EVALUATION FOR ANTIOXIDANT POTENTIAL AND HEMOLYTIC EFFECT OF *COSMOS SULPHUREUS* FLOWER EXTRACTS

Shital S. Phuse, Zia H. Khan

Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola Maharashtra

Email: ssphuse@gmail.com

ABSTRACT

Aims and objectives: *Cosmos* is a medicinal plant which has long traditional use in Brazil and Mexico for treatment of several diseases. Hence the present attempt had been made to describe antioxidant properties and hemolytic effect of polar and non polar extracts of *Cosmos sulphureus*. Flower part of the plant was studied.

Materials and Methods: Flowers were collected, air dried and individually subjected to extraction with ethyl acetate and acetone. These extracts were assessed for phenol and flavonoid contents. Further evaluations was done by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) method and anti-hemolytic activity assay against standard Ascorbic acid and quercetin respectively.

Result and conclusion: IC_{50} values for antioxidant potential of ethyl acetate and acetone flower extracts were found to be 146.84µg/ml and 74.66µg/ml respectively. IC_{50} values for antihemolytic activity were shown to be 154.44 µg/ml of ethyl acetate and 165.6 µg/ml of acetone flower extracts respectively. The present work revealed that flower part of acetone extract possesses maximum percentage of free scavenging activity and exhibits highest antihemolytic activity. This observation revealed that extracts exhibited great potential antioxidant property and antihemolytic activity. Thus it can be consider as very useful plant to treat and control several diseases.

Key words

Cosmos sulphureus, Extracts, Antioxidants, Anti hemolysis, DPPH, Ascorbic acid, Quercetin

Introduction:

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform as important biological functions and to defend against attack. Plant derived substances have recently become of great interest owing to their versatile applications in curing many diseases.

Cosmos sulphureus is commonly known as sulfur cosmos and yellow cosmos from the Asteraceae family. Flower in an infused oil as a topical ointment. This plant contains butein which has reported antioxidant and anti-inflammatory activities. It also contains dimethoxychalcone, flavonoid, Quercetin which is useful for many health issues like fibromyalgia, eczema and antiviral properties. It is also useful on sore muscles and skin issues.

Normal body functions such as breathing physical activity and other lifestyle habits produce substances called free radicals. These are the atoms with unpaired electron which can damage the cells, tissues present in our body when come in contact; to prevent this damage, body has inbuilt defense system of antioxidants which can safely interact with free radicals and terminate the process of damage by inhibition of oxidation. (Rajani GP *et al.,* 2009).

As the target compounds may be non-polar to polar, the suitability of the methods of extraction must be considered. Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures. (Sasidarshan *et al*, 2011). A Soxhlet extraction is a procedure for extracting non-volatile and semi-volatile organic compounds from solids. Acetone dissolves many hydrophilic and lipophilic components from the plants used. It is volatile and has a low toxicity to the bioassay used. 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 (Rajani GP *et al.,* 2009).

Antioxidant activity can be monitored by DPPH radical scavenging assay. DPPH is a common abbreviation for the organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl. It is a dark-colored crystalline powder composed of stable free-radical molecules. Toxicity of active molecule is a key factor during drug designing and hemolytic activity represents a useful starting point in this regard. Hemolytic activity of any compounds is an indicator of general cytotoxicity towards normal healthy cells (Gaurav Kumar *et al.,* 2011). Many plants contain chemical substances that might have a hemolytic or anti-hemolytic effect on human erythrocytes. Therefore, many of the commonly used plants need to be evaluated for their potential hemolytic activity (Vinjamuri *et al,* 2015*).* Very little work has been done on the antioxidant activity and hemolytic effect of the above selected plant part. Hence the present attempt has been made to evaluate the antioxidant potential and antihemolytic activity of the Ethyl acetate and Acetone extract of *Cosmos sulphureus* flowers.

Material and Methods

Plant material extraction: *Cosmos sulphureus* flowers were collected from nearby local area of Shegaon of Buldhana district. Plant material was washed, air dried, ground into a uniform powder. Extracts prepared with non-polar and polar solvents such as Ethyl acetate and acetone by Soxhlet apparatus as per respective boiling point until the extract turned to colorless. Dried extracts were used for analysis.

