Evaluation of the Protective Anti-Haemolytic Effect of Gymnema sylvestre Against Ouret lanata (L.) Kuntze-Induced Erythrocyte Membrane Damage

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***Abstract*—Erythrocyte membrane stability is widely used as an in-vitro index of the membrane-protective and anti-inflammatory potential of plant extracts, since the erythrocyte membrane shares several structural similarities with the lysosomal membrane. The present study evaluated the protective anti-haemolytic effect of Gymnema sylvestre against Ouret lanata (L.) Kuntze-induced erythrocyte membrane damage. Objective: To prepare ethanolic extracts of G. sylvestre leaves and O. lanata flowers, screen them phytochemically, and evaluate the haemolytic potential of O. lanata and the membrane-protective potential of G. sylvestre on human erythrocytes in vitro. Methods: Extracts were obtained by Soxhlet extraction and subjected to preliminary phytochemical screening. O. lanata (25–400 µg/mL) was evaluated for haemolytic activity on washed human erythrocytes; 100 µg/mL O. lanata, corresponding to 46.0% haemolysis, was selected to establish a standardized erythrocyte injury model. Low, medium and high concentrations of G. sylvestre were then co-incubated with the injury model, and haemoglobin release was measured spectrophotometrically at 540 nm. Results: O. lanata produced concentration-dependent haemolysis, rising from 13.0% at 25 µg/mL to 84.0% at 400 µg/mL. G. sylvestre extract alone showed concentration-dependent membrane stabilization (30–82% protection across 25–400 µg/mL), approaching the 85% protection shown by the reference standard. Against O. lanata-induced injury, G. sylvestre reduced haemolysis from 46.0% to 35.0%, 23.0% and 11.0% at low, medium and high concentrations respectively, corresponding to 23.9%, 50.0% and 76.1% membrane protection. Conclusion: Gymnema sylvestre exhibits concentration-dependent erythrocyte membrane-stabilizing activity against Ouret lanata (L.) Kuntze-induced membrane damage, supporting its potential as a membrane-protective and anti-inflammatory agent warranting further pharmacological investigation.**

***Index Terms*—Gymnema sylvestre; Ouret lanata (L.) Kuntze; erythrocyte membrane stabilization; anti-haemolytic activity; membrane protection; Soxhlet extraction.**

I. INTRODUCTION

Erythrocytes (red blood cells) are anucleate, biconcave cells whose membrane composition and flexibility are essential for their survival in circulation and for their principal function of transporting respiratory gases [1]. Because the erythrocyte membrane is readily accessible and structurally comparable to the lysosomal membrane, agents that stabilize erythrocytes against induced lysis are widely used as an in-vitro surrogate for evaluating membrane-protective and anti-inflammatory potential [2].

Haemolysis is the premature rupture of the erythrocyte membrane with release of intracellular haemoglobin into the surrounding medium, and can be produced experimentally by hypotonic stress, heat, oxidative agents or chemical/plant-derived agents [3]. Spectrophotometric quantification of released haemoglobin provides a simple, reproducible index of membrane damage, and has been used extensively to screen plant extracts for both haemolytic and membrane-stabilizing (anti-haemolytic) activity [4], [5].

Ouret lanata (L.) Kuntze [6] [the currently accepted taxonomic name for Aerva lanata (L.) Juss. ex Schult.] is a traditionally used medicinal herb reported to possess antioxidant, antimicrobial, hepatoprotective, anti-urolithiatic and diuretic properties, attributed to alkaloids, flavonoids, tannins, saponins and triterpenoids in its extracts [7]–[11]. Gymnema sylvestre (Retz.) R.Br. ex Sm. [12]

is a well-known antidiabetic herb whose leaves contain gymnemic acids, gymnemasaponins, flavonoids and phenolic compounds, and which has also been reported to show antioxidant, lipid-lowering, hepatoprotective and membrane-stabilizing activities [13]–[19].

While the individual pharmacological properties of both plants are documented, no study to date has evaluated Gymnema sylvestre as a protective agent against Ouret lanata (L.) Kuntze-induced erythrocyte membrane injury. The present study was therefore undertaken with the aim of evaluating the protective anti-haemolytic effect of Gymnema sylvestre against Ouret lanata (L.) Kuntze-induced erythrocyte membrane damage, with specific objectives to (i) prepare and standardize ethanolic extracts of both plants, (ii) perform preliminary phytochemical screening, (iii) establish an in-vitro erythrocyte injury model using Ouret lanata (L.) Kuntze, and (iv) evaluate the concentration-dependent membrane-protective activity of Gymnema sylvestre against this induced injury.

