IJPHT Journal
Papers Having Good Impact

International Journal of Pharmacy and Herbal Technology (Online)

Home Page: https://www.ijprdjournal.com/

Assessment of *In-Vitro* Hemolytic Activity of Senna Sulfurea

Siddharth R. Chandanshive,* Amol V. Pore, Sanjay K. Bais
Fabtech College of Pharmacy, Sangola
Tal-Sangola, Dist.-Solapur
Maharashtra -413307

ABSTRACT

Senna sulfurea is a plant species known for its traditional medicinal uses, including its potential as a laxative. This research aimed to assess in-vitro hemolytic action taken by Senna sulfurea extracts, which is crucial for evaluating its safety and pharmacological effects. Fresh blood samples were obtained combined with RBCs from healthy donors and were separated and incubated with various concentrations of Senna sulfurea extracts. Hemolysis was evaluated by determining the supernatant's absorbance to quantify hemoglobin release. The results revealed a concentration- dependent hemolytic effect of Senna sulfurea extracts, with higher concentrations inducing greater hemolysis compared to controls. Furthermore, comparison with positive and negative controls confirmed the hemolytic activity of Senna sulfurea extracts. Mechanistic studies suggested that bioactive compounds present in the extracts, such as saponins and alkaloids, may contribute to RBC lysis. These findings indicate the potential cytotoxic effects of Senna sulfurea on RBCs and raise concerns regarding its safety profile. Additional investigation is necessary to clarify the underlying mechanisms of hemolysis and to assess the safety and efficacy of Senna sulfurea extracts for therapeutic use. Overall, this study provides valuable insights into the pharmacological properties of Senna sulfurea and highlights the importance of considering its hemolytic activity in medicinal applications.

Keywords: *senna sulfurea, Organic extracts, hemolytic activity, therapeutic plants, and phytochemical screening.*

*Corresponding Author Email: - Siddharthchandanshive5101@gmail.com
Received on 06 July, 2024, Accepted 15 July, 2024

Please cite this article as: Chandanshive Siddharth et al Assessment of In-Vitro Hemolytic Activity of Senna Sulfurea
International Journal of Pharmacy And Herbal Technology 2024.

INTRODUCTION

Senna sulfurea, commonly known as "Fogfruit Senna" or "Fountain Senna", is a species of plant that is part of the Fabaceae family. It is native to tropical regions, particularly found in South America, including countries like Brazil, Paraguay, and Argentina. Senna sulfurea has a long history of traditional medicinal use, especially as a laxative. Its leaves and stems have been employed in folk medicine for the treatment of various ailments, including constipation, gastrointestinal disorders, and skin conditions. The pharmacological properties of Senna sulfurea are attributed to its rich phytochemical composition. Studies have identified several bioactive compounds present in the plant, including anthraquinones, flavonoids, saponins, alkaloids, and tannins. Numerous biological properties, including laxative, antibacterial, antioxidant, anti-inflammatory, and anticancer effects, are exhibited by these substances. However, along with these therapeutic benefits, certain bioactive constituents of Senna sulfurea may also possess cytotoxic properties, potentially affecting RBCs and resulting in hemolysis. Hemolysis is the process of RBC destruction, resulting in hemoglobin's release onto its surrounding environment. Assessing the hemolytic activity of botanical extracts is essential for understanding their safety profile and potential adverse effects. While Senna sulfurea is primarily valued for its laxative properties, there is limited research on its hemolytic activity. Therefore, the purpose of this work is to examine the hemolytic activity of Cassia sulfurea extract in vitro, which will provide valuable insights into its safety and pharmacological effects.[1]

Definition of Hemolysis

Hemolysis, taken via Greek words "hemo" (blood) and "lysis" (breaking), hemoglobin is released into the extracellular fluid or surrounding plasma when red blood cells rupture or are destroyed. It is a critical phenomenon in various physiological and pathological conditions and has significant implications in clinical medicine, pharmacology, and biology.

