Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

Phytochemical Screeing On Sida Spinosa Linn Leaves for in – Vitro Anti-Ulcer, Anti-Oxidant and Antiinflammatory Activities

T.Venkatachalam[1], K.Dhivya[1] ,P.Kalaiselvi[1], N.Senthilkumar[1], M.Dhanalakshmi[2]

[1]JKKMMRFs –Annai JKK Sampoorani ammal college of Pharmacy,B.Komarapalayam,Namakkal Dt,Tamil nadu,The Tamil nadu Dr MGR medical university, Chennai

[2]Dean, department of Pharmaceutical chemistry, SMVEC school of Pharmacy, Pondicherry

Correspondence address: Dr.T.Venkatachalam , M.Pharm,Ph.D Professor & HOD [1]JKKMMRFs –Annai JKK Sampoorani ammal college of Pharmacy,B.Komarapalayam Namakkal –Dt

(Received: 05 January 2026 Revised: 15 February 2026 Accepted: 05 March 2026)

KEYWORDS

Sida spinosa, Medicinal plant, Phytochemical constituents, Pharmacological activities, Traditional medicine

ABSTRACT:

Sida spinosa L., commonly known as prickly sida or spiny sida, is an important medicinal plant belonging to the family Malvaceae. It is a small, erect, perennial herb widely distributed in tropical and subtropical regions of Asia, Africa, and the Americas. The plant is characterized by its hairy stems, small yellow flowers, and spiny appearance, which contribute to its identification in the field. Sida spinosa has been traditionally employed in various systems of medicine, including Ayurveda, African traditional medicine, and folk healing practices, where it is used to treat ailments such as fever, inflammation, wounds, respiratory disorders, and gastrointestinal problems. Phytochemical screening of Sida spinosa has revealed the presence of a wide range of bioactive constituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, and steroids. These compounds are believed to be responsible for the plant's diverse pharmacological activities. Several experimental studies have demonstrated that extracts of Sida spinosa possess significant antimicrobial, anti-inflammatory, antioxidant, antipyretic, analgesic, antidiabetic, and wound-healing properties. In addition, the plant has shown potential in combating oxidative stress by scavenging free radicals, thereby contributing to its protective role in various degenerative diseases. The biological activities of Sida spinosa have been evaluated using both in vitro and in vivo models, with promising results that validate many of its traditional uses. Despite its extensive medicinal potential, scientific research on this species remains relatively limited compared to other members of the Sida genus. Further detailed studies on its phytochemistry, pharmacokinetics, toxicological profile, and clinical efficacy are essential to establish standardized formulations and to support its safe therapeutic application. In conclusion, Sida spinosa represents a valuable source of natural bioactive compounds with multiple pharmacological potentials. Continued investigation into this plant could lead to the discovery of novel natural products beneficial in modern drug development and complementary medicine.

INTRODUCTION:

Herbal medicine represents one of the oldest and most widely practiced systems of healthcare in human history, utilizing plants and plant-derived products for the prevention and treatment of diseases. Since ancient times, medicinal plants have played a central role in traditional healing systems such as Ayurveda, Siddha, Unani, and Traditional Chinese Medicine. Even in the modern era, the World Health Organization estimates that nearly 80% of the global population relies on herbal medicines either as a primary or complementary form of healthcare, particularly in developing countries[1]. The renewed interest in herbal drugs is driven by their natural origin, cultural acceptance, affordability, and comparatively lower incidence of adverse effects when used appropriately.

Medicinal plants are known to be rich sources of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, and steroids. These phytoconstituents are responsible for a wide range of pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-ulcer, antidiabetic, hepatoprotective, and immunomodulatory effects[2]. Unlike synthetic drugs, which often act on a

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

single molecular target, herbal medicines usually exert therapeutic effects through multiple pathways due to the synergistic action of their bioactive constituents. This multi-target approach is particularly advantageous in managing chronic and multifactorial disorders.

Extraction of bioactive compounds is a critical step in the scientific evaluation and development of herbal medicines. Extraction involves the separation of therapeutically active constituents from plant materials using suitable solvents and techniques. The efficiency of extraction depends on factors such as solvent polarity, extraction temperature, duration, particle size of the plant material, and chemical nature of the phytoconstituents[3]. Common extraction techniques include maceration, infusion, decoction, percolation, and Soxhlet extraction. Among these, Soxhlet extraction is frequently employed in phytochemical investigations due to its ability to extract a broad range of compounds efficiently. Standardization of extraction procedures is essential to ensure batch-to-batch consistency, reproducibility, and quality of herbal extracts.

Peptic ulcer disease is a common gastrointestinal disorder characterized by localized damage to the mucosal lining of the stomach or duodenum. It results from an imbalance between aggressive factors such as gastric acid, pepsin, Helicobacter pylori infection, and non-steroidal anti-inflammatory drugs, and defensive mechanisms including mucus, bicarbonate secretion, prostaglandins, and mucosal blood flow[4]. Lifestyle factors such as alcohol consumption, smoking, stress, and irregular dietary habits further contribute to ulcer development. Although synthetic anti-ulcer drugs such as proton pump inhibitors, H_2-receptor antagonists, and antacids are widely used, their long-term use is associated with adverse effects, drug interactions, high cost, and recurrence of ulcers after discontinuation[5]. This has created a strong demand for safer, cost-effective, and plant-based anti-ulcer agents.

