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ASSESSMENT OF IN-VITRO HEMOLYTIC ACTIVITY OF GUILANDINA BONDUC.L

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ABSTRACT

Gulandina bonduc, also known as Caesalpinia bonduc or Gray Nicker, is a plant species with a rich history of traditional medicinal use. This review focuses on the in vitro hemolytic activity research work conducted on Gulandina bonduc. Hemolysis is the process of red blood cell destruction, which can have implications for various health conditions.Research studies have investigated the potential hemolytic activity of Gulandina bonduc extracts and isolated compounds. The findings suggest that certain components of the plant may exhibit hemolytic properties in vitro. These studies have utilized red blood cell models to assess the impact of Gulandina bonduc on cell membrane integrity and hemoglobin release.

Understanding the hemolytic activity of Gulandina bonduc is crucial for evaluating its safety profile and potential therapeutic applications. Further research is needed to elucidate the specific bioactive compounds responsible for the observed hemolytic effects and to determine the mechanisms underlying these activities.

Overall, investigating the in vitro hemolytic activity of Gulandina bonduc contributes to the comprehensive understanding of its pharmacological properties and aids in assessing its suitability for therapeutic use. Additional studies are warranted to explore the implications of these findings in the context of human health and disease management.

Keywords: *Caesalpinia bonduc, Gray Nicker, hemolytic activity, red blood cells, bioactive compounds, pharmacological properties, therapeutic use*

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INTRODUCTION

Gulandina bonduc, also known as Caesalpinia bonduc or Gray Nicker, is a medicinal plant that has been traditionally used in various cultures for its therapeutic properties. One of the notable pharmacological activities of Gulandina bonduc is its antihemolytic activity, which involves the protection of red blood cells from damage and hemoglobin release inhibition. This activity is of interest due to its potential applications in the treatment of various hemolytic disorders and conditions.Research on the antihemolytic activity of Gulandina bonduc has gained attention in recent years, as scientists have sought to explore the bioactive compounds present in the plant and their effects on red blood cells. In vitro studies have been conducted to investigate the mechanisms underlying the antihemolytic activity of Gulandina bonduc and to assess its potential therapeutic benefits.[1]

Definition of heamolysis

Heamolysis is the process of red blood cell destruction, causing hemoglobin to be released into the surrounding fluid. It can be induced by various factors such as toxins, drugs, and certain plant extracts. Heamolysis can have adverse effects on health, including anemia and organ damage.[2]

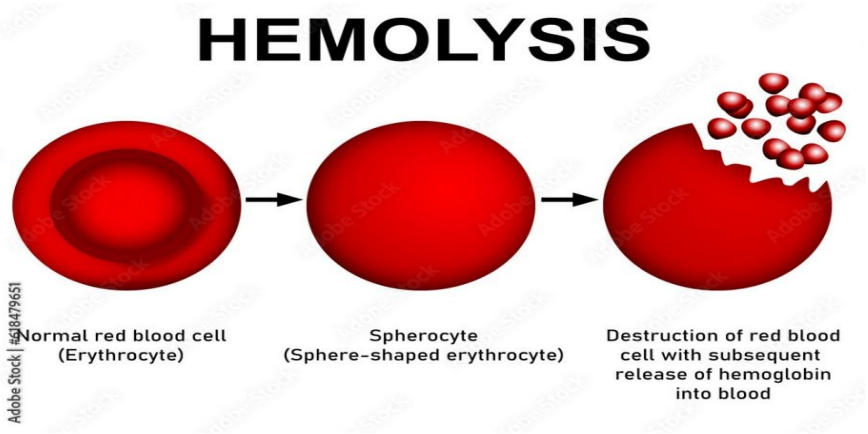


Figure No.1: Hemolysis

Plant Profile:

Figure. No.2: Gulandina boduc

Synonym

Caesalpinia Bonduc,Nicker Nut

Biological source

Guilandina Bonduc is a tropical plant found in various regions including Africa, Asia, and the Americas. The biological source of Gulandina boduc includes the seeds, roots, and leaves of the Caesalpinia bonduc plant.[3]

Family

Fabaceae (Leguminosae)

Chemical Constituents

Guilandina Bonduc contains various bioactive compounds, including

Alkaloids

bonducine, caesalpinine, and nickerin

Flavonoids

quercetin and kaempferol[4]

Tannins

Saponins

Sterols

Fatty acids

Description

Plant

It is a large, thorny, straggling, evergreen shrub or climber that can grow up to 15 meters.[5]

Leaves

The leaves are bipinnate with small leaflets.