II. MATERIALS AND METHODS

A. Collection and Authentication of Plant Material

Fresh flowers of Ouret lanata (L.) Kuntze were collected from Elavampatti village, Thirupathur, and dried leaves of Gymnema sylvestre were procured commercially. The plant material was authenticated by Dr. S. Jagatheskumar, Assistant Professor of Botany, Sri Vijay Vidyalaya College of Arts and Science, Nallampalli (T.K.), Dharmapuri –636807, Tamil Nadu, India. The material was shade-dried and pulverized to a coarse powder using a mechanical grinder; approximately 500 g of each coarse powder was used for extraction.

B. Extraction of Plant Material

Coarse powdered material of both plants was extracted separately by continuous hot extraction in a Soxhlet apparatus using ethanol as solvent [20]. Briefly, 100 g of powdered material was packed in the thimble and extracted with 1000 mL ethanol for 18 h at 50 °C. The extract was filtered, and the solvent was removed by distillation and further concentrated under vacuum; the process was repeated for two additional 18 h cycles with fresh solvent to ensure exhaustive extraction. The concentrated dried extracts were stored in a desiccator until further use.

C. Preliminary Phytochemical Screening

Ethanolic and aqueous preparations of both extracts were subjected to standard qualitative phytochemical tests for alkaloids (Mayer's and Wagner's tests), flavonoids (Shinoda test/alkaline reagent test), phenolic compounds (ferric chloride test), tannins (gelatin test), saponins (foam/froth test), terpenoids/triterpenoids (Salkowski test), steroids/phytosterols (Liebermann–Burchard test), cardiac glycosides (Keller–Killiani test), proteins and amino acids (Ninhydrin test), and carbohydrates (Molisch's test) [21], [22].

D. Preparation of Erythrocyte Suspension

Human erythrocytes were obtained from an institutionally approved blood source with human ethics clearance and handled under standard biosafety procedures. Blood was centrifuged to separate erythrocytes from plasma, and the cells were washed three times with isotonic phosphate-buffered saline. A standardized erythrocyte suspension was freshly prepared for each experiment to ensure consistency of haemoglobin release measurements across groups.

E. Determination of Percentage Haemolysis and Percentage Protection

Percentage haemolysis was calculated from the absorbance of released haemoglobin at 540 nm (OD_{540}), using the negative control (isotonic buffer, representing 0% haemolysis) and the positive control (complete haemolysis, representing 100% haemolysis) as reference points:

$$\% \text{ Haemolysis} = [(OD_{sample} - OD_{negative}) / (OD_{positive} - OD_{negative})] \times 100$$

Percentage membrane protection afforded by Gymnema sylvestre was calculated relative to the corresponding haemolysis value:

$$\% \text{ Protection} = 100 - \% \text{ Haemolysis}$$

F. Membrane-Stabilization Activity of Gymnema sylvestre Extract Alone

The ethanolic extract of Gymnema sylvestre (25, 50, 100, 200 and 400 µg/mL) was independently evaluated for erythrocyte membrane-stabilizing activity against hypotonicity-induced haemolysis,

alongside a reference standard membrane-stabilizing agent, using the OD_{540} method described above.

G. Phase I – Haemolytic Activity of Ouret lanata (L.) Kuntze

Washed human erythrocytes were incubated with Ouret lanata (L.) Kuntze extract at 25, 50, 100, 200 and 400 µg/mL (groups A1–A5), alongside negative and positive controls. Absorbance was measured at 540 nm and percentage haemolysis was calculated for each group as described in Section II-E.

H. Selection of the Injury Concentration

Based on the Phase I concentration-response data, 100 µg/mL Ouret lanata (L.) Kuntze (producing 46.0% haemolysis, a reproducible sub-maximal injury) was selected to establish the standardized erythrocyte injury model for Phase II, since this concentration provided measurable membrane damage while retaining sufficient viable erythrocytes to demonstrate a protective effect.