Oxidative stress plays a significant role in the pathogenesis of peptic ulcers and inflammatory disorders. Excessive production of reactive oxygen species leads to lipid peroxidation, protein oxidation, and DNA damage, thereby compromising cellular integrity and delaying tissue healing. Antioxidants counteract oxidative stress by scavenging free radicals and enhancing endogenous defense mechanisms. Medicinal plants rich in flavonoids, phenolic compounds, and tannins exhibit strong antioxidant properties, which contribute to their gastroprotective and cytoprotective effects[6]. Hence, evaluation of antioxidant activity is an essential component of herbal drug research, particularly in diseases associated with oxidative damage.

Inflammation is a protective physiological response to tissue injury, infection, or irritation. However, chronic or uncontrolled inflammation contributes to the development of several pathological conditions, including ulcers, arthritis, cardiovascular diseases, and cancer. Synthetic anti-inflammatory drugs such as non-steroidal anti-inflammatory agents provide symptomatic relief but are associated with adverse effects such as gastric irritation, ulceration, and renal toxicity upon prolonged use. Plant-derived anti-inflammatory agents are considered safer alternatives, as they modulate inflammatory mediators while preserving mucosal integrity and physiological balance[7]. Therefore, identifying medicinal plants with combined antioxidant and anti-inflammatory properties is of great therapeutic importance.

Sida spinosa Linn., commonly known as prickly sida, belongs to the family Malvaceae and is widely distributed in tropical and subtropical regions. In traditional systems of medicine, different parts of the plant are used to treat fever, inflammation, ulcers, diarrhea, wounds, respiratory disorders, and urinary ailments. Phytochemical studies have reported the presence of alkaloids such as ephedrine, vasicine, and vasicinone, along with flavonoids, phenolic compounds, tannins, saponins, and phytosterols[8]. These constituents are believed to be responsible for the diverse pharmacological activities attributed to the plant.

Several experimental studies have demonstrated the antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and gastroprotective potential of Sida species. However, systematic scientific evaluation of Sida spinosa leaves, particularly focusing on in vitro anti-ulcer, antioxidant, and anti-inflammatory activities, remains limited. In vitro models provide a rapid, cost-effective, and ethically acceptable approach for preliminary screening of biological activities and understanding possible mechanisms of action.

Therefore, the present study aims to carry out phytochemical screening of Sida spinosa Linn. leaves

and to evaluate their in vitro anti-ulcer, antioxidant, and anti-inflammatory activities. This investigation seeks to provide scientific validation for the traditional use of Sida spinosa and to explore its potential as a source of safe and effective herbal therapeutic agents.

MATERIALS AND METHODS:

Collection of plant material:

The leaves of Sida spinosa were collected in the month of April from the gardens in Erode and nearby places in the morning & evening and authenticated by Dr. P. Radha., Research Officer (Botany), Sci-II, I/C Sidha Medicinal Plants Garden (Central Council For Research In Sidha), Ministry of Ayush, Govt. of India), Mettur Dam – 636 401 bearing a reference number S011125135S. The aerial parts of the plant were cleaned and dried under shade over night. Then these were subjected drying in a hot air oven at 40°C for 24 hours and subsequently ground in to a powder with a Preethi mixer grinder. The powdered material was stored in air tight poly bag till further use[9] .

Extraction:

The powdered plant material was subjected to extraction using the solvents; petroleum, chloroform, and ethanol. 500g of the powdered plant sample was weighed and transferred into the thimble of the Soxhlet apparatus for successive extraction. According to these polarity of the solvents, they were measured and poured into the thimble and round bottom flask. Then thimble is filted with the condense round bottom flask is placed in the heating mantle. These were kept for 48 hours and then filtered via filter paper. The crude extract gotten was weighed and stored in a vial bottle for analysis[10] .

Phyto chemical Screening:

Qualitative Analysis for the Petroleum ether, Chloroform, and Ethanol extract wasted for the following natural products like carbohydrates, proteins, amino acids, alkaloids, glycosides, steroids, saponins, and phenols by using different types of chemical tests.

In-vitro anti – ulcer activity:

Acid – Neutralizing Capacity:

The in-vitro anti-ulcer activity of the ethanolic extract of *Sida spinosa* Linn leaves was evaluated by determining its acid-neutralizing capacity (ANC). This method is based on the ability of the extract to neutralize gastric acid, thereby reducing acidity in the stomach. The procedure was carried out according to the United States Pharmacopoeia (USP) back-titration method with slight modifications[11].