Total Phenolic Content: Phenolic Quantification Assay has been performed based on Folin-Ciocalteu method (Kesian-Sasic *et al,* 2012).

Total Flavonoid Content: It has been done with Aluminium chloride ($AlCl_3$) colorimetric method. (Anelise Samara Nazari Formagio *et al*, 2014).

Antioxidant activity assay:

0.5 ml of the fraction solutions (10, 20, 40, 60, 80, 100, 200, and 400 µg/ml in ethanol) was added to 0.5 ml of a DPPH solution (0.1mM in ethanol). After a 30 min of reaction at room temperature, the absorbance of the solution was measured at 517 nm. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample). Ascorbic acid was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging activity (%) = [(Ac – At) /Ac] ×100, Where Ac is the absorbance of the control and At is the absorbance of test sample. (Charles Lekhya Priya *et al*, 2010) (Shisode *et al*, 2011)

The half maximal inhibitory concentration (IC_{50}) 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. IC_{50} values denote the concentration of sample required to scavenge 50% of DPPH radical.

Hemolytic effect:

Preparation of erythrocytes suspension: Five milliliters of blood was collected from a healthy individual in EDTA vacutainer. The blood was centrifuged at 3000 rpm for 10 minutes in a laboratory centrifuge. Plasma (supernatant) was discarded and the pellet was washed 3-4 times with sterile phosphate buffer saline solution (pH 7.2±0.2) by centrifugation at 3000 rpm for 10 min. The cells were resuspended in 0.9 % normal saline.

Anti-hemolytic activity assay: In vitro anti-hemolytic activity was performed by spectrophotometer method with some modifications. A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (10, 50, 100, 200 and 250 µg/ml concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 37˚C in an incubator. The mixture was centrifuged at 3000 rpm for 10 min in a laboratory centrifuge. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as minimal and maximal hemolytic controls. Each experiment was performed in triplicates at each concentration. The level of percentage hemolysis by the extracts was calculated according to the following formula:

Anti-hemolytic activity (%) = O. D. of Control – O.D. of Sample/ O. D. of Control) × 100

(Bhaskara Rao *et al*, 2011), (Pallavi Thakur *et al*, 2016), (C.U. Rajeshwari et al, 2012).

Result and discussion:

Plants and herbs contain more number of secondary metabolites as they are responsible for several biological activities in human beings and animals. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases (Shanmugapriya *et al*, 2014).

Total Phenolic and Flavonoid contents:

Total Phenolic and Total Flavonoid Contents of *Cosmos sulphureus* flowers were evaluated according to the Folin-Ciocalteu method and Aluminum chloride assay respectively. Result showed a significant difference in total phenolics and Flavonoids were noticed of flower extracts (CSF EA= 0.176 ±0.0088 mg/ml for phenolics, 0.175 ±0.0087 mg/ml for flavonoids and CSF A=0.188 ±0.0094 mg/ml for phenolics and 0.175 ±0.0087 mg/ml for flavonoids) and both the standards (0.2 mg/ml).

Antioxidant activity:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) analysis is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity. The donation of H+ to the DPPH radicals made a corresponding change from violet colour to pale yellow in the solution.

Table 1: Percentage of DPPH radical scavenging activity in Flower extracts

Concentration(µg/ml) of Dried extract	STD	CSF EA	CSF A
10	25.5±0.23	24.37±0.22	27.6±0.25
20	41.4±0.37	28.5±0.26	36.2±0.33
40	57.2±0.51	34.4±0.31	40.6±0.37
60	66.3±0.6	41.7±0.38	51.2±0.46
80	71.2±0.64	50±0.45	60.8±0.55
100	74.5±0.67	58.5±0.53	69.1±0.62
200	84.1±0.76	62.9±0.57	72.2±0.65
400	91.5±0.82	70.4±0.63	78±0.7

STD: Standard, CSF EA: *Cosmos sulphureus* Flower Ethyl acetate, CSF A: *Cosmos sulphureus* Flower Acetone