Flowers

The flowers are yellow, arranged in racemes.

Fruits

The plant produces pods that are about 5-7.5 cm long, containing 1-2 large seeds.

Seeds

The seeds are globular, hard, and grayish in color.

Colour

The seeds are typically gray or light gray in color.

Odour

The seeds are generally odorless.[6]

Cultivation and Collection

Cultivation

Climate and Soil

Climate

Guilandina bonduc thrives in tropical and subtropical climates. It prefers a warm and humid environment with a good amount of sunlight.[7]

Soil

The plant grows well in sandy, loamy, and well-drained soils. It can tolerate poor soil conditions but performs best in fertile, well-drained soils.[8]

Propagation

Seeds

Guilandina bonduc is typically propagated by seeds. The seeds have a hard coat, which requires scarification (scratching or nicking the seed coat) or soaking in warm water for 24 hours to enhance germination [9].

Planting

Sow the seeds directly in the soil or in seed trays. Plant the seeds about 1-2 cm deep. Keep the soil moist but not waterlogged.

Germination

Seeds generally take about 2-4 weeks to germinate. Optimal germination temperatures are between 20°C and 30°C (68°F - 86°F).[10]

Growth and Care

Watering

Regular watering is essential during the early stages of growth. Once established, the plant is fairly drought-tolerant.

Fertilization

Apply a balanced fertilizer to promote healthy growth. Organic compost or well-rotted manure can also be used.

Pruning

Regular pruning helps in maintaining the shape and size of the plant and promotes better air circulation.[11]

Collection

Harvesting

The main parts of Guilandina bonduc used are the seeds and sometimes the roots and leaves.

Seeds

Harvest the pods when they are mature and brown. This usually occurs several months after flowering. The pods are spiny and should be handled with care. Wear gloves to avoid injury.

Processing Seeds

After harvesting, dry the pods in the sun until they split open. Extract the seeds and further dry them to reduce moisture content for storage.[12]

Post-Harvest Handling

Cleaning

Clean the seeds by removing any debris or remaining pod material.

Storage

Store the seeds in a cool, dry place. Properly dried seeds can be stored for several years if kept in an airtight container to protect from moisture and pests.

Material and Methodology

Plant Collection and Authentication

The best time for collection of Gulandina Bonduc.L is generally during the flowering and fruiting seasons. Gulandina Bonduc.L plant was collected from Bhivghat, Sangli, Maharashtra, India. The plant was authenticated by Mr. Tebhurne R.R. M. Sc. B.Ed Botany plant physiology.

Preparation of plant extracts gulandina boduc

Collection of Plant Material

Harvest fresh leaves, young shoots, or other plant parts of gulandina boduc during the appropriate season as mentioned earlier.[13]

Cleaning and Drying

Make sure all dirt and trash are gone from the gathered plant material. Until the plant material is completely dry, let it air dry in a place with good ventilation and protection from the sun. This step helps to prevent the growth of mold and bacteria during storage.

Grinding or Crushing

Once dried, grind or crush the plant material into smaller pieces using a mortar and pestle or a grinder. This increases the surface area of the plant material, facilitating the extraction process.[14]

Preparation of Ethanolic Extract

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

Preparation of phosphate buffer solution

To prepare a phosphate buffer solution, you will need to mix solutions of two types of sodium phosphate are dibasic (Na2HPO4) and monobasic (NaH2PO4). in appropriate proportions to achieve the desired pH. Here's a general procedure for preparing a phosphate buffer solution.