Different concentrations of the ethanolic extract (100 mg/mL, 200 mg/mL, 500 mg/mL, and 1000 mg/mL) were prepared using distilled water. A standard antacid preparation containing aluminium hydroxide and magnesium hydroxide (500 mg/mL) was used as the reference standard. For each test and standard preparation, 5 mL of the sample was taken in a conical flask and the total volume was adjusted to 70 mL using distilled water. To this mixture, 30 mL of 1 N hydrochloric acid (HCl) was added and the contents were stirred continuously for 15 minutes to ensure complete reaction between the acid and the antacid or plant extract.

After incubation, 2–3 drops of phenolphthalein indicator were added, and the excess unreacted acid was titrated against 0.5 N sodium hydroxide (NaOH) until a stable pale pink color was observed, indicating the end point. The volume of NaOH consumed was noted. The acid-neutralizing capacity was calculated and expressed as milliequivalents (mEq) of acid neutralized per gram of extract.

The moles of acid neutralized is calculated by,

Total m Eq of acid Consumed = (vol. of HCl× Normality of HCl)– (vol. of NaOH × Normality of NaOH)

Acid Neutralizing Capacity (ANC) per Gram of Antacid = Mole of HCL Neutralized Grams of Antacid or Extract

A higher ANC value indicates stronger anti-ulcer potential due to greater acid neutralization capacity[12].

H+/K+- ATPase Inhibition Activity:

To prepare H+/K+- ATPase enzyme sample the fresh goat stomach has purchased from the local slaughter house, the gastric mucosa of the fundus was cut- off and opened, the inner layer of the stomach has scrapped out for the parietal cell. The parietal cell obtained from the stomach has homogenized in16m MTS is buffer with PH of 7.4, which has 10% Triton X-100 and centrifugAssessment of H+/K+ ATPase inhibition: Per-incubated for 60 min at 37 ºC for the reaction mixture of the sample containing 0.1ml of enzyme extract (300µg) and plant extract with different concentration (20µg,

40µg,60µg,80µg,100µg). There action was initiated by adding substrate 2m MATP (200µL), in addition to this 2m M MgCl2 (200µL) and 10mM KCl (200µL) has added. After 30 min of incubation at 37 ºC there action was stopped by 4.5% ammonium molybdate, and 60% per chloric acid was added and centrifuged at 2000 rpm for10min, and in spectro photo metrically inorganic phosphate was released and measured at 660nm by following the Fiske- Subbarow method.

Briefly, at10 min at room temperature, 1ml of supernatant 4ml of Milli pore water, 1 ml of 2.5% of ammonium molybdate, 0.4ml of ANSA was added. At 660nm inorganic phosphate, absorbance has been measured at various doses of the extract; the enzyme activity has been calculated as micro moles of Pi released per hour. Results were compared with the known anti-Ulcer PPA inhibit or Omeprazole and expressed as Mean ± SEM 16% enzyme inhibition has calculated using the formula[13]:

$$\text{Percentage inhibition} = \frac{\text{Activity(control)} - \text{Activity (test)}}{\text{Activity(control)}} \times 100$$

In- vitro anti- oxidant activity:

2,2- Di phenyl -1 –Picryl Hydrazyl (DPPH) Free Radical Scavenging Assay:

The antioxidant activity of plant extract was analyzed using the DPPH assay. Various concentrations (10µg/ mL, 20µg/mL, 30µg/mL, 40µg/mL, and50µg/mL) of the extract were mixed with 1mL of DPPH (0.1mM) in methanol and 450µg/mL of 50m MT ris -HC l buffer at pH7.4. The mixture was then incubated in a dark room for 30 minutes. The reduction in the quantity of the DPPH free radical was assessed by measuring the absorbance at 517nm. This measurement indicated the antioxidant capacity of the various plant extracts. Ascorbic acid was used as standard in this assay. By evaluating the absorbance at 517 nm, this assay determined the antioxidant activity of the sida spinosa plant extracts [42]. The percentage of the inhibition was determined from the following equation: Percentage inhibition =Absorbance of control - Absorbance of test sample X100 Absorbance of control Then Plant extract /Positive control concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition concerning control against concentration.

Hydroxyl free radical scavenging assay:

Freshly prepared solutions were used for the experiment. In are action mixture of 1.0 mL, the following components were added: 100µL of a 28mM solution of 2-deoxy - 2- ribose dissolved in phosphate buffer at a pH7.4, 500µL of a solution containing different concentrations of the plant extract (ranging from10 to50 µg), 200 µL of a 200 µM ferric chloride (FeCl3) and 1.04 mM ethylene di amine tetra acetic acid (EDTA) mixture in a 1:1 volume ratio, 100 µL of H2O2 (1.0mM), and 100µL of ascorbic acid (1.0mM). The reaction mixture was incubated for 1 hour at 37°C. The extent of deoxy ribose degradation after the incubation period, was determined by thio barbituric acid (TBA) reaction. The mixture was further incubated for 1 hour at 37°C, and the absorbance at 532nm was measured against a blank solution. For comparison, vitamin E served as the positive control, and ascorbic acid was use as the standard. By measuring the absorbance at 532 nm, the hydroxyl radical scavenging activity of the extract of sida spinosa was assessed. The percentage of hydroxyl radical scavenging activity was calculated according to the following formula:

% hydroxyl radical scavenging activity = [A0−(A1−A2] × 100 / A0

Where,

A0 – is the absorbance of the control without a sample.