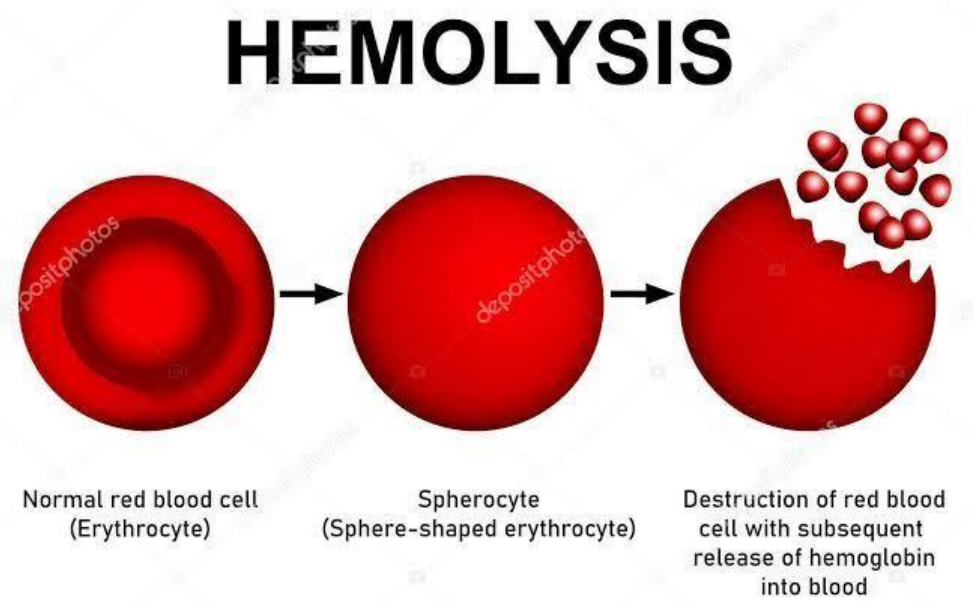


Figure No. 1: Hemolysis

Physiology of RBCs

The most common form of blood cells in vertebrates, red blood cells, or erythrocytes, are essential for the movement of carbon dioxide from tissue to the lungs and oxygen through the lungs to the tissues. Their unique biconcave shape helps them to bend when they pass through tiny capillaries and enhances the surface area available for gas exchange. RBCs they are able to carry more hemoglobin, the iron-containing protein that is responsible for binding and transporting oxygen, since they lack a nucleus and the majority of organelles.[2]

Types of Hemolysis

Hemolysis can occur through various mechanisms and can be classified into three main types: osmotic, mechanical, and chemical hemolysis.

Osmotic Hemolysis

Osmotic hemolysis occurs when RBCs are exposed to solutions with extreme osmotic gradients, whereas hypertonic solutions force water to exit the cell, causing shrinkage and crenation, solutions with a low pH induce water to get inside the cell, causing swelling and eventually rupture. Osmotic hemolysis is commonly observed in conditions such as hereditary spherocytosis and in laboratory settings during osmotic fragility tests.

Mechanical Hemolysis

Mechanical hemolysis occurs when RBCs are subjected to physical forces that disrupt their membrane integrity. This can include shear stress from turbulent blood flow in narrowed vessels, trauma from prosthetic heart valves or hemodialysis, or physical agitation during blood collection or processing. Mechanical hemolysis can result in the formation of schistocytes, fragmented RBCs with irregular shapes. [3]

Chemical Hemolysis

Chemical hemolysis occurs when RBCs are exposed to chemical agents that disrupt their membrane structure or function. These agents can include toxins, drugs, complement proteins, and certain plant extracts. Chemical hemolysis is of particular interest in pharmacology and toxicology, as it can affect drug safety and efficacy.