I. Phase II – Anti-Haemolytic (Protective) Activity of Gymnema sylvestre

Erythrocytes were divided into five groups: Group I (normal RBC control), Group II (Ouret lanata (L.) Kuntze injury model, 100 µg/mL), and Groups III–V (Ouret lanata (L.) Kuntze injury model co-incubated with low, medium and high concentrations of Gymnema sylvestre, respectively). Absorbance was measured at 540 nm, and percentage haemolysis and percentage protection were calculated for each group relative to the injury model (Group II).

III. RESULTS

A. Preliminary Phytochemical Screening

Qualitative screening of the ethanolic and aqueous extracts of Gymnema sylvestre showed the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, phytosterols, cardiac glycosides, proteins/amino acids and carbohydrates, while saponins were not detected by the foam test (Table I). The ethanolic and aqueous extracts of Ouret lanata (L.) Kuntze showed the presence of flavonoids, tannins, alkaloids, steroids, triterpenoids, glycosides and carbohydrates, while saponins were similarly not detected (Table II).

TABLE I. PRELIMINARY PHYTOCHEMICAL SCREENING OF GYMNEMA SYLVESTRE EXTRACT

Phytoconstituent	Test	Ethanol	Aqueous
Alkaloids	Mayer's test	+	+
Alkaloids	Wagner's test	+	+
Flavonoids	Alkaline reagent test	+	+
Flavonoids	Shinoda test	+	+
Phenolic compounds	Ferric chloride test	+	+
Tannins	Gelatin test	+	+
Saponins	Foam/Froth test	−	−
Terpenoids	Salkowski test	+	+
Phytosterols	Liebermann–Burchard test	+	+
Cardiac glycosides	Keller–Killiani test	+	+
Proteins & amino acids	Ninhydrin test	+	+
Carbohydrates	Molisch's test	+	+

TABLE II. PRELIMINARY PHYTOCHEMICAL SCREENING OF OURET LANATA (L.) KUNTZE EXTRACT

Phytoconstituent	Test	Ethanol	Aqueous
Saponins	Foam test	−	−
Flavonoids	Shinoda test	+	+
Tannins	Ferric chloride test	+	+

Alkaloids	Mayer's test	+	+
Steroids	Liebermann–Burchard test	+	+
Triterpenoids	Salkowski test	+	+
Glycosides	Keller–Killiani test	+	+
Carbohydrates	Molisch's test	+	+

Note: "+" indicates presence; "–" indicates absence.

B. Membrane-Stabilization Activity of Gymnema sylvestre Extract Alone

The ethanolic extract of Gymnema sylvestre exhibited concentration-dependent membrane-stabilizing activity, with percentage protection increasing from 30% at 25 µg/mL to 82% at 400 µg/mL, approaching the 85% protection shown by the reference standard (Table III, Fig. 1).

TABLE III. MEMBRANE-STABILIZATION (ANTI-HAEMOLYTIC) ACTIVITY OF GYMNEMA SYLVESTRE EXTRACT ALONE

Concentration	OD_{540}	% Membrane protection
25 µg/mL	0.70	30%
50 µg/mL	0.60	40%
100 µg/mL	0.48	52%
200 µg/mL	0.32	68%
400 µg/mL	0.18	82%
Standard*	0.15	85%

*Reference standard membrane-stabilizing agent — to be specified by the author.

