SCREENING OF ANTIOXIDANT FREE RADICAL SCAVENGING ASSAY AND HEMOLYSIS POTENTIAL OF METHANOLIC FLOWER EXTRACT OF Crinum asiaticum

Kempegowda M.S & Rabin Chandra Paramanik
Department of Botany, Rural degree college, Kanakapura,Bangalore rural.

Abstract:

Crinum asiaticum is commonly called Spider lily, belonging to the family Amaryllidaceae. Fresh flowers were made into fine paste with methanol solvent using pestle and mortar. The fine paste of flower was soaked in methanol solvent for 4–5 h in water bath at 50°C. After hot extraction, the extract was filtered then allowed to evaporate. The condensed extract was subjected to antioxidant free radical scavenging assay and hemolysis activity. Determination of antioxidant activity of methanolic flower extract was performed using two assays such as 2,2,-diphenyl-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) free radical scavenging assay using quercetin as standard. In both DPPH, ABTS radical scavenging assays, methanol extract showed remarkable antioxidant activity with an IC50 value of 600µg/ml. Hemolysis activity revealed positive result for methanolic flower extract tested for their hemolytic potential. From the result it could be concluded that the *C. asiaticum* flower extract owned significant antioxidant and antihemolytic potential.

Key words: *C.asiaticum,* Methanol, Antioxidants, DPPH, ABTS, Hemolysis, Quercetin, IC50, Triton X-100, Phosphate buffer saline.

INTRODUCTION

Plants are a rich source of antioxidants help to counter the detrimental effects of oxygen free radicals which arise during oxidation process in cells of our body. Antioxidants are capable of stabilizing or deactivating free radicals [1]. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation (as reducing agents), metal ion chelation (thereby eliminating potential free radicals), sparing of antioxidants (co-antioxidants) [2]. Antioxidants lower the burden of free radicals and they have the ability to take up the free radicals and reduce the free radical and make it stable. Free radicals are generally very reactive molecules possessing an unpaired electron which are produced continuously in cells either as by-products of metabolism or by leakage from mitochondrial respiration [3]. The free radicals produced in-vivo include the active oxygen species such as super-oxide radical O_2^-, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). During metabolism, oxygen consumption involves the constant generation of free radicals and reactive oxygen species (ROS). H_2O_2 and O_2 can

interact in the presence of certain transition metal ions to yield a highly- reactive oxidizing species, the hydroxyl radical (OH) [4]. The hydroxyl radical, one of the ROS, is an extremely reactive free radical formed in biological systems and reacts rapidly with molecules found in living cells, for example, sugars, lipids, DNA bases, amino acids [5]. Oxygen free radicals have been shown to be responsible for many pathological conditions [6]. Free radicals and Reactive Oxygen Species (ROS) cause DNA damage, lipid per-oxidation, protein damage. They are known to be involved in the pathogenesis of a wide variety of clinical disorders as cancer, cardiovascular diseases, inflammatory diseases, asthma and aging [7,8]. Plants are synthesizing many antioxidants which can prevent the oxidative stress. *Crinum asiaticum* is one of the important ornamental plant selected for this research study. According to literature studies leaves,bulbs of this plant have been used for various medical treatments. The leaves of this plant is used for treating various ailments like, anthracia and swelling toxicity, adenolymphitis, laryngopharyngitis, headache, arthralgia spasm and numbness, falls and bruises, fractures, venomous snake bites. Bulb part is used to treat superficial infections, swelling sores, sarcoptidosis, mammary abscess, laryngalgia, toothache, pain of rheumatic joints, injuries caused by falls, fractures, venomous snake bites [9]. Antioxidant, Anticancerous, Antimicrobial activities have been done from leaves and bulb parts of this plant. However antioxidant and hemolysis assays from flower extract of *C.asiaticum* has not been done. Hence during the present investigation methanol extract obtained from flowers of *C. asiaticum* to screen the DPPH, ABTS radical scavenging assay in detail. Hemolysis is the disruption of erythrocyte membranes, which causes the release of hemoglobin. In the hemolysis assay, human red blood cells and test materials are co-incubated in buffers at defined pHs. The percentage of red blood cell (RBC's) disruption is then quantified relative to positive control samples lysed with a detergent [10]. Therefore, plants are need to be evaluated for their potential hemolysis activity. The present analysis was carried out the determination of antihemolysis activity of flower extract of *C. asiaticum.*

MATERIALS AND METHODS

Collection of Plant Materials

Crinum asiaticum flowers were collected in July at Kengeri satellite town. The latitude, longitude, and elevation of this area is 12.9231°N, 77.4847°E, and 814.68m (2672.83 feet) respectively. The voucher specimen was deposited in the form of herbarium at the Department of Botany, St. Joseph's College (Autonomous),Bengaluru. The plant was authenticated by Dr. K P Srinath, Professor, Department of Botany, BUB. The plant material was made into herbarium.

Chemicals Used

Methanol, DPPH, ABTS, Quercetin, Erythrocyte suspension, Phosphate buffer saline, NaCl solution, Triton X-100

Preparation of Flower Extract by Decoction Method

The freshly collected flowers of *C.asiaticum* were washed under running tap water to remove dirt and weighed 20g of fresh flowers and were made into fine paste with 5-10ml of methanol using pestle and mortar.The fine paste of flowers was soaked in 75ml of methanol solvent for 4–5 h in water bath at 50°C. The extract was filtered through the Whatman No.1 filter paper. The extract was then allowed to evaporate. The condensed extract was stored in eppendorf vials at 4°C till further investigation [11].

Methodology

Antioxidant Free Radical Scavenging Assay

DPPH radical scavenging assay

The effect of given samples on DPPH radical was estimated according to the procedure described by Von Gadow *et al.* (1997). Two ml of 6 $\times 10^{-5}$ M methonolic solution of DPPH was added to 50 µl of a methonolic solution (10 mg ml^{-1}) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compound [quercetin] were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min in duration. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994) [12,13].

$$\mathbf{IP = [(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100}$$

Where $A_{C(0)}$ is the absorbance of the control at $t = 0$ min; and $A_{A(t)}$ is the absorbance of the antioxidants at $t = 16$ min.

ABTS radical scavenging assay

The free radical scavenging capacity of sample was tested using ABTS radical cation decolorization assay (Re *et al.*, 1999). ABTS dissolved in water to get 7 mM concentration. ABTS radical cation ($ABTS^{*+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark room temperature for 12-16 hrs before use. The free radical was stable for more than two days, when stored in the dark room temperature. For the study of the test samples, the $ABTS^{*+}$ solution was diluted with absolute ethanol to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30°C. Control (quercetin) reading was taken (A_0). After addition of 2.0 mL of diluted $ABTS^{*+}$ solution (A 734 nm = 0.700 (±0.02) to 20 µL of test sample, the absorbance reading was taken at 30°C exactly 6 min after initial mixing (A_t). Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times. The percentage inhibition of absorbance at 734 nm was calculated using the above formula and decrease of the absorbance between A_0 and A_t [14].

12. Von Gadow A, Joubert E, Hansmann CF. 1997. Comparison of antioxidant activity of aspalathin with that of other plant phenols of Rooibosed tea (*Aspalathon linearis*), α-tocopherol, BHT and BHA. *Journal of Agricultural and Food Chemistry,* **45:** 632-638.

13. Yen GC, Duh PD. 1994. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *Journal of Agricultural and Food Chemistry*, **42:** 629-632.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26:** 1231-1237.

15. D. Malagoli. A full-length protocol to test hemolytic activity of palytoxin on human erythrocytes. ISJ 2007; 4, 92-4.