Clinical Significance of Hemolysis

Hemolysis can have significant clinical implications depending on its extent and underlying cause. Mild hemolysis may not produce noticeable symptoms, while severe hemolysis can lead to life-threatening complications such as anemia, jaundice, hemoglobinuria, and acute kidney injury. Hemolysis can also result in the release of intracellular contents, including potassium, LDH, and bilirubin, which can cause electrolyte imbalances, tissue damage, and organ dysfunction. [4]

Laboratory Diagnosis of Hemolysis

Laboratory diagnosis of hemolysis typically involves the evaluation of blood samples for signs of RBC destruction and the measurement of markers indicative of hemolysis. Common laboratory tests used to assess hemolysis include

Complete Blood Count (CBC)

The Complete Blood Count provide details regarding RBC count, hemoglobin concentration, hematocrit, and hemoglobin (MCH), the average corpuscular volume (MCV), and the average corpuscular haemoglobin concentration (MCHC) are examples of RBC indicators. Abnormalities in these parameters, such as decreased RBC count or hemoglobin concentration, can indicate hemolysis.

Peripheral Blood Smear

Examination of peripheral blood smears under a microscope allows for the visualization of RBC morphology and the identification of abnormal forms such as spherocytes, schistocytes, and spherocytes, indicative of hemolysis.[5]

Reticulocyte Count

The amount of immature red blood cells in the circulatory system is determined by the reticulocyte count. Raising reticulocyte numbers might be a sign of a compensatory reaction to hemolysis or bleeding-related RBC loss.

Bilirubin Levels

Bilirubin is a breakdown product of heme metabolism and is typically elevated in hemolytic conditions due to increased RBC destruction. Elevated serum bilirubin levels can result in jaundice, a yellowing of the skin and eyes.[6]

Lactate Dehydrogenase (LDH) Levels

LDH is an enzyme present in RBCs and other tissues. Elevated serum LDH levels are a nonspecific marker of tissue damage and can indicate hemolysis, as RBC destruction releases LDH into the bloodstream.

MATERIAL AND METHODOLOGY

Plant Collection and Authentication

The best time for the collection of Senna sulfurea is generally during the flowering and fruiting seasons. Senna sulfurea plant were collected from Pandharpur, Maharashtra, India. The plant was authenticated by Mr. Tebhurne R.R. M.Sc.B.Ed Botany plant physiology.

Plant Profile

Figure No.2 : Senna Sulfurea

Synonym

Cassia sulfurea

Biological source:

The biological source of Senna sulfurea is a plant with blossoms of the family Fabaceae. It is native to tropical regions of South America, particularly found in countries like Brazil, Paraguay, and Argentina.

Senna sulfurea is commonly known by various names such as "Fogfruit Senna" or "Fountain Senna". It typically grows in open areas, grasslands, and disturbed habitats. The plant is characterized by its slender stems, pinnate leaves, and clusters of bright yellow flowers. The leaves and stems of Senna sulfurea are harvested for medicinal and therapeutic purposes, including their potential laxative effects.[7]

Family

Fabaceae

Description

Stem and Leaves: Slender, erect stems reaching heights up to one meter. Pinnate leaves with numerous small leaflets arranged alternately along the stem. Leaflets have smooth borders and a lanceolate or elliptical form.

Flowers: Clusters of bright yellow flowers. Profuse blooming during the flowering season. Flowers are small, with five petals and a prominent central stamen. Arranged in terminal racemes or panicles.[8]

Fruit: Elongated seed pods produced after flowering. Cylindrical pods reach lengths of up to 10 centimeters. Pods split open along the seams when ripe, releasing the seeds.

Color: The flowers of Senna sulfurea are characterized by their bright yellow color. The petals of the flowers typically have a vibrant yellow hue, adding to the plant's ornamental appeal. In addition to the flowers, the leaves of Senna sulfurea may have a green coloration, which is common among many plant species.