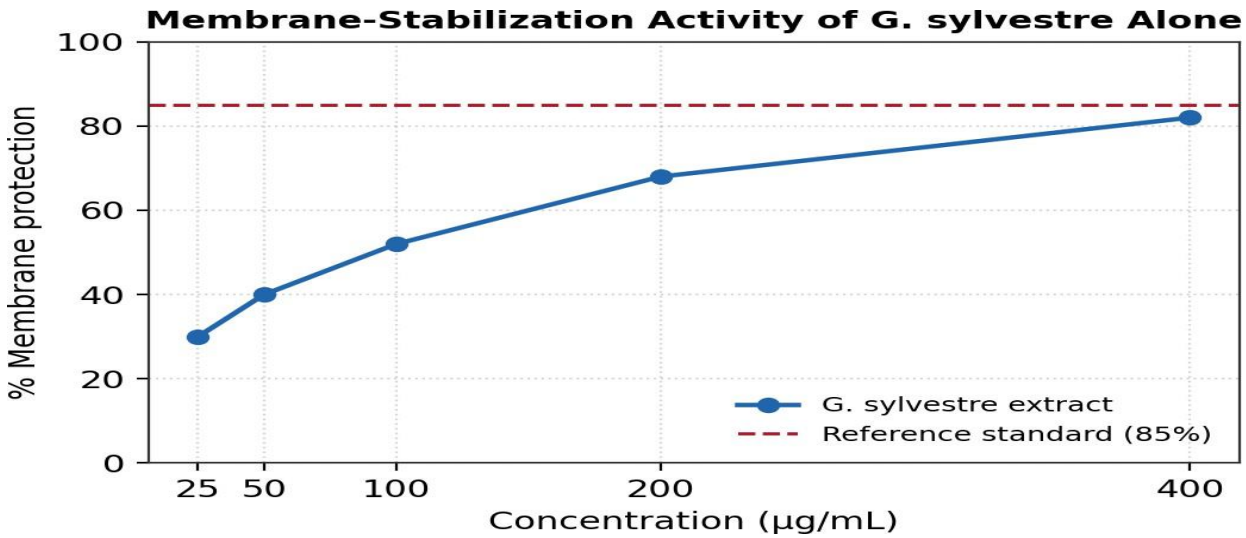

Fig. 1. Concentration-dependent membrane-stabilizing (anti-haemolytic) activity of Gymnema sylvestre extract alone, compared with the reference standard.

C. Phase I: Haemolytic Activity of Ouret lanata (L.) Kuntze

The negative control showed a mean OD of 0.050 (0% haemolysis) and the positive control showed a mean OD of 1.050 (100% haemolysis). Ouret lanata (L.) Kuntze extract produced a clear concentration-dependent increase in haemolysis, rising from 13.0% at 25 µg/mL (A1) to 84.0% at 400 µg/mL (A5), an over sixfold increase across the tested range (Table IV,

Fig. 2). This progressive, graded response confirmed the suitability of Ouret lanata (L.) Kuntze as a haemolytic injury-inducing agent for the subsequent protection study.

TABLE IV. PHASE I – CONCENTRATION-DEPENDENT HAEMOLYTIC ACTIVITY OF OURET LANATA (L.) KUNTZE

Group	Concentration	Mean OD_{540}	% Haemolysis
Negative control	—	0.050	0%
Positive control	Complete haemolysis	1.050	100%
A1	25 µg/mL	0.180	13.0%
A2	50 µg/mL	0.320	27.0%
A3	100 µg/mL	0.510	46.0%
A4	200 µg/mL	0.720	67.0%
A5	400 µg/mL	0.890	84.0%

