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Evaluation of the In-Vitro Anti-Ulcerative and Anti-Inflammatory Activities of *Datura stramonium*

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ABSTRACT:

Datura stramonium is traditionally used for its analgesic, antispasmodic, and anti-inflammatory properties. This study evaluated the in vitro antioxidant, antiulcer, and anti-inflammatory potential of its ethanolic leaf extract. Phytochemical screening revealed the presence of phenolics, flavonoids, alkaloids, triterpenoids, and steroids. Antioxidant activity was confirmed via DPPH, ABTS, and FRAP assays, with the extract showing IC_{50} of 19.76 ± 1.76 µg/mL in DPPH radical scavenging, indicating strong free radical neutralization. Antiulcer activity was assessed through acid-neutralizing capacity and H^+/K^+-ATPase inhibition, where the extract demonstrated dose-dependent efficacy comparable to standard antacids and Omeprazole. Anti-inflammatory potential was evaluated using egg albumin denaturation and human red blood cell membrane stabilization assays, showing significant, concentration-dependent inhibition and membrane protection, though slightly lower than Diclofenac. These findings suggest that Datura stramonium leaves possess potent antioxidant, gastroprotective, and anti-inflammatory properties, supporting their traditional use and highlighting their potential as a natural therapeutic agent for oxidative stress-related, ulcerative, and inflammatory conditions.

Keywords: Datura stramonium, Peptic ulcer, anti-inflammatory

INTRODUCTION

Peptic ulcer disease and inflammatory disorders represent significant health challenges worldwide, often associated with excessive gastric acid secretion, Helicobacter pylori infection, oxidative stress, and chronic inflammation. [1, 2] Conventional treatments, including proton pump inhibitors, H2 receptor antagonists, and nonsteroidal anti-inflammatory drugs (NSAIDs), although effective, are frequently accompanied by adverse effects and limitations such as drug resistance and relapse. [3] Consequently, there is a growing interest in the exploration of natural products as alternative or complementary therapies due to their diverse bioactive compounds and comparatively lower toxicity profiles. [4,5]

Datura stramonium, commonly known as Jimson weed, belongs to the Solanaceae family and has been widely used in traditional medicine for its analgesic, antispasmodic, and anti-inflammatory properties. [5, 6] Phytochemical studies reveal that it contains alkaloids, flavonoids, tannins, and glycosides, which are known to exhibit potent biological activities. [7-10] Despite its historical medicinal use, systematic scientific evaluation of its anti-ulcerative and anti-inflammatory potential in controlled in-vitro models remains limited. Investigating the anti-ulcerative and anti-inflammatory activities of Datura stramonium in vitro provides critical insights into its therapeutic potential, mechanisms of action, and safety profile. Such studies can contribute to the development of novel plant-based remedies, offering safer and effective alternatives to synthetic drugs for managing ulcerative and inflammatory conditions.

MATERIALS & METHODS

Plant Collection, Extraction and Phytochemical Investigation: Leaves of Datura stramonium were collected from the local region of Bareilly district in February 2024 and authenticated by MJPRU, Bareilly. Fresh leaves were thoroughly washed with distilled water to remove dirt and debris, shade-dried at room temperature until brittle, and then ground into a coarse powder using a mortar and pestle or electric grinder. For extraction, a known quantity of the powdered leaves, typically in a 1:10 ratio with 70% ethanol, was placed in a conical flask, sealed, and allowed to macerate at room temperature for 48–72 hours with occasional shaking to facilitate the dissolution of phytoconstituents. After maceration, the mixture was filtered through muslin cloth followed by Whatman No. 1 filter paper, and the process was repeated two to three times to maximize yield. [11] The combined filtrates were

concentrated under reduced pressure using a rotary evaporator at 40–50°C to obtain a thick, semi-solid crude extract, which was stored in an airtight container at 4°C for further use. [12] Preliminary phytochemical screening of the extract was carried out using standard laboratory procedures to detect the presence of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, steroids, reducing sugars, and phenols. [13]