Odor: Senna sulfurea is not known for having a strong or distinctive odor. The plant generally lacks any significant fragrance, particularly in comparison to aromatic plants known for their pleasant scents. While individual preferences may vary, the odor of Senna sulfurea is typically described as mild or neutral.[9]

Cultivation and Collection

Choosing a Planting Site

Pick a spot that receives full to partial sunshine and good drainage. Senna sulfurea thrives in tropical and subtropical climates and prefers sandy or loamy soils.

Propagation

Senna sulfurea can be propagated from seeds or cuttings. Seeds are often sacrificed (scratched or nicked) to improve germination rates. Plant seeds or cuttings in prepared soil beds or containers.[10]

Soil Preparation

To promote fertility and drainage, prepare the soil by removing it to a depth of 15 to 20 cm and adding organic matter, like compost or well-rotted manure.[11]

Planting

Plant seeds or cuttings at a depth of 1-2 cm in rows or spacing them according to the desired planting density. Water thoroughly after planting.

Hydrating

Particularly throughout the sprouting and growing stages, keep the soil uniformly wet. Once established, Senna sulfurea is relatively drought-tolerant but may benefit from supplemental irrigation during dry periods.[12]

Fertilization

Apply a balanced fertilizer or organic amendments as needed to promote healthy growth. Senna sulfurea generally does not need frequent fertilisation, however it could benefit from a little bit of food now and again during the growth season.[13]

Pruning

Trim back any dead or damaged foliage as needed to maintain plant health and appearance.

Harvesting

Harvest the leaves and stems of *Senna sulfurea* when they reach maturity, typically after several months of growth. Leaves can be harvested individually or by cutting back the entire plant.

Drying

Dry the harvested plant material in a well-ventilated area away from direct sunlight. Spread the leaves and stems in a single layer on screens or racks and allow them to air dry until crisp.

Storage

Once dried, store the *Senna sulfurea* leaves and stems in airtight containers away from moisture and light to preserve their potency and freshness. [14]

Chemical constituent

Bioactive compounds

Alkaloids, flavonoids, tannins, Anthraquinones and saponins.

USES

Laxative, Antimicrobial, Antioxidant, Antidiabetic.

Preparation of Plant extracts *senna sulfurea*

Once dried, grind or crush the plant material into smaller pieces using a mortar and pestle or a grinder. This makes the plant material's surface area larger, which makes the extraction process easier.

Preparation of Ethanolic Extract

The extraction preparation procedures differed slightly from those detailed in. The leaf sample was washed with ordinary water, allegedly from those detailed in the blender to be ground into powder. Various ratios are used to dry and then put into for the Soxhlet extraction procedure after 6 to 8 hours ethanol is used as gathered Utilise a muslin cloth to filter it. Centrifuge the collected extract for 15 minutes at 4,000 rpm and 25 °C. After being collected, the supernatant was kept for drying.[15]

Phytochemical Investigation: [16]

Sr No.	Name of Test	Observation	Inference
1.	**Test For Phenol:** Extract combined with two millilitres of a 2% Fecl3 solution	Green/Blue Colour	Phenol present
2.	**Test for Saponin:** After placing the extract in a test tube, water was shaken briskly.	creation of a stable foam	Saponin present
3.	**Test for Tannins:** Blended Extract with 2%Fecl3	Black Colour	Tannin present
4.	**Test For Terpenoids:** Choloroform wascombined with the extract. then, 2 millilitres of concentrated sulfuric acidwere added and gently mixed.	rusty-brown tone observed in the interphase	Terpenoids Present