Calculate the Proportions

Determine the desired pH of the buffer solution using a buffer calculator or table. Then, calculate the appropriate proportions of monobasic and dibasic sodium phosphate solutions needed to achieve the desired pH. The Henderson-Hassel Balch equation can be used for this purpose.

Prepare Stock Solutions: Make stock solutions by dissolving the necessary quantities of each salt in distilled water. The salts are monobasic (0.2 M) and dibasic (0.2 M).

Ensure that the salts are completely dissolved.[16]

Mixing

Slowly add the calculated volumes of the monobasic and dibasic sodium phosphate stock solutions to a clean glass container, while stirring continuously. Continue mixing until the desired pH is reached.

Adjust pH (if Necessary)

Using a pH meter, find the buffer solution’s pH indicator paper. If the pH is not within the desired range, adjust it by adding small amounts of either monobasic or dibasic sodium phosphate solution as needed, and then recheck the pH.

Final Dilution (if needed)

Once the pH is adjusted to the desired range, adjust the volume of the buffer solution to the final desired volume by adding distilled water if necessary. Mix thoroughly.

Filtering (optional)

If desired, filter the buffer solution using a sterile filter to remove any particulate matter or impurities.

Sterilization (optional): If the buffer solution is to be used for biological applications, it may be sterilized by autoclaving or filtering through a sterile membrane filter.

Storage

Store the prepared phosphate buffer solution in a clean, sterile container with an airtight lid. It can be stored at room temperature for short-term use or refrigerated for longer-term storage.[17]

The process for making erythrocyte cells

Blood samples were obtained from willing, healthy volunteers. A solution of sterile saline phosphate buffer (PBS Buffer) was used to wash the pellet obtained after centrifuging 5 ml of blood. The cell suspension was added once more to a 0.5% solution of regular saline.

Perform the in-vitro hemolytic activity test

Make different concentration of solution and mixed with of the erythrocyte suspension and incubate and centrifuge it and free hemoglobin obtained, measured the absorbance using the UV-Vis spectrophotometer and calculate the % hemolysis.[18]

Phytochemical Investigation

Sr No.	Name of Test	Observation	Inference
1.	**Test For Phenol** Extract Mixed with 2 ml of 2% of Solution of $Fecl_3$	Blue/Green Colour	Phenol present
2.	**Test for Saponin** The Extract was taken in test tube and shaken vigorously with water	Formation of stable foam	Saponin absent
3.	**Test for Tannins** Extract Mixed with 2% of $Fecl_3$	No Black Colour	Tannin present
4.	**Test For Terpenoids** The Extract mixed with chloroform. then 2ml of conc. sulphuric acid was added carefully and shaken gently	Reddish brown colour observed in the interphase	Terpenoids Present
5.	**Test for flavonoids** Extract was treated with few drops of sodium hydroxide solution	Formation of intense yellow colour.which becomes colourless on the addition of dilute acid.	Flavonoids present
6.	**Test for glycosides** The extract was mixed with 2ml of glacial acetic acid containing few drops of 2% $Fecl_3$; mixture poured into another tube containing 2ml of conc. Sulphuric acid.	A brown ring at the interphase	Carbohydrate present
7.	**Test for protein** The extract treated with few drops of conc. Nitric acid	Formation of yellow colour.	Protein absent
8.	Test for alkaloids **Dragendroff's test** To a few ml of filtrate ,1 or 2ml of	Orange brown coloured ppt.	Alkaloids present

	Dragendorff reagent **Mayers test** To few ml of extract ,2drops of Mayers reagents. **Hager's test** To few ml of extract 1or2ml of Hager's reagent (saturated solution of picric acid) were added **Wagner's test** To few ml of the extract, few drops of Wagner's reagent (iodine in potassium iodide)	Cream coloured ppt. Yellow coloured ppt. Reddish brown coloured ppt.	Alkaloids present Alkaloids present Alkaloids present