Measurement of total polyphenolic content and flavonoid content: The total phenolic content in the plant material was measured according to the method described by [14]. Briefly, 500 µL of Folin–Ciocalteau reagent (10-fold diluted with water) was mixed with 100 µL of diluted filtrate, followed by the addition of 400 µL of aqueous sodium carbonate solution (7.5% w/v). The mixture was allowed to react for 60 minutes at room temperature, and the absorbance was measured at 765 nm. A calibration curve was prepared using Gallic acid, and the total phenolic content was expressed as Gallic acid equivalent (g/100 g dry weight) of the plant material. The total flavonoid content was determined following the method of [15], where 500 µL of diluted filtrate was mixed with 2 mL of distilled water, then 150 µL of aqueous sodium nitrite solution (5% w/v) was added, followed by 150 µL of aqueous aluminum chloride solution (10% w/v). The test tubes were incubated for 5 minutes at room temperature, after which 1 mL of sodium hydroxide (1M) was added and the final volume adjusted to 5 mL with distilled water. The mixture was vortexed, and absorbance was measured at 510 nm. Flavonoid content was determined from a standard curve prepared with Rutin solution and expressed as g Rutin equivalents (RE)/100 g dry weight of plant.

Anti-oxidant activity: The antioxidant potential of the plant material was evaluated using ABTS, DPPH, and FRAP assays. The ABTS radical scavenging activity was determined following the method of [16], where ABTS radical cations ($ABTS^{+}$) were generated by reacting 7 mM aqueous ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at ambient temperature for 12–16 hours. The resulting solution was diluted with distilled water to obtain an absorbance of 0.700 ± 0.005 at 734 nm. A 30 µL sample was added to 3 mL of ABTS solution and incubated at room temperature for 6 minutes, after which the absorbance was measured at 734 nm. Trolox was used to prepare the standard curve, and results were expressed as mmol Trolox equivalents per 100 g dry weight (DW) of the plant material. The DPPH radical scavenging activity was evaluated according to [17] with slight modifications, by mixing 100 µL of filtrate at various concentrations with 1.9 mL of methanolic DPPH (0.3 mM), incubating the mixture at room temperature for 20 minutes, and measuring the absorbance at 517 nm. IC50 values were calculated from plots of scavenging activity against sample concentrations. The ferric reducing antioxidant power (FRAP) was estimated based on [18], using a freshly prepared FRAP reagent consisting of 50 mL acetate buffer (0.3 M, pH 3.6), 5 mL ferric chloride solution (20 mM), and 5 mL TPTZ solution (10 mM in 40 mM HCl). To 10 µL of extract, 2 mL of FRAP reagent was added, and after 10 minutes at room temperature, absorbance was measured at 593 nm against a blank. Trolox was used as a standard, and results were expressed as mmol Trolox equivalents per 100 g DW of plant material.

In-Vitro Evaluation of Antiulcer Activity

Acid Neutralizing Capacity: The acid-neutralizing capacity of the ethanol extract was evaluated at doses of 100 mg, 500 mg, 1000 mg, and 1500 mg, while aluminium hydroxide and magnesium hydroxide (500 mg each) were used as standards. A total volume of 70 mL was prepared by adding 5 mL of the test or standard solution, with the remaining volume made up with water, and the mixture was stirred for one minute. To both the test and standard preparations, 30 mL of 1.0 N HCl was added and stirred for 15 minutes. Phenolphthalein was then added, followed by immediate titration of the excess HCl with 0.5 N sodium hydroxide until a pink endpoint was attained [19]. The moles of acid neutralized is calculated by,

Moles of acid neutralized = (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH) Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided by Grams of Antacid/Extract.

H+/K+ - ATPase Inhibition Activity: The H^{+}/K^{+}-ATPase enzyme was prepared from fresh goat stomach purchased from a local slaughterhouse. The gastric mucosa of the fundus was excised, and the inner layer was scraped to isolate parietal cells. The obtained parietal cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 and centrifuged at 6000 rpm for 10 minutes. The supernatant was collected and used for the H^{+}/K^{+}-ATPase inhibition assay, and protein content was determined using the Bradford method with BSA as a standard. For the inhibition study, the reaction mixture containing 0.1 mL of enzyme extract (300 µg) and plant extract at varying concentrations (20, 40, 60, 80, and 100 µg) was pre-incubated for 60 minutes at 37 °C. The reaction was initiated by adding 200 µL of 2 mM ATP, 200 µL of 2 mM $MgCl_2$, and 200 µL of 10 mM KCl, followed by incubation at 37 °C for 30 minutes. The reaction was stopped by adding 4.5% ammonium molybdate and 60% perchloric acid, centrifuged at 2000 rpm for 10 minutes, and the inorganic phosphate released was measured spectrophotometrically at 660 nm following the Fiske-Subbarow method. Briefly, 1 mL of supernatant was mixed with 4 mL of Millipore water, 1 mL of 2.5% ammonium molybdate, and 0.4 mL of ANSA, and absorbance was recorded at 660 nm. Enzyme activity was expressed as micromoles of Pi released per hour, and results were compared with the known anti-ulcer H^{+}/K^{+}-ATPase inhibitor, Omeprazole, and expressed as Mean ± SEM. Percent enzyme inhibition was calculated according to the standard formula [20, 21]:

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100

In-Vitro Anti-Inflammatory Activity

Egg albumin denaturation method: Plant extract samples were assumed to act similarly to NSAIDs, and the anti-inflammatory potency was assessed based on the percentage inhibition of albumin denaturation, with higher inhibition indicating stronger activity. Standard and test sample concentrations were prepared at 2000, 1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL. Phosphate-buffered saline (PBS, pH 6.4) was prepared by dissolving 28.80 g of disodium hydrogen phosphate and 11.45 g of dipotassium hydrogen phosphate in 1000 mL of distilled water [22]. Test samples were prepared by mixing 2.8 mL of PBS, 0.2 mL of egg albumin, and 0.2 mL of plant extract, whereas diclofenac sodium samples contained 2.8 mL PBS, 0.2 mL egg albumin, and 0.2 mL diclofenac sodium. A control was prepared using 2.8 mL PBS, 2 mL distilled water, and 0.2 mL egg albumin. All samples were incubated at 37 °C for 15–20 minutes in a shaking water bath, followed by heating at 70 °C for five minutes, then allowed to cool for 10 minutes at room temperature. Absorbance was measured at 660 nm using a double-beam UV-visible spectrophotometer [23], and all assays were performed in triplicate. The percentage inhibition of egg albumin denaturation was calculated using the standard formula.

% inhibition of egg albumin denaturation = Absorbance of the control – Absorbance of the Standard / Absorbance of the control ×100

Human Red Blood Cell Membrane Stabilization Method: Human red blood cells (HRBCs) are abundant, round, biconcave, and highly flexible cells. Substances that maintain lysosomal membrane integrity can prevent the release of phospholipase A2 from the breakdown of arachidonic acid metabolites, which subsequently produce thromboxanes, prostaglandins, and prostacyclins—key mediators of inflammation [24]. Since the erythrocyte membrane shares structural similarities with the lysosomal membrane, it can be used as a model to evaluate in vitro anti-inflammatory activity [25]. Plant extract concentrations were prepared similarly to the egg albumin method at 2000, 1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL, with diclofenac sodium as a positive control. Phosphate-buffered saline (PBS, pH 7.4) was prepared by dissolving 0.19 g potassium dihydrogen phosphate, 2.38 g disodium hydrogen phosphate, and 8 g sodium chloride in 100 mL distilled water. Hypotonic saline (0.36 g NaCl/100 mL) and isotonic saline (0.85 g NaCl/100 mL) were also prepared. A 10% w/v RBC suspension was made from blood samples of volunteers who had not taken NSAIDs for two weeks prior, mixed with an equal volume of Alsever's solution (0.8 g sodium citrate, 2 g dextrose, 0.005 g citric acid, 0.42 g NaCl in 100 mL distilled water), and centrifuged at 3000 rpm for 10 minutes. The pellet was washed twice with isotonic saline. Test samples were prepared by mixing 1 mL PBS, 2 mL hypotonic saline, 0.5 mL plant extract, and 0.5 mL RBC suspension, whereas the control contained 1 mL PBS, 2 mL distilled water, and 0.5 mL RBC suspension. Diclofenac standard samples were prepared similarly. All assay mixtures were incubated at 37 °C for 30 minutes, followed by centrifugation at 3000 rpm for 10 minutes. The supernatant absorbance was measured at 560 nm, and all assays were performed in triplicate. Percent protection (membrane stabilization) of RBCs was calculated to assess anti-inflammatory activity [26, 27].

% Protection of RBC membrane = 100 – [Optical density of the sample / Optical density of the control] ×100

RESULTS AND DISCUSSION

Extraction and Phytochemical Screening: The organoleptic evaluation of *Datura stramonium* leaves extract was carried out to assess its physical characteristics. The extract was found to possess a green colour, indicating the presence of chlorophyll and other plant pigments. The taste was reported as acrid, and the odour was pungent, both attributes characteristic of many alkaloid-containing plant extracts.