A1 -is the absorbance after adding the sample and 2–deoxy – D- ribose.

A2 - is the absorbance of the sample without 2- deoxy - D- ribose.

Then percentage of inhibition was plotted against concentration, and from the graph IC50 was calculated. The experiment was repeated three times at each concentration.

In vitro anti- inflammatory activity:

Protein denaturation assay:

Egg albumin's original conformation is disrupted during denaturation, changing its Physical characteristics, and causing it to lose its functional activity. The egg albumin denaturation assay measures a drug or compound's capacity to prevent or lessen egg albumin Denaturation to evaluate it anti-inflammatory effects. The egg albumin

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

denaturation assay is based on the idea that substances with anti-inflammatory qualities may be able to stabilize protein structures and prevent denaturation, which is frequently linked to inflammation and tissue damage. As a result, agents or chemicals that significantly decrease the denaturation of egg albuminin this assay may have potential anti-inflammatory properties. One of the causes of inflammation is assumed to be protein denaturation. NSAIDs prevent protein denaturation and inhibit the COX enzyme at the same time. The different concentrations of the test sample can be incubated with egg albumin solution in controlled experimental conditions and let the reactions happen and then the determination of absorbance to calculate the percentage inhibition And then IC50 values can be calculated using Graph Pad Prism software[14].

The anti – inflammatory activity of unknown crude extracts Can be determined in vitro for inhibition of the denaturation of egg albumin (protein).

0.2mL of 12% egg albumin solution (from fresh hen's egg/ or commercially available egg albumin powder), 2mL of sample extract or standard (Diclofenac sodium) at varying concentrations, and 2.8 mL of phosphate buffered saline (pH7.4) were mixed to form are action mixture of a total volume of 5mL.

A total volume of 5 mL of the control was created by combining 2 mL of triple- distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline.

The reaction mixtures were then incubated at 37±2°C for 30 min and will be heated in a water bath at 70±2°Cfor 15 min.

After cooling, the absorbance was measured at 280 nm by a suitable UV/ Vis spectrophotometer using triple distilled water as the blank.

The following equation was used to determine the % inhibition of protein denaturation,

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Fresh hen's eggs or egg albumin powder that is readily accessible in stores can be used to make a 1% egg albumin solution. Making egg- albumin solution using afresh hen's egg properly Involves carefully cracking an egg, transferring 1mL of the translucent portion to100 mL of w/V distilled water, and stirring thoroughly . The clear component of the egg is called egg albumin. The water should be cold when making the solution .Water will coagulate if it is heatedtoboil[15].

RESULTS AND DISCUSSION:

Phytochemical analysis:

Qualitative Analysis for the Petroleum ether, Chloroform, and Ethanol extracts of Sida spinosa was tested for the following natural products like carbohydrates, proteins, amino acids, alkaloids, glycosides, steroids, saponins, and phenols by using different types of chemical tests. The results were tabulated as follows:

Figure No: 1 Phytochemical Analysis of the plant extracts

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

Table No 1: Qualitative analysis of Plant Extracts.

Qualitative Tests	Petroleum Ether extract	Chloroform Extract	Ethanol Extract
Carbohydrates	+	+	-
Proteins	+	+	-
Lipids	+	+	-
Glycosides	+	+	+
Alkaloids	+	+	+
Steroids	-	-	+
Flavanoids	+	+	+
Phenols	-	-	+
Saponins	+	+	+

+ (Positive sign) indicates the Presence of the chemical constituents

- (Negative sign) indicates the Absence of the chemical constituents

The phytochemical screening of Sida spinosa extracts (Petroleum ether, Chloroform, and Ethanol) revealed the presence of various biologically active compounds. Carbohydrates, proteins, lipids, glycosides, alkaloids, flavonoids, and saponins were predominantly present in both petroleum ether and chloroform extracts, indicating that these solvents are efficient in extracting a wide range of phyto constituents. However, ethanol extract showed a slightly different profile, being positive for glycosides, alkaloids, flavonoids, phenols, saponins, and steroids, but negative for carbohydrates, proteins, and lipids. The presence of phenols and steroids specifically in the ethanol extract suggests that ethanol is more effective in extracting polar bioactive compounds.

This phytochemical diversity reflects the therapeutic potential of Sida spinosa as many of the identified compounds are known for their pharmacological properties. For instance, flavonoids and phenols are well-known for their antioxidant and anti-inflammatory activities, while alkaloids and glycosides have significant antimicrobial potential.

In-vitro anti-ulcer activity:

Acid neutrilising capacity assay:

The neutralizing effect of the ethanolic extract of sida spinosa was studied for four concentration (100mg, 500mg, 1000mg, 1500mg) and standard Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2] (500mg). The results obtained investigate that theextractatconcentration100mg, 500mg, 1000mg, and 1500mg showed a significant reduction in acid neutralizing capacity (ANC), i.e., 110.5, 35.5, 11.75, and 9.3, respectively, as compared to standard Al(OH)3+Mg(OH)2 (500 mg) which is 15.7. The extract at a concentrationof1500 mg has beenfound to neutralizeacid more significantlyascompared to standard. The results have tabulated in Table 2 & Graph 1.

