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In vitro antioxidant and antihemolytic potential of methanolic flower extract of *Crinum asiaticum*

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Abstract
Crinum asiaticum is a member belonging to the family Amaryllidaceae. Commonly called Spider lily, Fresh flowers were made into fine paste with methanol solvent using pestle and mortar. The fine paste of flower was soaked in respective solvent for 4–5 h in water bath at 50°C. After hot extraction, the extract was filtered through the Whatman No.1 filter paper and allowed to evaporate. The condensed extract was subjected to antioxidant free radical scavenging assay and antihemolytic 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 crude flower extract tested for their antihemolytic potential. Triton X-100 and Phosphate buffer saline were used as positive and negative control respectively. From the result it could be concluded that *C. asiaticum* flower extract owned significant antioxidant and antihemolytic potential.

Keywords: *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 [1, 16]. Free radicals are unstable atoms that carries an unpaired electron, due to the insufficiency of a stable number of electrons in an outer most shell these atoms are constantly searching for another molecules to become stable. In a living system free radicals are produced during an uncontrolled mitochondrial respiration or as by products of other metabolic processes [2]. Antioxidants are capable of stabilizing or deactivating free radicals and have the ability to scavenge the free radicals and make them stable and reduce the free radical molecules in biological system. Antioxidants may exert their effect on cellular system by different mechanism including electron donation (as reducing agents), metal ion chelation (thereby eliminating potential free radicals), sparing of antioxidants (co-antioxidants) [3, 17-18]. The free radicals produced *invivo* include the active oxygenspecies such as super-oxide radical O_2^-, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). O_2^- and H_2O_2, can react strongly in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical (OH) [4]. During metabolism, constant generation of free radicals and reactive oxygen species (ROS) occurs due to the utilization of oxygen. 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*etc* [5, 20]. Oxygen free radicals have been shown to be responsible for many pathological conditions [6, 19]. 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, 21]. 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 the literature studies leaves,bulbs of this planthave been used for various medical treatments. The leaves of this plant is used for treating various ailments like, 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, sarcoidosis, mammary abscess, laryngalgia, toothache, pain of rheumatic joints, injuries caused by falls, fractures, venomous snake bites [9, 22, 24]. 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 antihemolytic 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 are 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, Bangalore university, 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 were immediately made into fine paste by using pestle and mortar with 5-10 ml of methanol. The paste thus obtained was soaked in respective solvent for 4-5 hrs in water bath at 50°C. The extract was filtered through the Whatman No.1 filter paper and allowed to evaporate. The condensed extract was stored in micro centrifuge tube at 4˚C for subjecting it to antioxidant and antihemolytic assay [11].

Methodology

Antioxidant free radical scavenging assay

DPPH radical scavenging assay

DPPH radical scavenging assay was estimated according to the procedure described by Von Gadow *et al.* (1997). The reaction mixture was contained 2ml of 6×10^{-5} M methonolic solution of DPPH and 50µl of plant crude extract at different concentrations (200-1000µg/ml). Quercetin was used as a standard (1mg/ml). The reaction mixtures were incubated at room temperature for 16 mins. Absorbance was measured at 515nm. The scavenging effect was plotted against the time and the percentage of DPPH radical scavenging ability of the sample. This experiment was performed in triplicates. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994) [12, 13, 23].

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

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

ABTS radical scavenging assay was estimated according to (Re et al., 1999) [13]. Prepared 7 mM concentration of ABTS stock solution dissolved in water. ($ABTS^{*+}$) radical cation was produced by reacting 7 mM ABTS and 2.45 mM potassium persulphate solution and stored in the dark condition for 12-16 hrs before use. The free radical was stable for more than two days when stored in the dark room temperature and then $ABTS^{*+}$ solution was diluted with absolute ethanol to get an absorbance of 0.700 (±0.02) at 734 nm. Quercetin was a standard drug (Ao). 20 µL of plant sample (at different concentrations 200-1000 µg/ml) was mixed with 2.0 mL of diluted $ABTS^{*+}$ solution and then incubated at 30˚C for 2hrs. Absorbance was measured at 734 nm and reading was taken for 6 mins after initial mixing (At).Appropriate solvent blanks were run in each assay. All determinations were carried out in triplicates. The percentage inhibition of absorbance at 734 nm was calculated using the following formula and decrease of the absorbance between A_0 and A_t [14, 25].

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

Where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$is the absorbance of the antioxidant at t = 6 min.

Antihemolytic activity

Hemolysis activity was carried out by the slight modified method of Malagoli (2007) [14]. The sheep blood sample was collected in an anticoagulant coated tube from butcher shop. The blood sample was centrifuged at 5.000 rpm for five minutes. 2% supernatant erythrocyte suspension was prepared in sterile phosphate buffer saline for hemolytic assay.