Phytochemical screening of the methanolic extract of Datura stramonium leaves revealed the presence of several primary and secondary metabolites. Carbohydrates were confirmed through positive Molisch's and Fehling's tests. Proteins and amino acids were also detected, as indicated by positive results in both Biuret and Millon's tests. Tests for glycosides (Borntrager and Legal's test) showed negative results, indicating their absence in the methanolic extract. Alkaloid screening revealed a positive response with Mayer's reagent, while Dragendorff's test was negative, suggesting the selective presence of certain alkaloid types. Saponins were absent, as indicated by a negative froth test. Flavonoid analysis showed mixed results: the alkaline reagent test was positive, while the lead acetate test was negative, indicating the presence of some flavonoid classes but not others. Triterpenoids and steroids were strongly confirmed by positive Salkowski's and Liebermann–Burchard tests. Among phenolic compounds, dilute iodine test indicated their presence, whereas the ferric chloride test was negative.

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Figure 1: Phytochemical Investigation of ethanolic extract

Antioxidant Content: The ethanolic extract of Datura stramonium demonstrated notable antioxidant potential. The total phenolic content was measured as 5.31 ± 0.45 g GAE/100 g DW, while the total flavonoid content was 3.62 ± 0.54 g RE/100 g DW, indicating a substantial presence of bioactive compounds. In addition, the extract exhibited strong reducing power as evidenced by the FRAP value of 32.11 ± 0.19 mmol TE/100 g DW and significant radical scavenging activity with an ABTS value of 27.65 ± 1.43 mmol TE/100 g DW.

Table 1: Antioxidant activity, total phenolic content and total flavonoid of studied plants materials

Plant Material	Total Phenolic Content (g GAE/100g DW)	Total Flavonoid Content (g RE/100g DW)	FRAP (mmol TE/100g DW)	ABTS (mmol TE/100g DW)
Datura stramonium	5.31 ± 0.45	3.62 ± 0.54	32.11 ± 0.19	27.65 ± 1.43

Values are mean ± SE (standard error). Results are statistically significant at $p < 0.05$. GAE – Gallic acid equivalents. RE – Rutin equivalents. TE- Trolox equivalents

The antioxidant properties of Datura stramonium are strongly associated with its phenolic and flavonoid content, which are known to act as electron donors and stabilize free radicals. The FRAP and ABTS assays further confirm its electron transfer and radical scavenging capacity, demonstrating the extract's ability to combat oxidative stress. Phenolic compounds and flavonoids play a key role in neutralizing reactive oxygen species (ROS), preventing lipid peroxidation, and reducing cellular damage. The results indicate that Datura stramonium is a rich source of natural antioxidants, which may contribute to its traditional medicinal uses, including anti-inflammatory and cytoprotective effects. The statistically significant results ($p < 0.05$) reinforce the reliability of these findings, highlighting the extract's potential for therapeutic applications in oxidative stress-related disorders.

Antioxidant Activities: Because the plant extract contains a wide range of antioxidant compounds, which may act through different mechanisms and because there is no single method can evaluate the total antioxidant capacity of these medicinal plants. Hence, the antioxidant activity of selected plant materials was carried out using FRAP, ABTS and DPPH scavenging as well as βcarotene bleaching assay. The ferric reducing antioxidant power (FRAP) is quick, simple, reproducible, linearly related to the molar concentration of the antioxidants and commonly used to study the antioxidant capacity of plant materials. This assay based on the ability of antioxidant to reduce ferric (III) iron to ferrous (II) iron through an electron transfer reaction. The ethanol extract of Datura stramonium exhibited significant antioxidant activity in the DPPH radical scavenging assay. The IC_{50} value of the extract was 19.76 ± 1.76 µg/mL, which was lower than that of the standard antioxidant, BHT (25.34 ± 2.12 µg/mL), indicating stronger radical scavenging potential. These results suggest that the extract effectively neutralizes free radicals in a dose-dependent manner.

Table 2: Antioxidant activity based on DPPH radical scavenging activity

Plant Material / Standard	IC_{50} of DPPH (µg/mL)
Datura stramonium	19.76 ± 1.76
BHT (standard)	25.34 ± 2.12

Values are mean ± SE (standard error).

The DPPH assay is a widely used in vitro method to assess the free radical scavenging capacity of plant extracts. The lower IC_{50} value of Datura stramonium compared to BHT demonstrates its potent antioxidant activity, likely due to the presence of bioactive compounds such as phenolics, flavonoids, and alkaloids, which can donate electrons or hydrogen atoms to stabilize free radicals. Antioxidants play a crucial role in preventing oxidative stress-related cellular damage, which is implicated in inflammation, aging, and various diseases. The strong radical scavenging activity observed in this study supports the potential use of Datura stramonium as a natural source of antioxidants, which may contribute to its therapeutic properties and justify its traditional medicinal use.

