml, 13.29µg/ml respectively. The research had shown that the ethyl acetate fraction of the Cosmos leaves have more potential for antioxidant activity, but less as compare to standard Ascorbic acid. Thus this study concluded that, the result might be helpful for treatment of oxidative stress generated diseases.



## INTRODUCTION

Medicinal plants belong to the earliest known health care products that have been used by the mankind. Over three-quarters of the world population relay on the use of traditional medicine for their primary health care needs. Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants. In the last years, interest in medicinal plants as an alternative to synthetic drugs is more and more increasing because of safety concerns, particularly against oxidative stress. Oxidative stress is potential when there is an imbalance between ROS (Reactive Oxygen Species) production and cellular antioxidant activity. Oxidative stress is implicated in over hundred human disease conditions, such as cancer, cardiovascular diseases, aging and neurological disorders. This free radical induced oxidative stress can be prevented by the intake of sufficient amount of antioxidants.[1] Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions.[2]

The premier steps to utilize the biologically active compound from plant resources are Extraction. Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant material for further separation and characterization.[3] Efficacy of plant extracts may in some cases be dependent on extraction efficiency. As the target compounds may be non-polar to polar, the suitability of the methods of extraction must be considered. Ethyl acetate is used primarily as a solvent and diluents being favoured because of low toxicity and agreeable odor. It is use in the pharmaceutical industry as an extractant. Phenolic compound present in medicinal plants have been reported to possess powerful antioxidant activity. Flavonoids are a major class of phenolic compounds present in medicinal plants and are found to have a potential role in prevention of various diseases through their antioxidant activity.[4]

Nitric Oxide is one of the few gaseous signaling molecules known and is additionally exceptional due to the fact that it is a radical gas. It is a key vertebrate biological messenger, playing a role in a variety of biological processes. Reduction of inorganic nitrate may also serve to make nitric oxide. Nitric oxide is highly reactive yet diffuses freely across membranes.

*Cosmos sulphureus* is commonly known as sulfur cosmos and yellow cosmos**.** They are from the Asteraceae family. *Cosmos* have long traditional use in Brazil and Mexico for treatment of

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malaria.This plant contains butein which has reported antioxidant and anti-inflammatory activities.

Moreover, there is no information pertaining to the antioxidant potential of *Cosmos* leaves. Based on the traditional knowledge of medicinal system, the present study was carried out to estimate the antioxidant activity of ethyl acetate solvent extract of leaves of *Cosmos sulphureus.*

***Experimental section***

The present study was done at the department of Biochemistry, Shri Shivaji college of Arts, Commerce and Science, Akola.

***Collection and Extraction of leaves***

*Cosmos* leaves were collected from nearby local area of Shegaon of Buldhana district. Leaves were washed and air dried to complete removal of soil from it and ground into a uniform powder using a grinder and stored in plastic bottles at 4$^{o}$C for future use in experiment. Extracts prepared with non-polar solvent Ethyl acetate by Soxhlet apparatus at 77.1$^{o}$C boiling point until the extract turned to colourless. Dried extract was used for analysis.

***Estimation of Total Phenolic content***

The total phenolic content in the extract was determined with Folin-ciocalteu reagent by using UV- Spectrophotometer technique. 0.2 ml sample extract was mixed with 1.0 ml of 10% (v/v) Folin-ciocalteu reagent and was vortex for 3 min followed by addition of 0.8 ml of 7.5% (w/v) Sodium carbonate. This reaction mixture was incubated for 30 min at room temperature. The absorbance was measured at 765 nm. Same procedure was carried out for Gallic acid standard curve and results were expressed as mg Gallic acid equivalent/ ml of extract.[5]

***Estimation of Total Flavonoid Content***

The amount of total flavonoid in the extract was measured spectrophotometrically. Briefly, 500 µl of each extract was mixed with 1.50 ml of 95% ethanol, 0.10 ml of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 ml of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1M) and 2.80 ml of distilled water. After incubation for 40 min, absorbance was measured at 415 nm using a spectrophotometer. To calculate the concentration of flavonoid, a calibration curve was prepared using Quercetin as standard. The flavonoid concentration is expressed as Quercetin equivalents in mg per ml of extract. All assays were carried out in triplicate.[6]