Table no 2: effect of ethanolic extract of *sida spinosa* on acid neutralizing capacity

S.no.	Concentration(mg)	Volume of NaOH Consumed (ml)	MEq of Acid Consumed	ANC per gram of Antacid
1	100	37.9	13.05	110.5

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

2	500	29.5	17.25	35.5
3	1000	39.5	9.75	11.75
4	1500	42	12	9.33
5	500mg (Al(OH)3+Mg(OH)2)	45.3	7.85	15.7

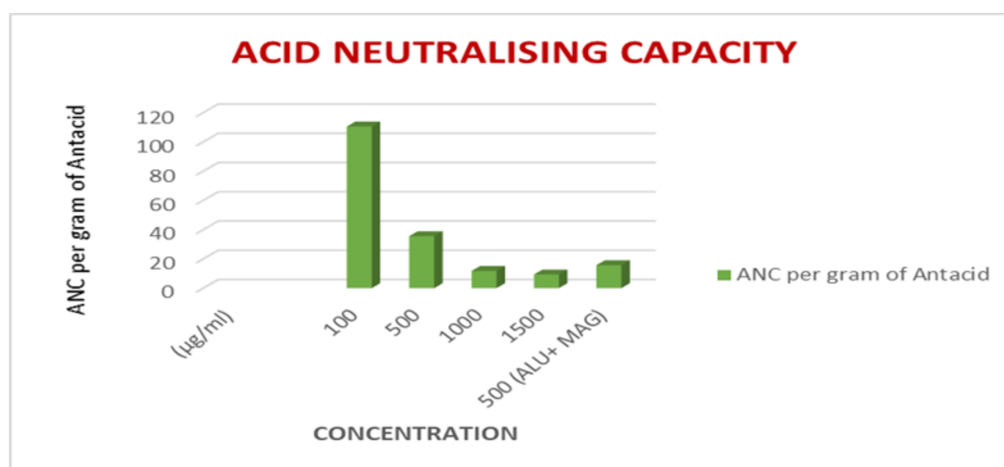

Figure 2: Effect of ethanolic extract of *sida spinosa* on acid neutralizing capacity

ANC was highest at the lowest extract concentration (100mg) and decreased as the dose increased. This inverse relationship could be due to the saturation of reactive functional groups or dilution of buffering compounds at higher volumes. However, the 100 mg extract showed significantly superior ANC (110.5 m Eq/g) than the standard antacid, indicating strong acid-neutralizing potential. The extract likely contains basic or weakly alkaline phyto constituents—such as flavonoids and alkaloids—that contribute to this effect. These compounds may. Directly bind to HCl, Raise local Ph in the gastric lumen, Act synergistically to buffer acid.

This result supports the traditional use of Sida spinosa for gastric discomfort, acidity, and ulcers. It also suggests that lower doses may be more effective, possibly due to optimized concentration of active constituents without dilution effects.

H+ /K+-ATPase Inhibition Activity:

The H+/ K+ - ATPase inhibition activity of aqueous extract at a various concentration (20µg, 40µg, 60µg, 80µg, 100µg) has compared with Omeprazole as standard. The extract significantly showed activity in a dose dependent manner. Maximum percentage inhibition of 62.18±0.54% has been observed for extract at a concentration of 100µg, and standard Omeprazole showed 69.56±1.72%. The results have been tabulated in Table 3 and Graph 2.

Table no 3:Effect of Aqueous extract of sida spinosa on in –vitro H+ /K+-ATPase Inhibition Activity

S.NO	Concentration(µg)	Percentage Inhibition(%)(Mean±SEM)	
		Standard Omeprazole	Ethanolic extract
1	20	-51.25±0.78	-30.12±0.26
2	40	-56.32±1.24	-18.84±1.86

3	60	36.58±1.58	31.64±0.68
4	80	58.62±0.24	55.36±1.54
5	100	69.56±1.72	62.18±0.54

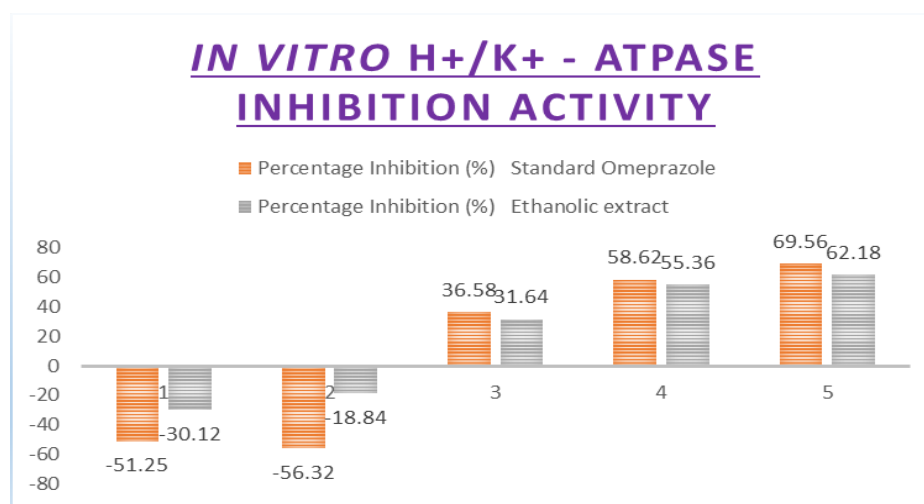

Figure 3: Effect of Aqueous extract of sida spinosa on in –vitro H+ /K+-ATPase Inhibition Activity