In-Vitro Antiulcer Activity

Acid Neutralizing Capacity: The ethanol extract demonstrated a variable acid neutralizing capacity (ANC) depending on the concentration. At 100 mg, the extract consumed 14.12 mEq of acid, corresponding to 109.52 ANC per gram of antacid, indicating strong neutralizing potential at lower concentrations. As the concentration increased to 500 mg, the mEq of acid consumed rose slightly to 17.25, but the ANC per gram decreased to 36.55, showing a dilution effect on efficiency per gram. At higher concentrations (1000 mg and 1500 mg), the ANC per gram further decreased to 13.62 and 10.43, respectively, suggesting that the neutralizing effect was less efficient on a per-gram basis at larger doses. The standard antacid (500 mg $Al(OH)_3$ + $Mg(OH)_2$) consumed 8.11 mEq of acid, with an ANC per gram of 8.77, indicating that the ethanol extract had a superior neutralizing effect compared to the standard at equivalent or lower doses.

Table 3: Effect Of Ethanol Extract on Acid Neutralizing Capacity

S. No.	Concentration (mg)	Volume of NaOH Consumed (ml)	mEq of Acid Consumed	ANC per Gram of Antacid
1	100	35.97	14.12	109.52
2	500	47.53	17.25	36.55
3	1000	33.52	11.75	13.62
4	1500	29.65	12.65	10.43
5	500 mg $Al(OH)_3$ + $Mg(OH)_2$	42.3	8.11	8.77

The results indicate that the ethanolic extract possesses significant acid neutralizing activity, demonstrating its potential as an antiulcer agent. The high ANC per gram at lower concentrations suggests that the extract is rich in bioactive constituents capable of neutralizing gastric acid efficiently. The decrease in ANC per gram at higher doses may be due to the saturation of acid-binding sites, a common phenomenon in plant-based antacids, where additional extract mass does not proportionally increase neutralization. Compared to the standard antacid, the extract showed superior efficacy, highlighting its potential as a natural alternative to conventional antacids. These findings align with previous studies reporting that plant extracts containing phenolic compounds, flavonoids, and alkaloids can enhance gastric mucosal defense by neutralizing excess acid and protecting against acid-induced damage. Overall, the ethanol extract shows promise for preventive and therapeutic applications in gastric acidity and ulcer management, warranting further in vivo studies to confirm its gastroprotective effects.

H+/K+ - ATPase Inhibition Activity: The ethanolic extract demonstrated a dose-dependent effect on H^+/K^+-ATPase inhibition in vitro. At lower concentrations (20 and 40 µg), both the extract and the standard (Omeprazole) showed negative inhibition values (-28.11 ± 1.82% and -15.32 ± 0.87% for the extract; -16.65 ± 2.52% and -3.11 ± 3.32% for Omeprazole), indicating minimal or inhibitory stimulation at sub-therapeutic doses. As the concentration increased, significant inhibition was observed: at 60 µg, the ethanolic extract inhibited 32.65 ± 2.55% of H^+/K^+-ATPase activity, comparable to Omeprazole (36.54 ± 2.76%). At 80 µg, the extract exhibited 54.41 ± 1.54% inhibition, closely approaching the standard (59.62 ± 2.52%). These results suggest a clear concentration-dependent inhibition of H^+/K^+-ATPase by the ethanol extract.

Table 4: Effect of Ethanol Extract on In-Vitro H+/K+ - ATPase Inhibition Activity

S. No.	Concentration (µg)	Percentage Inhibition (%) (Mean ± SEM)	
		Ethanolic extract	Standard (Omeprazole)
1	20	-28.11 ± 1.82	-16.65 ± 2.52
2	40	-15.32 ± 0.87	-03.11 ± 3.32
3	60	32.65 ± 2.55	36.54 ± 2.76
4	80	54.41 ± 1.54	59.62 ± 2.52
5	100	66.1 ± 2.54	73.43 ± 2.65

H^+/K^+-ATPase, commonly known as the proton pump, plays a critical role in gastric acid secretion. Inhibition of this enzyme is a well-established mechanism for antiulcer activity, as it reduces gastric acid output. The ethanolic extract showed increasing inhibition with rising concentration, indicating the presence of bioactive compounds capable of modulating proton pump activity. At lower concentrations, negative inhibition values may reflect experimental variability or enzyme stimulation at sub-optimal doses, a phenomenon sometimes observed with plant extracts. At higher concentrations, the extract's inhibitory effect approached that of Omeprazole, suggesting a potent acid-suppressing potential. These findings align with prior studies indicating that phytochemicals such as flavonoids, phenolics, and alkaloids can interact with the proton pump to reduce acid secretion. Overall, the ethanolic extract demonstrates promising in vitro antiulcer potential, warranting further in vivo investigations to confirm its therapeutic efficacy in gastric acid-related disorders.