The plant sample was taken at different concentrations (0.5-10 mg/ml), to this 0.85% NaCl solution and 2% erythrocyte suspension were added. Here, sterile phosphate buffer saline and 0.1 % Triton X-100 were served as negative and positive control respectively. The reaction mixtures were incubated at room temperature for 30 mins, absorbance was measured at 540 nm. The average value was calculated from triplicate. Hemolysis percentage for each sample was calculated by dividing sample's absorbance on positive control absorbance (complete hemolysis) multiplied by100 [15].

$$\% \text{ Hemolysis} = \frac{(\text{Control OD} - \text{Sample OD})}{(\text{Control OD})} \times 100$$

Results

DPPH assay

The methanolic flower extract of *C. asiaticum* showed remarkable antioxidant activity with an IC50 value of 600µg/ml. The result of DPPH assay is tabulated in Table 1. and Graphically represented in figure 1.

Table 1: Results of 2, 2,-diphenyl-picryl hydrazyl (DPPH) antioxidant assay

SL. no.	Sample Conc. (µg/mL)	Optical density (OD)	Inhibition %
1.	0	0.654	0.00
2.	200	0.547	16.36
3.	400	0.411	37.16
4.	600	0.297	54.59
5.	800	0.189	71.10
6.	1000	0.114	82.57

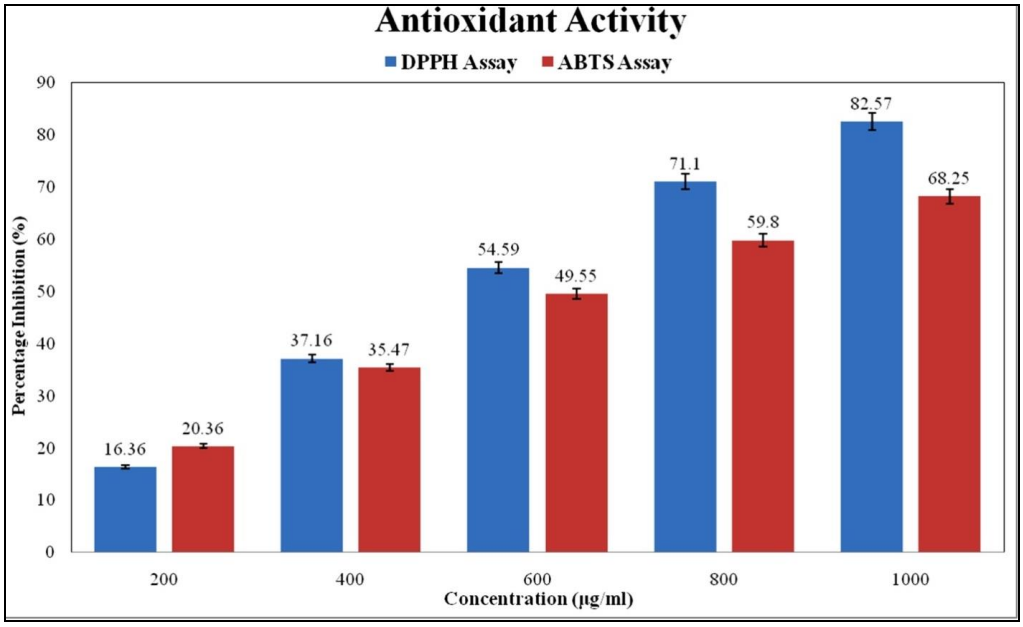

Fig. 1: Results of Antioxidant activity for methanolic flower extract of *C. asiaticum*

ABTS assay

The crude flower extract of *C. asiaticum* revealed significant antioxidant activity with an IC50 value of 600µg/ml.

The result of ABTS assay is tabulated in Table 2. And Graphically represented in figure 1.

Table 2: Results of 2,2' azinobis 3 ethyl benzothiazoline 6 sulfonic acid (ABTS) antioxidant assay

Sl. no.	Sample conc. (µg/mL)	Optical density (OD)	Inhibition %
1.	0	0.781	0.00
2.	200	0.622	20.36
3.	400	0.504	35.47
4.	600	0.394	49.55
5.	800	0.314	59.80
6.	1000	0.248	68.25

Antihemolytic activity

The flower extract of *C. asiaticum* was tested for anti-hemolytic potential demonstrated maximum hemolysis at higher concentrations (5, 10 mg/ml) compared to the lower concentrations (0.5, 1.0, 2.0, mg/ml). Triton X 100 showed 100 % hemolysis. The percentage of in hibition is tabulated in Table 3. and Graphically represented in figure 2.