The extract showed a high degree of inhibition, with only a modest difference from omeprazole, the gold standard. This implies that Sida spinosa contains bioactive molecules capable of interfering with the enzyme activity of the gastric proton pump. Flavonoids and tannins are known to possess enzyme inhibitory properties, and their presence in the extract supports this pharmacological activity. Additionally, phenolic compounds may alter enzyme conformation, reducing activity. This activity complements the acid-neutralizing effect, giving the extract a dual-action anti-ulcer mechanism: Immediate relief through buffering gastric acid (ANC) &Long-term protection by reducing acid secretion (ATPase inhibition). Together, these findings strongly support the antiulcer potential of Sida spinosa and justify further research for development into herbal formulations for gastric disorders.

In-vitro anti oxidant activity:

DPPH assay:

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is a well-established method to assess the free radical scavenging ability of plant extracts. DPPH is a stable free radical that exhibits a deep violet color in solution, which turns pale yellow or color less upon reduction by an antioxidant. The ethanolic extract of Sida spinosa demonstrated a dose-dependent increase in DPPH radical scavenging activity.Maximum inhibition: 57.48% at 50 µg/ml. IC_{50} (concentration required to inhibit 50% of DPPH radicals) : 43.81µg/ml. In comparison, the standard (ascorbic acid) showed: Higher inhibition (61.41% at 50 µg/ml); Lower IC_{50} (22.18 µg/ml), indicating stronger antioxidant potential. The results have tabulated in Table 4& Graph 3.

Table no 3: Effect of Aqueous extract of sida spinosa on DPPH assay

S.NO	Concentration	Absorbance			Percentage inhibition of ethanolic extract of Sida Spinosa	Percentage inhibition of standard
		control	sample	standard		

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

1.	**10**	0.972	0.9854	0.520	13.78	46.50
2.	**20**	0.972	0.7412	0.568	23.74	41.56
3.	**30**	0.972	1.245	0.458	28.08	52.88
4.	**40**	0.972	0.4951	0.416	49.06	56.99
5.	**50**	0.972	0.4132	0.375	57.48	61.41
					Sample IC_{50}	43.81
					Standard IC_{50}	22.18

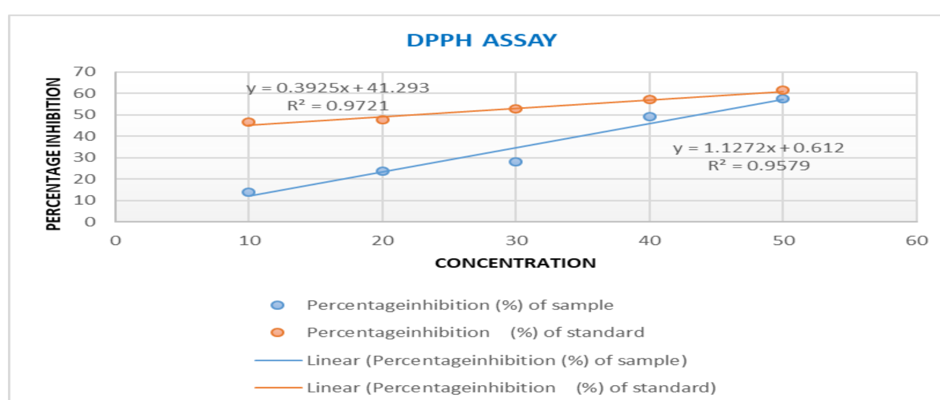

Figure 4: Effect of Aqueous extract of sida spinosa on DPPH assay

These findings indicate that the Sida spinosa ethanol extract contains compounds capable of donating hydrogen atoms or electrons to neutralize free radicals. The presence of flavonoids and phenolic compounds in the ethanol extract (as identified in phytochemical screening) likely contributes to this antioxidant effect. Though slightly less potent than ascorbic acid, the activity is still significant and highlights the therapeutic potential of Sida spinosa as a natural antioxidant source. Free radicals such as DPPH mimic oxidative stress in biological systems. The ability of Sida spinosa to scavenge these radicals suggests it may play a role in Preventing oxidative damage to biomolecules (DNA, proteins, lipids) and Slowing the progression of degenerative diseases like cancer, diabetes, and neurodegenerative disorders.