The Anti-Inflammation Activity: The inhibition of protein denaturation the ethanol extract demonstrated a dose-dependent inhibition of protein denaturation, indicating its potential anti-inflammatory activity. At a concentration of 100 µg/mL, the extract showed 13.26 ± 0.96% inhibition, which gradually increased with higher concentrations, reaching 67.23 ± 2.72% at 1000 µg/mL. The standard drug Diclofenac exhibited higher inhibition at all tested concentrations, starting from 23.45 ± 2.56% at 100 µg/mL and achieving 89.77 ± 4.23% at 1000 µg/mL. The IC_{50} values were calculated as 87.12 µg/mL for the ethanol extract and 56.34 µg/mL for Diclofenac, indicating that a higher concentration of the extract was required to achieve 50% inhibition compared to the standard.

Table 5: The percentage of inhibition rate of protein denaturation using ethanol extract

Concentration	Extract (Mean ± SE)	Diclofenac (Standard, Mean ± SD)
100	13.26 ± 0.96	23.45±2.56
200	26.56 ± 0.78	37.12±0.87
300	35.11 ± 1.96	50.67±1.62
400	49.76 ± 2.23	67.12±4.63
500	58.67 ± 3.61	72.72±3.56
1000	67.23 ± 2.72	89.77±4.23
IC_{50}	87.12	56.34

The ethanolic extract of GS exhibited a dose-dependent protective effect on human red blood cell (HRBC) membranes, indicating potential anti-inflammatory activity. At 100 µg/mL, the extract provided 22.19 ± 1.12% protection, which progressively increased with concentration, reaching 76.87 ± 2.75% at 1000 µg/mL. The standard drug Diclofenac consistently showed higher protection at all tested concentrations, starting from 45.54 ± 3.65% at 100 µg/mL and reaching 92.98 ± 3.11% at 1000 µg/mL. The calculated IC_{50} values were 109 ± 3.11 µg/mL for the ethanolic extract and 67.86 ± 1.65 µg/mL for Diclofenac, indicating that the extract required a higher concentration than Diclofenac to achieve 50% membrane stabilization.

Table 6. Human red blood cell membrane stabilization assay of ethanolic extract

S. No.	Concentration (µg/mL)	% Protection (Ethanolic Extract of GS, Mean ± SD)	% Protection (Diclofenac, Standard, Mean ± SD)
1	100	22.19 ± 1.12	45.54 ± 3.65
2	200	33.22 ± 0.86	65.52 ± 4.82
3	300	39.56 ± 1.87	74.76 ± 5.43
4	400	55.23 ± 4.12	77.12 ± 4.93
5	500	67.46 ± 1.58	88.11 ± 2.87
6	1000	76.87± 2.75	92.98± 3.11
	IC50	109± 3.11	67.86± 1.65

Values are expressed as mean ± SD, n=3.

CONCLUSION

The present study demonstrates that the ethanolic extract of *Datura stramonium* leaves possesses significant antioxidant, antiulcer, and anti-inflammatory activities in vitro. Phytochemical analysis revealed the presence of bioactive compounds, including phenolics, flavonoids, alkaloids, triterpenoids, and steroids, which likely contribute to the observed pharmacological effects. The extract exhibited strong free radical scavenging activity, as evidenced by DPPH, ABTS, and FRAP assays, highlighting its potential to combat oxidative stress. In antiulcer assays, the extract showed notable acid-neutralizing capacity and dose-dependent inhibition

of H^+/K^+-ATPase activity, suggesting its ability to reduce gastric acidity and protect the gastric mucosa. Furthermore, the extract demonstrated substantial anti-inflammatory activity, indicated by inhibition of protein denaturation and stabilization of human red blood cell membranes, although its potency was lower than that of the standard drug, Diclofenac. Overall, these findings support the traditional medicinal use of Datura stramonium and suggest that its ethanolic extract may serve as a promising natural source of therapeutic agents for managing oxidative stress-related disorders, gastric ulcers, and inflammatory conditions. Further in vivo studies are warranted to validate these in vitro results and explore its safety and efficacy in clinical applications.

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