***Nitric Oxide radical assay***

The extract was prepared from an mg/ml ethanol crude extract and serially diluted with ethanol to make concentrations from 10−400 µg/ml of leaves extract and the standard Ascorbic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 ml of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 ml of the different concentrations of the ethanol extracts (10−400 µg/ml) and incubated at 25°C for 180 mins. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control sample without the extract but with an equal volume of buffer was prepared in a similar manner as was done for the test samples. The colour tubes contained ethanol extracts at the same concentrations with no sodium nitroprusside. The absorbance was measured at 546 nm using UV-Vis spectrophotometer. Ascorbic acid was used as the positive control. The percentage inhibition of the extract and standard was calculated and recorded. IC50 values denote the concentration of sample required to scavenge 50% of Nitric Oxide radical. The percentage nitrite radical scavenging activity of the ethyl acetate extracts and Ascorbic acid were calculated using the following formula:

Nitric oxide scavenging Activity % = (Abs of Control – Abs of Sample/Abs of Control) × 100

The IC50 values were calculated using linear trendline, R-squared equation in excel where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of scavenging capacity.[7]

## RESULT AND DISCUSSION

Plant polyphenols are considered as a major source of biological active substances that contributed to the antioxidantactivity.[8] Total Phenolic and Total Flavonoid Contents of *Cosmos sulphureus* leaves were evaluated according to the Folin-Ciocalteu method and Aluminium chloride assay respectively which proved to be a convenient, simple, and rapid method. Table 1 showed a significant difference in total phenolics and Flavonoids were noticed between leaves extract and standard. The phenolic contents of leaves extract were found to be 0.164 mg/ml and Flavonoids contents was found to be 0.169 mg/ml. There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of this compounds.[9]

**Table 1** Average values of Total Phenolic and Total Flavonoids contents in *Cosmos* leaves extract and Standards

| Contents | Phenolic | Flavonoid |
|---|---|---|
| STD | 0.2 | 0.2 |
| CSL EA | 0.164 | 0.169 |

(STD: Standard, CSL EA: *Cosmos sulphureus* in Ethyl acetate)

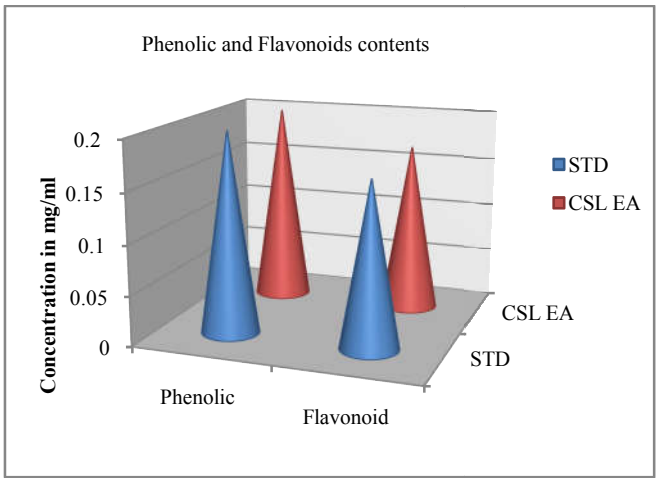



**Figure 1** Graphical representation of phenolic and Flavonoids contents in *Cosmos* leaves and Standards

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, and inhibition of platelet aggregation and regulation of cell mediated toxicity. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions.[10] Therefore, researchers have paid more attention to discovering natural antioxidants that may act as potent inhibitors of NO production in relation to the treatment of chronic inflammatory diseases.[11] The free radical scavenging activity of *Cosmos sulphureus* leaves extract was detected and compared with standard compound ascorbic acid. Figure-2 and table 2 & 3 illustrates a significant decrease in absorbance of Nitric Oxide radical due to the scavenging ability of extract and ascorbic acid. The Ethyl acetate extract showed maximum activity of 98.7% at 400 µg /ml; whereas ascorbic acid at the same concentration exhibited 80.2% inhibition.