Table No.1: Phytochemical constituents of gulandina boduc exract

Experimental Work

Procedure

Preparation of Plant Extracts Gulandina bonduc

The extraction preparation procedures differed slightly from those detailed in. The leaf sample wasowashed with ordinary water, alleshtly from those detailed in The blender to be ground into powder. Various ratios ored to dry, and then put into for the Soxhlet extraction procedure. 6 to 8 hours after ethanol are used as gathered Utilize a muslin cloth to filter it. Centrifuge the far the extract has been gathered at 25 °C and 4,000 rpm, for 15 minutes. The supernatant was separated and then stored for drying. [19]

Preparation of Phosphate Buffer Solution

In order to create a phosphate buffer solution, you must combine dibasic and monobasic sodium phosphate (Na2HPO4) in the right amounts to get the required pH. This is the standard protocol:

Calculate Buffer Ratio

To get the ratio of monobasic to dibasic sodium phosphate required to reach the required pH, use the Henderson-Hasselbalch equation. pH is equal to pKa + log ([A-]/[HA]), where [HA] denotes the weak acid concentration and [A-] denotes the conjugate base concentration.

Prepare Stock Solutions

By dissolving the required quantities of each salt in distilled water, separate stock solutions of monobasic sodium phosphate (0.1 M) and dibasic sodium phosphate (0.2 M) may be prepared. Make sure everything dissolves completely.

Mixing

Mix the stock solutions together in the calculated ratio to achieve the desired pH. For example, to prepare a pH 7.4 buffer,400 ml of 0.2 M dibasic sodium phosphate and 100 ml of 0.1 M monobasic sodium phosphate should be combined.

Adjust pH (if necessary)

Using a pH meter, find the buffer solution's pH indicator strips. If the pH is not within the desired range, adjust it by adding small amounts of either monobasic or dibasic sodium phosphate solution as needed, and then recheck the pH.

Final Dilution (if needed)

Once the pH is adjusted to the desired range, adjust the volume of the buffer solution to the final desired volume by adding distilled water if necessary. Mix thoroughly.

Filtering (optional)

If desired, filter the buffer solution using a sterile filter to remove any particulate matter or impurities.

Sterilization (optional)

If the buffer solution is to be used for biological applications, it may be sterilized by autoclaving or filtering through a sterile membrane filter.

Storage

Store the prepared phosphate buffer solution in a clean, sterile container with an airtight lid. It can be stored at room temperature for short-term use or refrigerated for longer-term storage.

Preparation of Erythrocyte Cell

Preparation of erythrocytic cells, also known as red blood cells, involves isolation from whole blood.

Blood Collection

Collect whole blood from a suitable animal species or human donor using sterile techniques. Use anticoagulants such as EDTA or heparin to prevent blood clotting during processing.

Centrifugation: Transfer the collected blood into centrifuge tubes and centrifuge at low speed (e.g., 200-300 x g) for 10-15 minutes. This separates the blood into layers, with erythrocytic cells settling at the bottom.[20]

Plasma Removal

Carefully remove the upper layer containing plasma using a pipette or vacuum aspirator. Be careful not to disturb the erythrocytic cell layer at the bottom of the tube.

Figure No.3: Separation of Serum and RBCs

Washing

Wash the erythrocytic cell pellet multiple times with an using an isotonic buffer solution (such as PBS, phosphate-buffered saline) to eliminate any remaining plasma proteins and platelets. Centrifuge the cells after each wash and carefully remove the supernatant.

Resuspension

After the final wash, resuspend the erythrocytic cells in the desired buffer solution or medium for further experimentation. Adjust the cell concentration as needed using a hemocytometer or automated cell counter.

Storage

Store the prepared erythrocytic cell suspension in aliquots at appropriate temperatures. Erythrocytes are normally frozen at -20°C or -80°C for long-term preservation, or kept at 4°C for short-term usage. To preserve the integrity of the cells, avoid doing repeated freeze-thaw cycles.[21]

Heamolytic Activity test

Hemolytic activity testing is a common assay used to assess the ability of substances to cause the lysis or rupture of red blood cells (erythrocytes). Here's a general overview of the hemolytic activity test:

Preparation of Red Blood Cells (RBCs)

Obtain fresh whole blood from a suitable animal species or human donor using sterile techniques. Centrifuge the blood to separate the RBCs from plasma and buffy coat.