Table 3: Results of Hemolysis Assay

SL. No	Sample Conc. (mg/mL)	Optical Density (OD)	Inhibition %
1.	Triton X 100	0.789	100
2.	0	0.024	3.04
3.	0.5	0.032	4.06
4.	1.0	0.048	6.08
5.	2.0	0.056	7.10
6.	5.0	0.074	9.38
7.	10	0.115	14.58

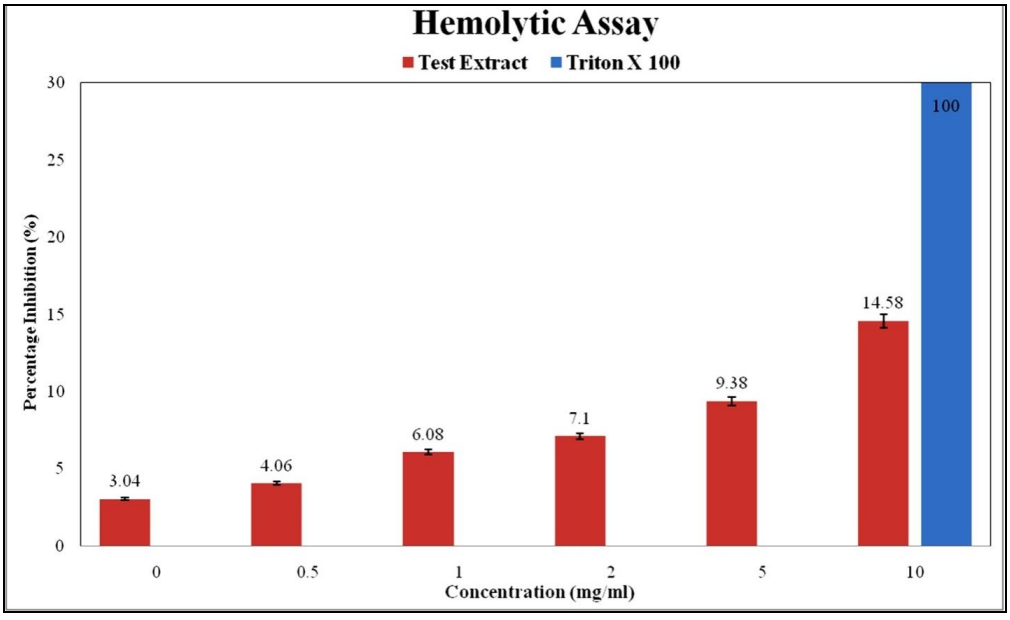

Fig 2: Results of Hemolysis assay for methanolic flower extract of *C. asiaticum*

Discussion

Crinum asiaticum flower was Selected for evaluation of antioxidant free radical scavenging assay and antihemolytic assay. DPPH [1, 1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple colour. When Antioxidants reacts with DPPH, that reduces 1,1-diphenyl-2-picryl hydrazine to colourless compound which is measured at an absorbance of 515nm. ABTS assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process which is associated with a change in absorption the relatively stable ABTS radical has a green colour and is quantified spectrophotometrically at 734nm. Parasite invasion and subsequent RBC rupture contributed to pathogenesis of hemolysis. With regard to preventing hemolysis of RBCs were treated with the plant extract. Determination of antioxidant activity of *C. asiaticum* flower extract was performed using two assays namely 2,2,-diphenyl-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) free radical scavenging assays, using quercetin as standard. In both the DPPH and ABTS radical scavenging assay, crude methanolic flower extract showed notable antioxidant activity. Antihemolytic activity revealed positive result at lower concentrations of methanolic flower extract.

Conclusion

The methanolic flower extract of *C. asiaticum* was tested for antioxidant free radical scavenging assays such as DPPH and ABTS. In both the assays methanolic flower extract exhibited potential antioxidant potential with an IC50 value of 600µg/ml. Hemolysis activity of flower extract demonstrated maximum hemolysis of erythrocytes (RBCs) at higher concentrations (5, 10 mg/ml) but minimum hemolysis of erythrocytes (RBCs) at lower concentrations (0.5, 1.0, 2.0, mg/ml). Hence, *C.asiaticum* can be used to ascertain the medicinal properties of the plant.

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Conflicts of Interest

None declared.