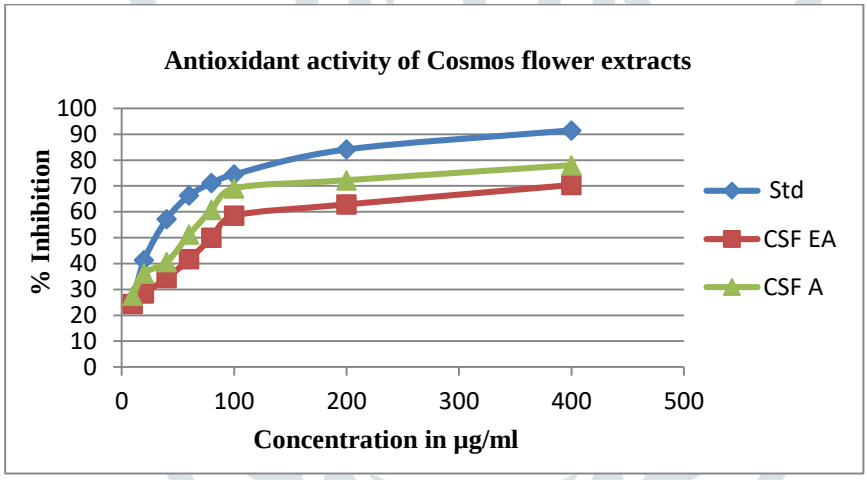

Fig 1: Graphical representation of percent scavenging activity of DPPH radical in *Cosmos sulphureus* flowers

As shown in Table 1 and figure 1, the highest % free radical scavenging potential were at conc.400µg /ml, for both the extracts. The activities increase with increasing concentration of all the extracts, hence a dose dependent manner of activity was observed for all of the extracts. The highest DPPH scavenging activity was observed in CSF A (78±0.7) as compared to CSF EA (70.4±0.63) and moderately lower than Standard ascorbic acid (91.5±0.82)

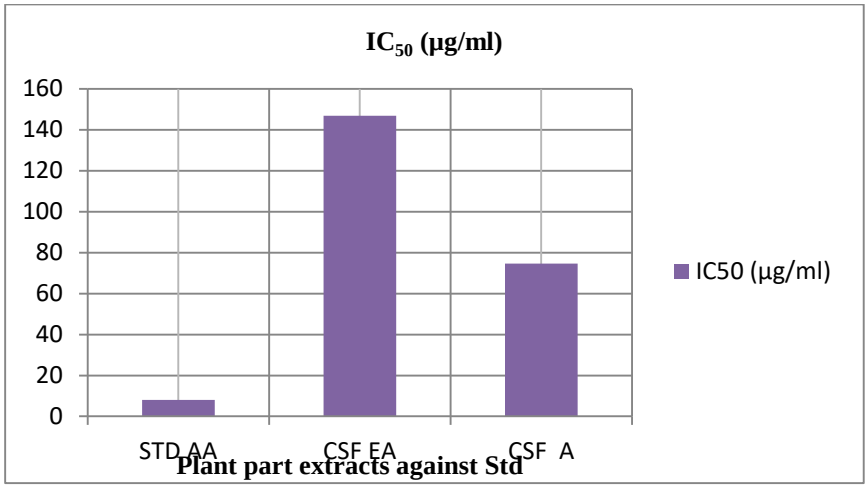

Fig 2: IC_{50} values of antioxidant activity of DPPH radical

IC_{50} values for DPPH assay of CSF (EA) and CSF (A) showed higher than IC_{50} value of Ascorbic acid standard.

Toxic effect towards human erythrocytes of the Ethyl acetate and Acetone extracts in flowers of *Cosmos sulphureus* plant were measured by Anti- Hemolytic Activity assay. The results are expressed in % inhibition of hemolysis. The results are expressed in % inhibition of hemolysis. It was found to be increased as dose increases. Both extracts exhibits very low hemolytic activity and can consider as safe for the human erythrocytes.