Washing of RBCs

Wash the RBC pellet multiple times with an isotonic buffer solution (e.g., phosphate-buffered saline, PBS) to remove any residual plasma proteins and platelets. Centrifuge the RBCs after each wash and carefully remove the RBCs.

Preparation of Test Samples

Prepare the test samples with different concentrations of the relevant chemical. (In the saline phosphate buffer, 25, 50, 75, and 100 g/ml). The substance could be a natural product extract, synthetic compound, or pharmaceutical formulation.

Incubation with RBCs

For a predetermined amount of time, usually one to two hours, incubate the RBC suspension with the test samples under physiological conditions (e.g., 37°C, pH 7.4).

Centrifugation

After the incubation period, centrifuge the RBC suspension to separate the intact RBCs (pellet) from any lysed or ruptured RBCs (supernatant).

Figure No.4: Free Hemoglobin

Measurement of Hemolysis

Measure the absorbance of the supernatant at a suitable wavelength (e.g., 540 nm) using a UV-vis spectrophotometer. The degree of hemolysis is shown by the absorbance, which is directly proportional to the amount of hemoglobin released.

Calculation of Hemolytic Activity: Use the following calculation to get the percentage of hemolysis: The formula for hemolysis (%) is (test sample absorbance – negative control absorbance) / (positive control absorbance – negative control absorbance) × 100.

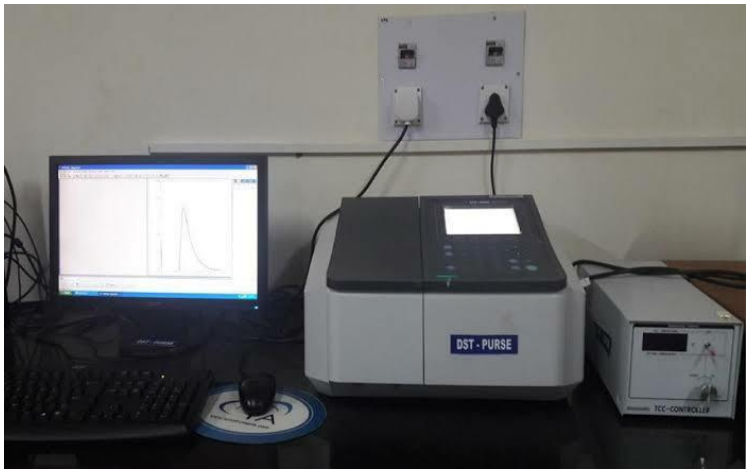


Figure No.5: Checking Absorbance of Sample

The Following Formula used to determine the proportion of hemolysis.[22]

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

Where,

At: absorbance of the test sample

An: minimal control absorbance (phosphate buffered saline solution PBS)

Ac: maximum control absorbance (distilled water)

Observation

Sr No	Concentration	Absorbance	Hemolysis %	Protection
1	25ml	0.018	91%	0.91
2	50ml	0.062	69.15%	0.69
3	75ml	0.175	12.93%	0.12
4	100ml	0.325	6.15%	0.061

Table No.2: Absorbance of sample

RESULT

Phytochemical screening is an effective method for figuring out the molecular composition of various plant extracts and for discovering bioactive chemicals used in medication production. Results of phytochemical analysis performed on ethanolic extracts of Gulandina bonduc's leaves and stem The screening of gulandina bonduc revealed the absence of reducing sugar but the presence of moisture and elemental components such as carbon, hydrogen, nitrogen, and sulfur. The existence of these chemicals indicates the plant's therapeutic potential. As the stem and leaf of the plant do not contain any reducing sugar, tests may be conducted to identify the various phenolic compounds, amino acids, and medicinal value of the plant.

