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Research Article

Assessment of hemolytic effect of *Cassia* flower extracts on human RBCs

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ABSTRACT

RBC membrane can be affected by consumption of bioactive compounds from herbs and medicinal plants. This study aimed to assess hemolytic effect of crude ethyl acetate and acetone extract from *Cassia glauca* flowers. Both the extracts of *Cassia* flowers were prepared, using Soxhlet apparatus. RBCs were washed with phosphate buffered saline and resuspended in 0.9% normal saline. These RBCs were added to different concentrations of the extracts and then incubated. After centrifugation, absorbance of the supernatant was determined by UV spectrophotometer at 540 nm. The present work shows that the fractions exhibited anti-hemolytic potential as extracts of *Cassia* flower showed very less percent of hemolysis when compared to standard quercetin. IC₅₀values were found to be 23.77µg/ml for (CF EA) and 12.50µg/ml for *Cassia* flower in acetone(CF A)against standard which was found to be 41.75µg/ml. Extracts of *Cassia* flower exhibited very low hemolytic activity. Hence, it can be considered as safe to human RBCs. In future recommend further *in vitro* and *in vivo* studies to evaluate the clinical efficacy of *Cassia glauca* extracts for treated several diseases.

Keyword: Extract, Acetone, Ethyl acetate, *Cassia glauca* flowers, Hemolytic effect, RBCs

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1. INTRODUCTION

Cassia is a genus of flowering plants in the family Fabaceae. It has been used as an anti-inflammatory, analgesic, laxative, purgative, antimicrobial and anti-ulcerative. It has also been used in traditional Brazilian medicine for the treatment of flu and cold. In Ayurvedic system of medicine, these plants were also used for the treatment of fever and headache.

Hemolysis also known by several other names, is the rupturing of red blood cells and the release of their contents into surrounding fluid. It may occur *in vivo* or *in vitro*. 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 cells¹.

Many plants contain chemical substances that might have a hemolytic or anti-hemolytic effect on human erythrocytes. Several reports indicate that the membranes of human erythrocytes from blood types have varying stability as determined from the mean corpuscular fragility. Plant extracts can positively affect the red cell membrane and many plants have serious adverse effects, which include induction of hemolytic anemia. Therefore, many of the

commonly used plants need to be evaluated for their potential hemolytic activity².



Cassia Glauca Flower

Various models exist for evaluation of membrane toxicity of surfactants including single cell models using erythrocytes, erythrocyte ghosts, or liposomes. The erythrocyte model has been widely used as it presents a direct indication of toxicity of injectable formulations as well as general indication of membrane toxicity. Another advantage of erythrocytes model is that blood is readily available and that cells are easy to isolate from the blood;

moreover, its membrane has similarities with other cell membrane³.

Erythrocytes, which are the most abundant cells in the human body, possessing desirable physiological and morphological characteristics, are exploited extensively in drug delivery. Oxidative damage to the erythrocyte membrane (lipid/protein) may be implicated in hemolysis associated with some hemoglobinopathies, oxidative drugs, transition metal excess, radiation, and deficiencies in some erythrocyte antioxidant systems⁴.

The half maximal inhibitory concentration (IC_{50}) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The values are typically expressed as molar concentration. It is commonly used as a measure of antagonist drug potency in pharmacological research. According to the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition *in vitro*.

The aim of this study was to screen the ethyl acetate and acetone extract of the flower of *Cassia glauca*, for anti hemolytic activity towards human RBCs.

2. MATERIALS AND METHODS

2.1 Collection of flower: *Cassia* flowers were collected from nearby local area of Shegaon of Buldhana district. The plant material was washed under running tap water to remove the surface pollutants and air dried under the shade. To complete removal of soil from it, material was also dried in incubator at 40° C. Dried sample was grounded into a uniform powder using a grinder. The extraction was carried out separately by the solvents ethyl acetate and acetone until the extract turned to colourless.

2.2 Preparation of erythrocytes suspension:

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

2.3 Anti-hemolytic activity assay:

In vitro hemolytic activity was performed by spectrophotometer method with some modifications following the method of Shabbir *et al.* 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 $\mu g/ml$ 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:

$$\% \text{ Hemolysis} = 100 - (\text{Sample} / \text{Control}) \times 100^1$$

Statistical Analysis:

All tests were conducted in triplicate. Data are reported as means \pm standard deviation ($n=3$). Results were analyzed statistically by using Microsoft Excel 2007.

The 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 hemolysis.

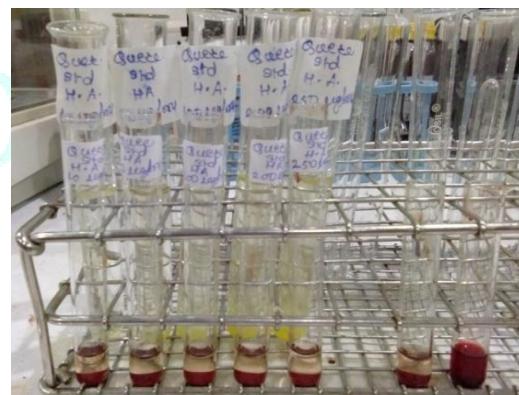
3. RESULT AND DISCUSSION

Erythrocytes are considered as major target for the free radicals owing to the presence of both high membrane concentration of polyunsaturated fatty acids (PUFA) and the oxygen transport associated with redox active hemoglobin molecules, which are potent promoters of activated oxygen species⁴.