Table 2: % Inhibition of hemolysis in *Cosmos sulphureus* flower extracts

Concentration(µg/ml) of Dried extract	STD	CSF EA	CSF A
10	14.53±0.06	30.12±0.12	25.78±0.10
50	26.50±0.10	35.68±0.14	31.68±0.12
100	35.63±0.14	45.03±0.18	44.67±0.17
200	45.85±0.18	52.05±0.20	53.27±0.21
250	54.72±0.21	65.48±0.26	62.92±0.25

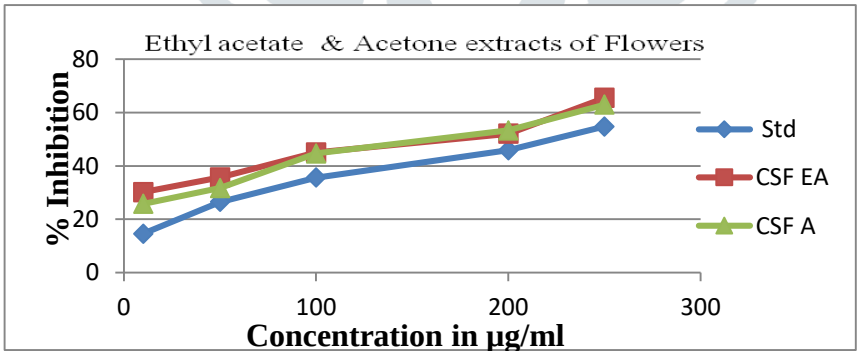

Fig 3: Percent inhibition of hemolysis in plant flower extracts

The hemolysis induced by extracts in red blood cells was concentration-dependent but all extract showed lower hemolytic effect on human red blood cell as compared to standard of all concentrations tested. As a result shown in table 2, the inhibition of hemolysis percent increased with increasing concentration of all the extracts. Flower extracts of acetone exhibited slightly lowest % inhibition of hemolysis than that of ethyl acetate extracts of plant flower. Standard Quercetin showed increased % inhibition of hemolysis from 14.53±0.06- 54.72±0.21at five different concentrations as 10- 250µg/ml.

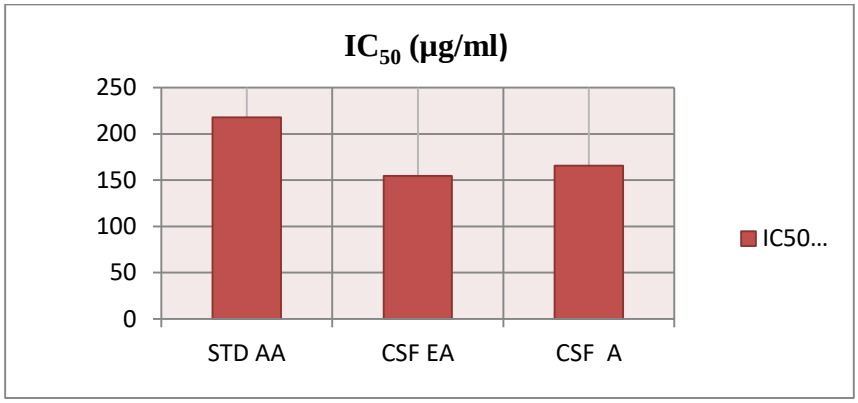

Fig 4: IC_{50} Values of percent inhibition of hemolysis in flower extracts of *Cosmos sulphureus*

IC_{50} value of standard Quercetin was found to be 217.9 µg/ml as highest anti hemolytic activity. IC_{50} values of CSF (EA) and CSF (A) showed moderate antihemolytic activity.

Conclusion: Based on the results obtained, the acetone extracts which are more polar solvent was more effective antioxidants compared to the non polar ethyl acetate extract in DPPH assay.

Antioxidant potential of extract or fraction is directly related to the scavenging of hydroxyl radical and consequently the inhibition of lipid peroxidation. Flower extracts of ethyl acetate and acetone exhibited lower hemolytic activity. Extracts or fractions used in this experiment exhibit the scavenging of DPPH radical suggesting the presence of primary antioxidants which possess anti hemolytic.

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