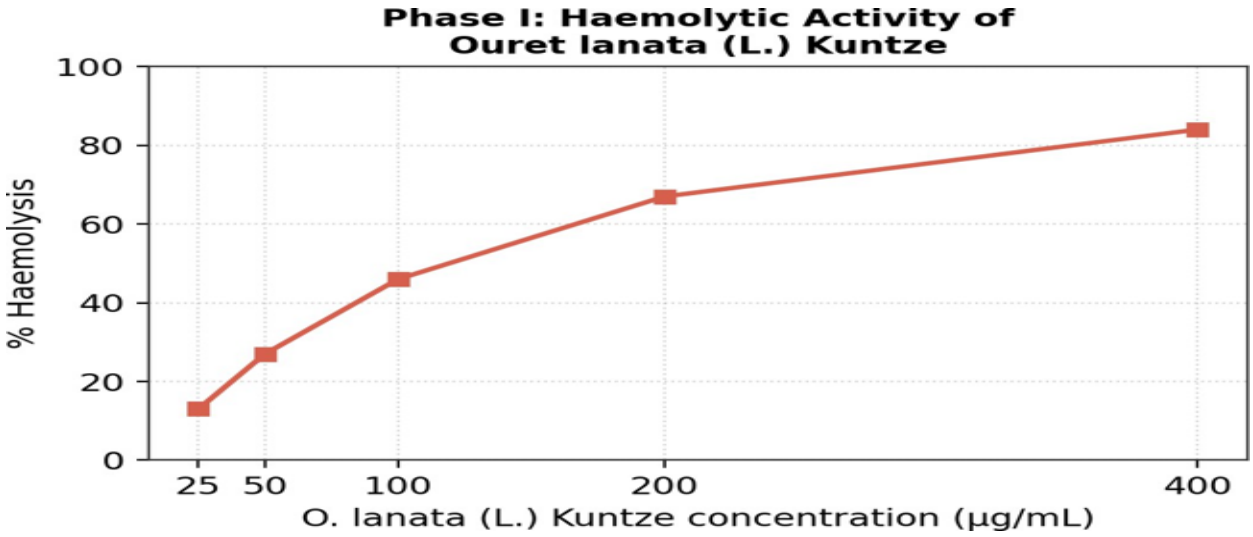

Fig. 2. Concentration-dependent haemolytic activity of Ouret lanata (L.) Kuntze on human erythrocytes (Phase I).

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D. Phase II: Protective Effect of Gymnema sylvestre Against Ouret lanata (L.) Kuntze-Induced Haemolysis

The normal RBC group (Group I) showed a mean OD of 0.050 (0% haemolysis). The Ouret lanata (L.) Kuntze injury group (Group II, 100 µg/mL) showed a mean OD of 0.510, corresponding to 46.0% haemolysis, confirming substantial induced membrane damage. Co-treatment with Gymnema sylvestre produced a clear, concentration-dependent reduction in haemolysis: the mean OD fell to 0.400, 0.280 and 0.160 at low, medium and high concentrations respectively, corresponding to 35.0%, 23.0% and 11.0% haemolysis, and to 23.9%, 50.0% and 76.1% membrane protection relative to the injury model (Table V, Fig. 3). At the highest concentration tested, the OD value (0.160) approached the normal, uninjured baseline (0.050) far more closely than the injury control (0.510).

TABLE V. ANTI-HAEMOLYTIC AND ERYTHROCYTE MEMBRANE-PROTECTIVE EFFECT OF GYMNEMA SYLVESTRE AGAINST OURET LANATA (L.) KUNTZE-INDUCED HAEMOLYSIS

Group	Treatment	Mean OD_{540}	% Haemolysis	% Protection
I	Normal RBC	0.050	0%	—
II	O. lanata injury (100 µg/mL)	0.510	46.0%	0%

III	Injury + Low G. sylvestre	0.400	35.0%	23.9%
IV	Injury + Medium G. sylvestre	0.280	23.0%	50.0%
V	Injury + High G. sylvestre	0.160	11.0%	76.1%