5.	**Test for flavonoids:** Some droplets of the sodium hydroxide solution were added to the extract.	formation of a bright yellow hue.which, when diluted acid is added, turns colourless.	Flavonoids present
6.	**Test for glycosides:** A second tube was filled with 2 ml of concentrated sulfuric acid after the extract and 2 ml of glacier acetic acid, which contained a few droplets of 2% Fecl3, were combined.	A brown ring at the inerphase	Carbohydrate present
7.	Protein test: The extract was exposed to a few drops of concentrated nitric acid.	creation of the colour yellow.	Protein present
8.	**Test for alkaloids:** **A) Dragndroffs test:** Add one or two millilitres of the Dragendorf reagent to a few millilitres of filtrate. **B) Mayers test:** To few ml of extract ,2drops of mayers reagents. **C) Hagers test:** A few millilitres of extract or two millilitres of Hagersreagent—a saturated picricacid solution—were added. **D) Wagners test:** Add a couple of droplets from Wagner reagent (Iodine that in potassium iodide) to a few millilitersthe of the extract.	Cream coloured ppt . Yellowish coloured ppt. Reddish brown coloured ppt.	Alkaloids present Alkaloids present Alkaloids present

Table No.1: Phytochemical constituents of *senna sulfurea* exract

RESULT

The Following Formula is used to determine the proportion of hemolysis.

Percentage Hemolysis = [(At-An) / (Ac-An)] ×100

Where,

At: test sample absorbance.

An: PBS (phosphate buffered saline solution) minimum control absorbance Ac: maximal absorbance under control (distilled water)

Observation:

Sr.no	Concentration	Absorbance	Hemolysis %	Protection
1	25ml	0.028	86.06%	0.08
2	50ml	0.135	32.83%	0.032
3	75ml	0.215	6.96%	0.69
4	100ml	0.340	6.20%	-0.620

Table No.2: Absorbance of sample

Phytochemical screening is a useful technique for identifying bioactive compounds utilized in drug synthesis and for determining the molecular makeup of different plant extracts. The findings of phytochemical analysis conducted on ethanol-based extracts of the stem and leaves of *senna sulfurea* Screening of *senna sulfurea* indicated the presence of moisture, elemental components like carbon, hydrogen, nitrogen, and sulfur, but not reducing sugar. The plant's medicinal potential is demonstrated by the presence of these compounds. Since there is reducing sugar in the plant's stem or leaf, tests can be performed to determine the different phenolic compounds, amino acids, and therapeutic value of the plant.

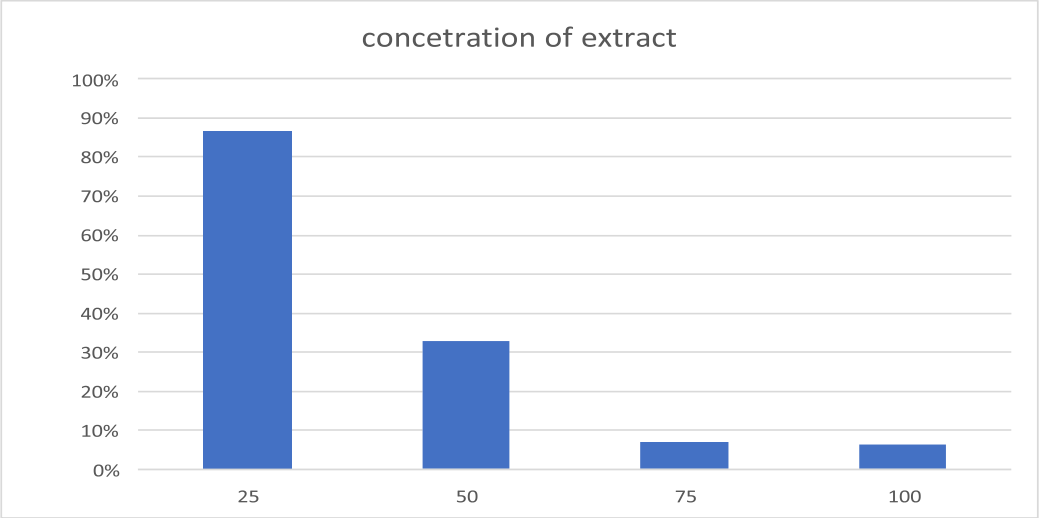
Sr.no.	Constituents	Observation
	Ethanolic Extract	
1	Saponins	+
2	Phenols	+
3	Tannins	+
4	Terpenoids	+
5	Flavonoids	+
6	Proteins	+
7	Carbohydrates	+
8.	Alkaloids	+

Table No.3: Result of Preliminary Phytochemical Screening of *senna sulfurea*

(-) denotes the lack of a compound

(+) shows that the Compound is present.