**Table 2** Percentage Inhibition in Absorbance of Nitric Oxide radical at 546 nm with half maximal inhibitory concentration ($IC_{50}$) for Standard Ascorbic acid.

| Concentration | Absorbance | % Inhibition | IC50 in µg/ml |
|---|---|---|---|
| 10 | 0.569 | 15.7 | |
| 20 | 0.383 | 43.3 | |
| 40 | 0.302 | 55.2 | |
| 60 | 0.227 | 66.3 | 13.29 |
| 80 | 0.161 | 76.1 | |
| 100 | 0.126 | 81.4 | |
| 200 | 0.032 | 95.2 | |
| 400 | 0.012 | 98.7 | |

**Table 3** Percentage Inhibition in Absorbance of Nitric Oxide radical at 546 nm with half maximal inhibitory concentration ($IC_{50}$) for *Cosmos* leaves extract

| Concentration | Absorbance | % Inhibition | IC50 in µg/ml |
|---|---|---|---|
| 10 | 0.569 | 18.1 | |
| 20 | 0.383 | 27.9 | |
| 40 | 0.302 | 36.7 | |
| 60 | 0.227 | 42.7 | 129.21 |
| 80 | 0.161 | 50.6 | |
| 100 | 0.126 | 59.1 | |
| 200 | 0.032 | 67.8 | |
| 400 | 0.012 | 80.2 | |

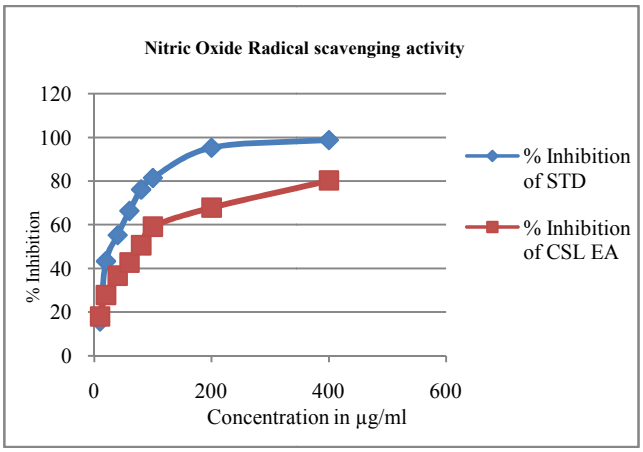
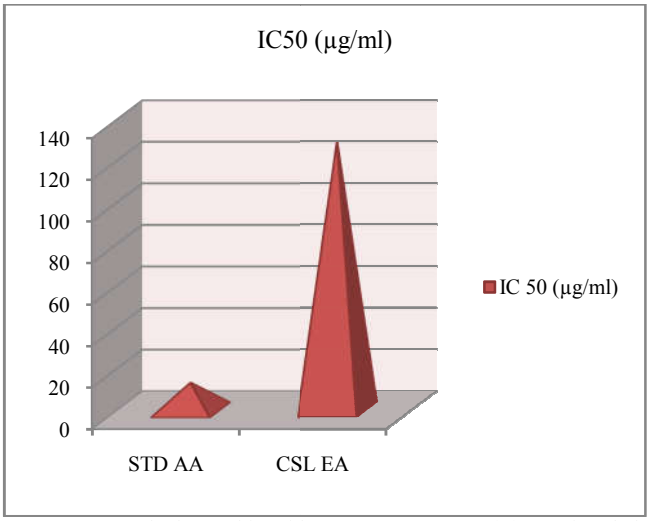



**Figure 2** Nitric oxide radical scavenging assay of Standard Ascorbic acid and *Cosmos* leaves extract




(STD AA: Standard Ascorbic acid, CSL EA: *Cosmos sulphureus* in Ethyl acetate)

**Figure 3** IC50 values of Ethyl acetate extract of *Cosmos* leaves and Standard Ascorbic acid

Above figure revealed that CSL EA (*Cosmos sulphureus* leaves in ethyl acetate) had been highest potential of antioxidant activity as it inhibited maximum nitric oxide radical scavenging activity as compare to standard Ascorbic acid (AA).

## CONCLUSION

The present study concluded that, that the CSL EA contained the highest amount of total phenolic and flavonoids content which provided the greatest scavenging activity as well as inhibited Nitric Oxide production in comparison of standard ascorbic acid. Further studies *in vivo* are required to confirm these findings. Thus, ethyl acetate extract of *Cosmos* leaves can be considered as new sources of natural antioxidants hence, it can be suggest that *Cosmos* leaves are a valuable bioactive natural product for dietary supplementation to enhance human nutrition via their phenolic composition and antioxidant activity.

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**How to cite this article:**

Shital S. Phuse and Zia. H. Khan. 2018, Estimation of Free Radical Scavenging Activity of Cosmos Leaves Extract. *Int J Recent Sci Res.* 9(8), pp. 28355-28358. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0908.2445


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