In the present study, anti-hemolytic activity of the extracts of flowers of *Cassia glauca* was screened against normal human erythrocytes. *Cassia* flower in ethyl acetate (CF EA) and *Cassia* flower in acetone (CF A) extracts inhibited hemolysis with 54.1% and 53.1% anti-hemolytic activity of 10 $\mu g/ml$. Percent of hemolysis found to be decreased as the concentration increases. These two extracts represented the strongest efficiency.

IC_{50} values were shown to be 41.75 $\mu g/ml$ for standard, 23.77 $\mu g/ml$ for CF EA and 12.50 $\mu g/ml$ for CF A respectively. Result of IC_{50} revealed that, *Cassia* flower extracts were shown to be low percent of hemolysis at half of the concentration in comparison of standard.

Since red blood cell membrane contains high amount of polyunsaturated fatty acids, they are vulnerable to oxidative stress. In patients with β -thalassemia, sickle cells anemia and renal disease, ROS (Reactive Oxygen Species) production is higher than normal⁷. Thus, this plant might be used for preventing harmfulness in many oxidative diseases.



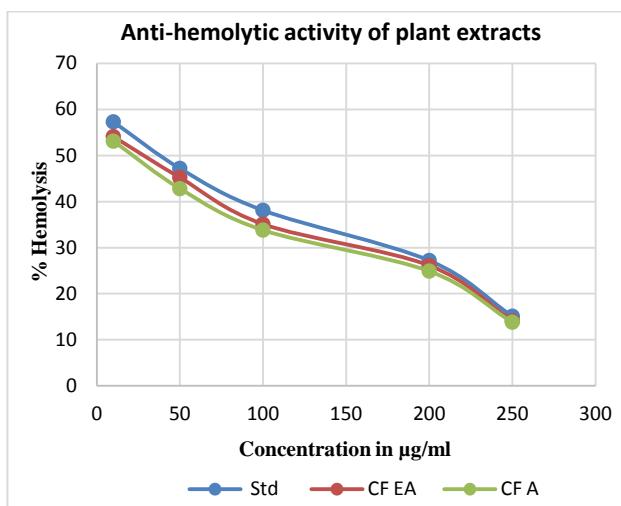
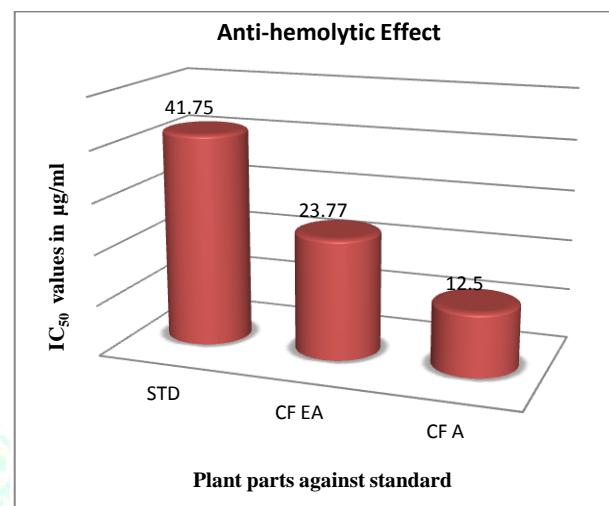
Inhibition of Hemolysis in Standard Quercetin against Control



Inhibition of Hemolysis in *Cassia* Flower extracts against Control

Table 1: Percent Hemolysis and IC_{50} Values of Standard Quercetin and *Cassia glauca* Flower extracts in different solvents

Concentration of dried extracts in $\mu\text{g}/\text{ml}$	STD	CF EA	CF A
10	57.3 \pm 0.22	54.1 \pm 0.21	53.1 \pm 0.21
50	47.2 \pm 0.18	45.2 \pm 0.18	42.8 \pm 0.17
100	38.1 \pm 0.15	35.1 \pm 0.14	33.8 \pm 0.13
200	27.2 \pm 0.11	26.1 \pm 0.10	24.9 \pm 0.10
250	15.1 \pm 0.06	14.1 \pm 0.05	13.8 \pm 0.05
IC_{50} in $\mu\text{g}/\text{ml}$	41.75	23.77	12.50

STD: Standard, CF EA: *Cassia* Flower Extract in Ethyl acetate, CF A: *Cassia* Flower Extract in Acetone.Figure 1: Graph showing percent hemolysis in Standard and *Cassia* flowersFigure 2: Graphical representation of IC_{50} values in *Cassia* flower extracts against Standard

4. CONCLUSION

It can be concluded from results that both extracts exhibited great anti-hemolytic activity as per the increased concentrations. Thus, study revealed that both the extracts from the flowers of *Cassia glauca* are protective to the human erythrocytes; lead us to propose that it can be used in pharmaceutical fields. The extracts possesses anti-hemolytic effect, for this, the further study of this plant parts can be suggested before such uses could be proposed with confidence which will make it possible to prevent the risks of a possible anemia.

Conflict of Interest: The authors report no conflict of interest.

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