Hydroxyl radical scavenging assay:

The hydroxyl radical (•OH) is considered one of the most reactive and damaging ROS (reactive oxygen species) in the body. It can attack virtually all biological molecules and is associated with inflammation, cancer, and tissue injury. The hydroxyl radical scavenging assay evaluates the extract's ability to protect deoxy ribose from oxidative degradation caused by these radicals. Ethanolic extract showed increased scavenging activity with concentration Maximum inhibition: 73.97% at 100 µg/ml; IC_{50}:55.54µg/ml. Standard (Vitamin E) showed Inhibition: 90.72%at100µg/ml& IC_{50}: 70.01 µg/ml .Interestingly, while Vitamin E is a strong antioxidant, the Sida spinosa extract exhibited lower IC_{50}, suggesting better efficacy at lower concentrations in this assay. This could be due to Synergistic action of multiple phytochemicals (e.g., flavonoids, alkaloids, phenols). Higher solubility or binding affinity of the extract's

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

components in the assay medium. The results have tabulated in Table 5& Graph 4.

Table no 4: Effect of Aqueous extract of *sida spinosa* on Hydroxyl radical scavenging activity

S.No	Concentration(µg/ml)	Percentage inhibition (%) of sample	Percentage inhibition (%) of standard
1.	10	9.7	15.01
2.	20	17.94	25.79
3.	40	31.77	37.58
4.	60	45.3	49.04
5.	80	48.98	63.8
6	100	73.97	90.72
		Sample IC50	55.544
		Standard IC50	70.017

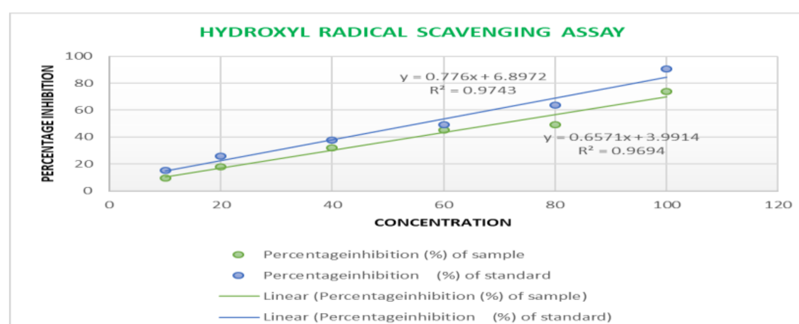

Figure 5: Effect of Aqueous extract of sida spinosa on Hydroxyl radical scavenging activity

This implies that Sida spinosa not only neutralizes free radicals like DPPH but also scavenges highly reactive species such as hydroxyl radicals, indicating broad-spectrum antioxidant potential. Biological Relevance: Hydroxyl radicals are generated via the Fenton reaction in vivo and are implicated in DNA strand breaks, Lipid peroxidation & Protein denaturation. Scavenging these radicals may reduce cellular injury in oxidative stress-associated disorders. Thus, the ethanol extract of Sida spinosa may serve as a protective agent against oxidative damage in chronic diseases.

In vitro anti inflammatory activity:

Protein denaturation assay:

The ethanolic extract of Sida spinosa demonstrated significant inhibition of protein denaturation, a standard marker for anti-inflammatory activity. At 50µg/ml, it achieved an inhibition of 82.35%, which is impressive though still lower than the standard (Diclofenac sodium) at95.78%.The calculated IC_{50} for the sample (25.574µg/ml) compared to Diclofenac (5.872µg/ml) confirms a potent, though less efficient, anti-inflammatory effect. The effectiveness of the extract could be attributed to the presence of flavonoids and phenolic compounds, which are known to inhibit inflammatory mediators and stabilize protein structures. The results have tabulated in Table 6 & Graph 5

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 581-593 | ISSN:2251-6727

Table no 4: Effect of Aqueous extract of *sida spinosa* on Protein denaturation assay

S.No	Concentration(µg/ml)	Percentage inhibition(%)ofsample (Ethanolic extract of *Sida spinosa*)	Percentage inhibition(%)of standard(Diclofenac Sodium)
1.	10	26.63	56.02
2.	20	48.92	61.13
3.	30	52.25	79.34
4.	40	68.94	86.41
5.	50	82.35	95.78
		Sample IC50	25.574
		Standard IC50	5.872

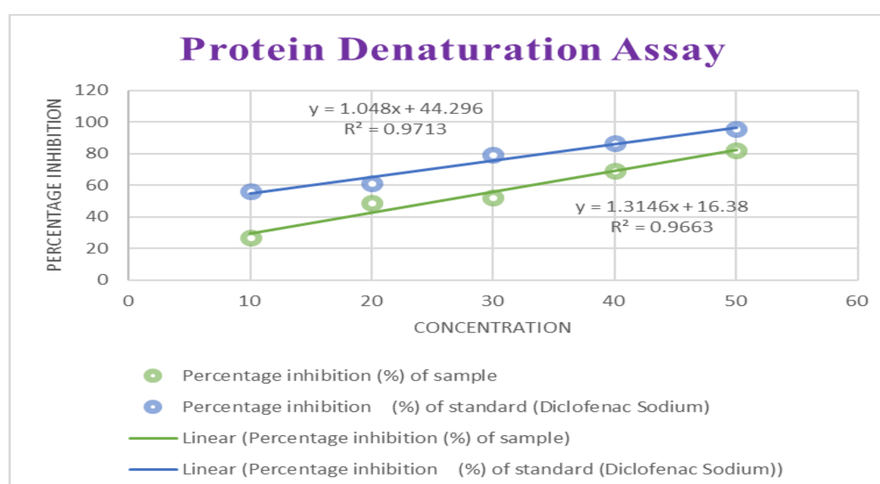

Figure 5: Effect of Aqueous extract of *sida spinosa* on Protein denaturation assay