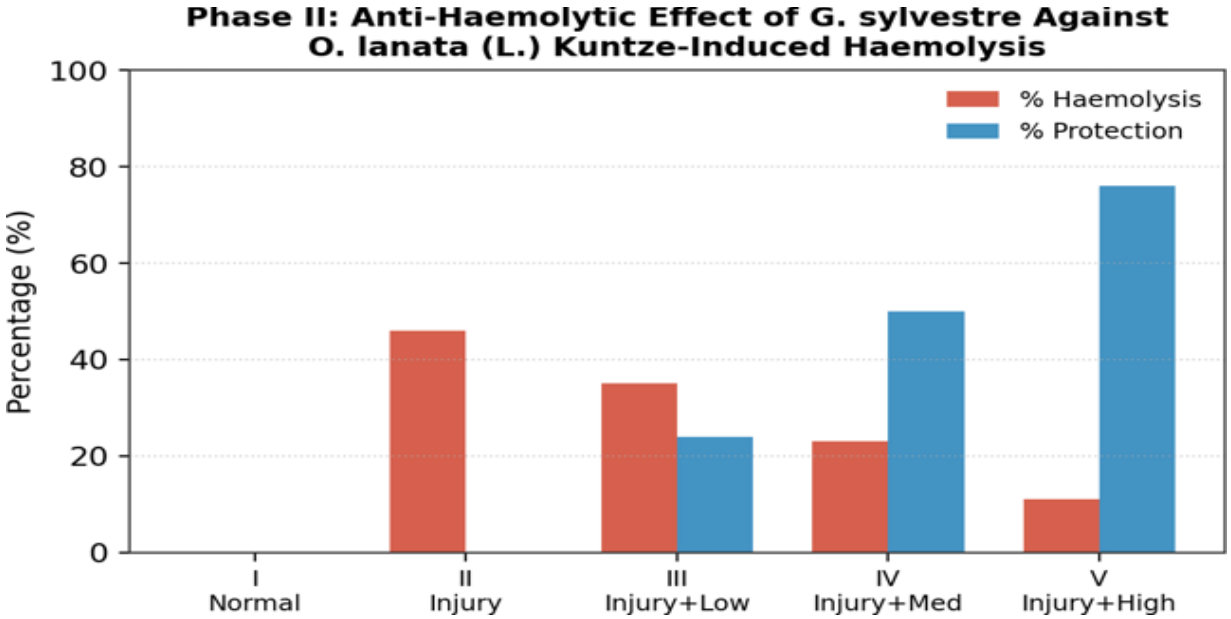

Fig. 3. Percentage haemolysis and percentage membrane protection across Phase II groups (I–V), showing the concentration-dependent protective effect of Gymnema sylvestre against Ouret lanata (L.) Kuntze-induced injury.

IV. DISCUSSION

Qualitative phytochemical screening confirmed the presence of pharmacologically relevant secondary metabolites in both extracts — notably gymnemic-acid-type triterpenoid saponins, flavonoids and phenolic compounds in Gymnema sylvestre, and alkaloids, flavonoids, tannins, terpenoids and phytosterols in Ouret lanata (L.) Kuntze — consistent with previous phytochemical reports on these species [13], [16], [7].

The Phase I findings establish that Ouret lanata (L.) Kuntze extract induces haemolysis in a clearly dose-dependent fashion, rising from 13.0% at the lowest concentration to 84.0% at the highest. This progressive, graded membrane disruption — rather than an all-or-nothing effect — supported the use of Ouret lanata (L.) Kuntze as a suitable and reproducible haemolytic injury-inducing agent for the subsequent protection study, allowing the protective capacity of Gymnema sylvestre to be measured with sensitivity across a range of doses.

Building on this, the Phase II results demonstrate that Gymnema sylvestre extract exerts a concentration-dependent membrane-stabilizing (anti-haemolytic) effect against Ouret lanata (L.) Kuntze-induced injury. Protection rose from 23.9% to 76.1% as the dose of Gymnema sylvestre increased, with a corresponding fall in haemolysis from 46.0% (injury alone) to 11.0% at the highest dose, bringing the OD value (0.160) much closer to the normal, uninjured baseline (0.050) than to the injury control (0.510). This is consistent with the concentration-dependent membrane-stabilizing activity independently observed for Gymnema sylvestre extract alone against hypotonicity-induced haemolysis (30–82% protection, Table III), where the highest tested concentration approached the activity of the reference standard (85%).

Mechanistically, such protection is typically attributed to membrane-stabilizing phytoconstituents of Gymnema sylvestre — gymnemic acids, triterpenoid saponins, flavonoids and phenolic compounds — interacting with the erythrocyte membrane to resist the destabilizing/lytic action of Ouret lanata (L.) Kuntze, either by reinforcing the

lipid bilayer or by counteracting oxidative or osmotic stress at the membrane surface [16], [23]. Because erythrocyte membrane-stabilization assays are commonly used as an in-vitro proxy for anti-inflammatory potential, given the structural similarities between erythrocyte and lysosomal membranes [2], [24], [25], these results support further investigation of the anti-inflammatory activity of Gymnema sylvestre through complementary assays.

V. CONCLUSION

The present study evaluated the protective anti-haemolytic effect of Gymnema sylvestre against Ouret lanata (L.) Kuntze-induced erythrocyte membrane damage. Qualitative phytochemical screening confirmed the presence of pharmacologically relevant secondary metabolites in both extracts. Ouret lanata (L.) Kuntze produced a clear concentration-dependent increase in haemolysis (13.0–84.0%), confirming its suitability as a membrane-damaging agent for induction of a standardized erythrocyte injury model. Co-treatment with Gymnema sylvestre demonstrated a concentration-dependent membrane-stabilizing effect against this induced damage, with percentage protection increasing from 23.9% at the low concentration to 76.1% at the high concentration. These findings indicate that Gymnema sylvestre extract possesses notable erythrocyte membrane-stabilizing (anti-haemolytic) activity, likely attributable to its gymnemic acid, saponin and flavonoid constituents, and support its potential as a protective agent against oxidative/chemically induced membrane damage. Further studies involving IC_{50} determination, a larger number of biological replicates with appropriate statistical validation, and comparison with a defined standard reference drug are recommended to substantiate and extend these findings.

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