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References

1. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Clarendon Press, Oxford, 2nd edn, 1989, 416-419. http://www.bentham.org/cmciema1-1/vaya/vaya-ms.htm
2. De Zwart LL, Meerman JHN, Commandeur JNM, Vermeulen NPE. 'Biomarkers of free radical damage applications in experimental animals and in humans'. Free Rad.Biol.Med,1999:26(1/2):202-226.
3. Sagrario Martin Aragon, Belen Basabe, Juana M, Benedi, Angel M, Villar. Antioxidant activity of Vacciniummyrtillus L. Phytother. Res,1998:12:S104-S106.
4. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test tube assay-determination of rate constants for reaction of hydroxyl radicals. Anal.Biochem,1987:165:215-219.
5. Hemant R. Jadhav KK Bhutani. Antioxidant properties of Indian Medicinal plants. Phytother. Res,2002:16:771-773.
6. Slater TF,. 'Free radical mechanisms in tissue injury'.Biochem. J,1984:222:1-15.
7. Vani T, Rajini M, Sarkar S, Shishoo CJ. 'Antioxidant properties of the Ayurvedic formulation-Triphala and its constituents', Int.J.Pharmac,1997:35(5):313-317.
8. ^ *a b* "Plant files: Poison Bulb, Giant Crinum Lily, Spider lily. Crinum asiaticum". Dave's Garden, 2014. Retrieved 18 April
9. ^ Reference, Genetics Home. "Atypical hemolytic-uremic syndrome". Genetics Home Reference, 2020. Retrieved 2020-08-18
10. Saikia D, Baruah PS, Hasnu S, Natha S, Akhtar S, Tanti B. Phytochemical screening and antioxidant activity of leaf extracts of *Phlogacanthus thyrsiflorus* nees. -a medicinal plant of Assam, India. Biosci Discov,2018:9:237-43.
11. Von Gadow A, Joubert E, Hansmann CF. 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,1997:45:632-638.
12. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. Journal of Agricultural and Food Chemistry,1994:42:629-632.
13. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine,1999:26:1231-1237.
14. Malagoli D. A full-length protocol to test hemolytic activity of palytoxin on human erythrocytes. ISJ,2007:4:92-4
15. Mamta Tiwari, Pushpraj S Gupta, Nisha Sharma. A Preliminary Study on *In Vitro* Antioxidant and *In Vivo* Anti-Inflammatory Activity of Cissus quadrangularis Linn. Research Journal of Pharmacy and Technology,2021:14(5):2619-4. doi: 10.52711/0974-360X.2021.00461.
16. Gouri Sankar K, Venkateswarlu BS. Free radicals scavenging aptitude of Spilanthes acmella. Research Journal of Pharmacy and Technology,2021:14(5):2631-4. doi: 10.52711/0974-360X.2021.00463.
17. Mrinmoy Basak, Moksood Ahmed Laskar, Priyanka Goswami. Evaluation of Anti-oxidant and Anti-pyretic activity of fruits of Garcinia pedunculata. Research Journal of Pharmacy and Technology,2021:14(5):2635-8. doi: 10.52711/0974-360X.2021.00464.
18. Nitin A Mirgane, Arun Chandore, Vitthal Shivankar, Yashwant Gaikwad, Gurumeet C Wadhawa. Phytochemical Study and Screening of Antioxidant, Anti-inflammatory Typhonium Flagelliforme. Research Journal of Pharmacy and Technology,2021:4(5):2686-0. doi: 10.52711/0974-360X.2021.00474.
19. Ramya Kuber B. *In vitro* Antioxidant potential, Total Phenolic and Flavonoid contents in Justicia gendarussa leaf extracts. Research Journal of Pharmacy and Technology,2021:14(5):2707-3. doi: 10.52711/0974-360X.2021.00477.

20. Lavanya D, Pooja Rao, Soundarya S, Surabhi TS, Prashith Kekuda TR. Antibacterial, Antifungal and Antioxidant activity of leaf and fruit of Alangium salviifolium L. (Cornaceae). Research Journal of Pharmacy and Technology,2021:14(5):2745-9. doi: 10.52711/0974-360X.2021.00484.
21. Divya Yada, Sivakkumar T, Sudhakar M. Phytochemical Evaluation and In-vitro Antioxidant Potential of whole plant of Caralluma adscendens. Research Journal of Pharmacy and Technology,2021:14(5):2774-8. doi:10.52711/0974-360X.2021.00489.
22. Erwin, Djihan Ryn Pratiwi, Iwan Saputra, Alimuddin. Antioxidant Assay with Scavenging DPPH Radical of Artocarpus anisophyllus Miq Stem bark extracts and Chemical compositions and Toxicity Evaluation for the Most Active Fraction. Research Journal of Pharmacy and Technology,2021:14(5):2819-3. doi: 10.52711/0974-360X.2021.00497.
23. Monika Thakur, Kunjan Sharma, Sonia Mehta, Swati Rai, Isha Sharma, Astha Tripathi. Phytochemicals, Antimicrobial and Antioxidant Potential of Methanolic Extract of Berberis aristata roots. Research J. Pharm. and Tech,2020:13(12):5763-5767. doi: 10.5958/0974-360X.2020.01004.5.
24. Jitendra Gupta. Preliminary Phytochemical Investigation, Antioxidant and Antimicrobial Activity of Jasminum pubescence Leaves Extracts. Research J. Pharm. and Tech,202013(12):6073-6076. doi: 10.5958/0974-360X.2020.01058.6.