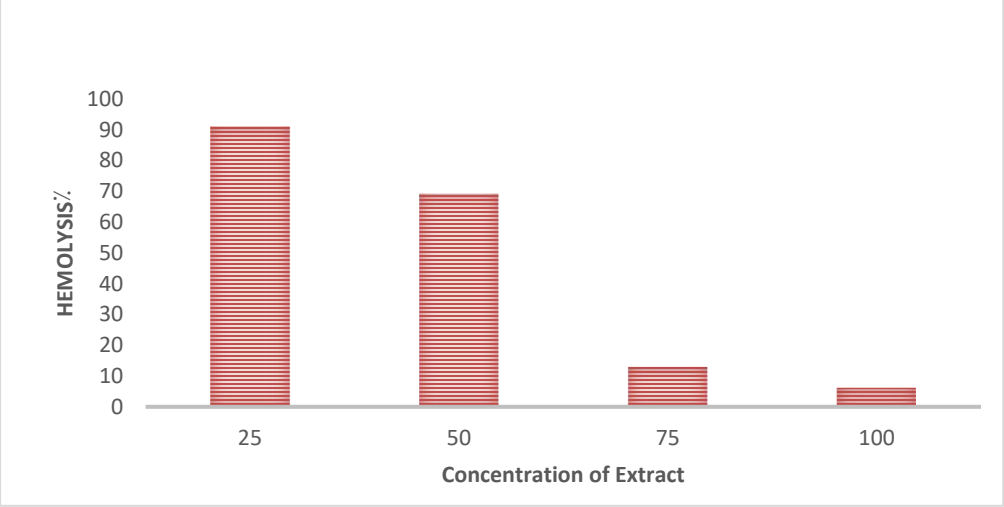
Sr No.	Constituents	Observation
	Ethanolic Extract	
1	Saponins	-
2	Phenols	+
3	Tannins	+
4	Terpenoids	+
5	Flavonoids	+
6	Protiens	-
7	Carbohydrates	+
8.	Alkaloids	+

Table No.3: Preliminary Phytochemical Screening of Gulandina bonduc
(-) indicates the absence of Compound
(+) indicates the presence of Compound

Using erythrocytes, the ethanolic extract of Gulandina bonduc was tested for hemolyzation capacity; the findings were represented as a percentage of hemolysis. The findings (table no.4) demonstrated that hemolysis was impacted by the extracts under study. At 100 g/ml, the ethanolic extract exhibits the least hemolytic activity; at 75 g/ml, 12.93%, 50 g/ml, and 69.15%, the least hemolytic activity is produced; at 25g/ml, or 91%, the highest hemolytic activity is produced. The outcomes also showedthat the extract concentration affects the extent of hemolysis. The various test extracts' hemolytic effects can be categorized using the following criteria: 25 g/ml to 100 g/ml, with a range of 50 g/ml to 75 g/ml.Many phytochemicals, including glycosides, phenol, flavonoids, and saponins, are present in medicinal plants and have unique pharmacological effects on human health.

Sr No.	Concentration	Hemolysis%
1	25	91%
2	50	69.15%
3	75	12.93%
4	100	6.15%

Table No.4: Percentage of Hemolysis



Graph No.1: Graphical Presentation of hemolysis %

DISCUSSION

The assessment of in-vitro hemolytic activity of guilandina bonduc presents an intriguing avenue for understanding the potential biological effects of this plant extract on red blood cells. Hemolysis, the rupture or destruction of red blood cells, can occur due to various factors including chemical agents, toxins, or natural compounds. guilandina bonduc, also known as the climbing wattle, possesses a diverse array of phytochemicals, making it a subject of interest for biomedical research.

CONCLUSION

In conclusion, the in vitro assessment of the hemolytic activity of Guilandina bonduc has provided compelling evidence of its potential therapeutic benefits in preventing red blood cell destruction. The antioxidant and membrane-stabilizing properties of the extracts make them promising candidates for the management of conditions associated with hemolysis, including hemolytic anemias, oxidative stress-related disorders, and complications of blood transfusions.

CONFLICTS OF INTEREST

Nil.

FUNDING

No financial interest.

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