The ability of the ethanolic extract of *senna sulfurea* to hemolyze was evaluated usingerythrocytes, and the results were given as a hemolysis percentage. Table No. 4 of the results revealed that the extracts under investigation had an effect on hemolysis. The ethanolic extract causes the least amount of hemolytic activity when it is concentrated at 100 g/ml; concentrations of 75 g/ml, 6.96%, 50 g/ml, and 32.83% cause the least amount of hemolytic activity, while 25 g/ml, or 86.6%, causes the most. The results additionally indicated that the degree of hemolysis is dependent on the extract concentration. The following categories can be used to group the hemolytic effects of the different test extracts: Value range: 25 g/ml to 100 g/ml, with 50 g/ml to 75 g/ml. The phytochemical components found in medicinal plants, such as flavonoids, phenol, saponin, and glycosides, are abundant and have specific pharmacological effects on the human body. among others, terpenoids and alkaloids. Hemolytic activity of any substance indicates that it is generally cytotoxic to healthy, normal cells. The four extracts' medium hemolytic reaction reveals their medium cytotoxicity against human erythrocytes. This assay helps identify whether or not cytotoxic action in linked to actual membrane damage.



Concentration of extract

Graph No.1: Graphical Presentation of Hemolysis %

Sr.no	Concentration	Hemolysis%
1	25	86.6%
2	50	32.83%
3	75	6.96%
4	100	6.20%

Table No.4: Percentage of Hemolysis

DISCUSSION

The assessment of the in-vitro hemolytic activity of *Senna sulfurea* yielded important insights into its potential cytotoxic effects on red blood cells (RBCs). The study demonstrated significant hemolytic activity of *Senna sulfurea* extracts in the in-vitro assay. This indicates that the plant contains compounds capable of disrupting RBC membranes and inducing hemolysis. The observed hemolytic activity aligns with the traditional use of Senna species as laxatives, where controlled hemolysis may contribute to their therapeutic effects by promoting bowel movement.

However, the hemolytic activity of *Senna sulfurea* raises safety concerns, especially regarding its potential cytotoxic effects. Excessive hemolysis can lead to anemia, hemoglobinuria, and other adverse effects, particularly in vulnerable populations. Therefore, caution is warranted in the therapeutic use of *Senna sulfurea* extracts, and further research is needed to establish safe dosage regimens and monitor potential sideeffects.

The methodology employed in the study, including the in-vitro assay used to evaluate hemolytic activity, was critical for interpreting the results accurately. However it's important to recognize several restrictions and possible confounding variables that could have affected the results. Variations in extraction methods, assay conditions, or sample purity could impact the reliability and reproducibility of the results.

Exploring the underlying mechanisms of hemolysis induced by *Senna sulfurea* extracts is crucial for understanding their pharmacological effects.

Anthraquinones, saponins, and alkaloids are examples of bioactive substances that may be involved in the plant's hemolytic action. To fully understand these chemicals' precise mode of action and how they interact with RBC membranes, more research is required.

CONCLUSION

The in-vitro assessment of the hemolytic activity of *Senna sulfurea* extract revealed a concentration-dependent effect on erythrocytes. At lower concentrations, the extract exhibited minimal hemolysis, indicating its potential safety at these levels. However, at higher concentrations, a significant increase in hemolytic activity was observed, suggesting a cytotoxic effect. These findings highlight the importance of dosage in thetherapeutic application of *Senna sulfurea*, as excessive amounts could lead to hemolytic side effects The report emphasises the necessity of more research into the specific bioactive compounds responsible for this activity and their mechanisms of action. Additionally, to fully comprehend *Senna sulfurea*'s safety record and therapeutic potential, in vivo investigations are crucial. Overall, while *Senna sulfurea* shows promise as a medicinal plant, careful consideration of its hemolytic propertiesis crucial for its safe use in clinical settings.

Overall, the assessment of the in-vitro hemolytic activity of *Senna sulfurea* provides valuable insights into its biological effects and therapeutic potential. Further research exploring its mechanisms of action and in-vivo effects is warranted to fully elucidate its pharmacological relevance.

REFERENCES

1. Prachi P. Gaikwad, Amol V. Pore, Assessment of in-vitro hemolytic activity of epiphyllum oxypetalum, World Journal of Pharmaceutical Research, 2023,3(2): 323-334
2. M. Rahman, M. A. Alam, M. N. Uddin, Comparative study of the antioxidant activity of methanolic extracts from different parts of Senna sulfurea, Journal of Coastal Life Medicine,2014, 2(1): 45–49.
3. K. M. Abas, K. Z. Al-Shujairi, A. B. Mohd Noor, Medicinal plants used for traditional medicine in the Kudat district of Sabah, Malaysia, Journal of Ethnobiology and Ethnomedicine,2019, 15(1): 15-23.
4. R. K. Kurup, S. R. Ansari, A. Patil, Extraction of active constituents of Senna tora L. using different solvents and its antibacterial activity, Pharmacognosy Journal,2019, 11(1):93-97.
5. T. M. Magwaza, J. L. Ndhlala, A. Ncube, Optimization of extraction conditions and preliminary characterization of phenolic constituents from the aerial parts of Psoralea pinnata L. South African Journal of Botany,2017, 110(4): 301-307.
6. B. F. Rodak, G. A. Fritsma, E. M. Keohane, Hemolytic Anemia, In Hematology: Clinical Principles and Applications Saunders,2013,4(7):386-423
7. G. J. Kato, M. H. Steinberg, M. T. Gladwin Intravascular Hemolysis and the Pathophysiology of Sickle Cell Disease, Journal of Clinical Investigation, 2017,127(3): 750-760.
8. Amol V. Pore, Sanjay K. Bais, Vaishnavi M. Kadam Aspects of Collection and Cultivation of Aromatic Plants and Medicinal Plants, IJPHT Journal, 2024,2(1): 967-978
9. Sanjay K Bais, Amol V. Pore, Shreya Kamavaram, A comprehensive review on commercial collection and cultivation aspects of medical and aromatic plants,IJPHT Journal 2023, 1(3): 297-308
10. J. C. Souza, D. F. Lopes, L. R. Souza, In-vitro hemolytic activity of Bauhinia cheilantha Steud extracts, Journal of Applied Pharmaceutical Science,2018, 8(4): 25-29.

11. B. J. Bain, Diagnosis from the Blood Smear Practical Haematology Elsevier Health Sciences,2005,10(2):25-57
12. G. T. Vuong, J. X. Nguyen, T. T. Nguyen, Standardization of Cassia fistula Linn. Extract and Evaluation of Its Hemolytic and In-Vitro Anthelmintic Activity, Journal of Pharmaceutical Sciences and Research, 2019,11(5): 1705-1711.
13. G. Lippi, M. Plebani, E. J. Favaloro, Hemolysis Index: Quality Indicator orClinical Artifact Clinical Chemistry and Laboratory Medicine, 2017,55(6): 940- 944
14. R. A. da Silva, R. S. Nunes, A. P. de Andrade, Hemolytic activity of Senna velutina leaves and stems: involvement of cell membrane structural alterations, Revista Brasileira de Farmacognosia,2018, 28(4): 442–446.
15. Schapkaitz E, Hemolysis and Hyperbilirubinemia: How to Interpret These Laboratory Tests, South African Family Practice,2014, 56(2): 92-95.
16. Dr. K.R. Khandelwal, Dr. Varuna Shetti, Practical Pharmacognosy Textbook of Nirali Prakashan, 2020,31:25.1-25.9.