CONCLUSION:

The present study extensively investigated the pharmacological potential of Sida spinosa using different solvent extracts (petroleum ether, chloroform, and ethanol). The research was designed to evaluate its phytochemical constituents and various in vitro biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anti-ulcer properties. The ethanol extract of Sida spinosa exhibited the richest phytochemical profile, particularly containing flavonoids, phenols, steroids, glycosides, saponins, and alkaloids. These compounds are recognized for their therapeutic effects, supporting the plant's use in traditional medicine. Both chloroform and ethanol extracts showed broad-spectrum antibacterial activity against Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, and Salmonella typhi. Ethanolic extract demonstrated stronger, dose-dependent inhibition, although not surpassing the standard antibiotic amoxicillin.

The ethanolic extract significantly inhibited protein denaturation in a concentration- dependent manner, reaching 82.35% inhibition at 50µg/ml with an IC_{50} of 25.57µg/ml, affirming its anti-inflammatory potential. In both DPPH and hydroxyl radical scavenging assays, the ethanol extract demonstrated substantial free radical

scavenging effects, with: DPPH IC_{50}: 43.81µg/ml; Hydroxyl radical IC_{50}: 55.54µg/ml These values indicate the plant's role in oxidative stress mitigation, likely due to its phenolic and flavonoid content. The extract neutralized acid significantly better than the standard at lower doses, with ANC of 110.5mEq/g at 100 mg/ml, outperforming aluminum-magnesium hydroxide combination. The ethanolic extract inhibited proton pump activity up to 62.18% at 100µg/ml, comparable to standard omeprazole (69.56%), suggesting both symptomatic and preventive anti-ulcer potential.

The findings of this study substantiate the traditional medicinal claims of Sida spinosa. The ethanolic extract, in particular, demonstrated that Strong phytochemical richness, Potent antioxidant and anti-inflammatory activity, Broad antibacterial effects, Effective acid-neutralizing and proton pump inhibitory properties. These activities are largely attributed to the presence of bioactive compounds such as flavonoids, alkaloids, phenols, and glycosides. Collectively, this work confirms that Sida spinosa is a multi-target therapeutic plant with significant pharmacological relevance. While these in vitro results are promising, further research is essential to Isolate and characterize the active compounds, Validate efficacy and safety through in vivo studies and Explore formulation strategies for clinical application. Hence, Sida spinosa holds potential as a natural, multi-functional remedy in the development of future plant-based pharmaceuticals, particularly for infections, inflammation, oxidative stress, and gastric disorders.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

ACKNOWLEDGMENTS:

REFERENCES:

1. Bhatt G, Kurmi P. New addition of <i>Sida spinosa</i> L. (Malvaceae) to the flora of Nepal. Banko Janakari [Internet]. 1970 Jan 26 ;20(2):53-54.doi.org/10.3126/banko.v20i2.4804.
2. Darwish FMM, Reinecke MG. Ecdysteroids and other constituents from Sida spinosa L. Phytochemistry [Internet]. 2003 Mar 25;62(8):1179–84. DOI: 10.1016/s0031-9422(03)00021-9
3. Rodrigues FC, De Oliveira AFM. The genus Sida L. (Malvaceae): An update of its ethnomedicinal use, pharmacology and phytochemistry. South African Journal of Botany [Internet]. 2020 Jun 26;132:432–62. doi.org/10.1016/j.sajb.2020.04.030
4. Jayasri P, A. Elumalai , Narendra Naik D and Kalugonda Murali Krishna, In-vitro antioxidant activity of Sida spinosa Linn., Journal Natural Products Plant Resources., 2011, 1 (4):35-39.
5. Is MdE, Naznin Ara Khatune, Md. Ekramul Haque. In vitro Antibacterial Activity of the Extracts and a Glycoside from Sida rhombifolia Linn. Journal of Medical Sciences(Faisalabad) [Internet]. 2002 Apr 15;2(3):134–6. DOI:10.3923/jms.2002.134.136
6. Kulkarni A, Vyawahare N, Patil D. Effect Of Hydroalcoholic Extract Of Sida Spinosa L. On 2,4,6-Trinitro-Benzenesulfonic Acid Induced Ulcerative Colitis In Rats. International Journal of Pharmaceutical Sciences and Research [Internet]. 2021 Jan 1;12(1). DOI: 10.13040/IJPSR.0975-8232.12(1).450-58
7. Monika K, Srivastava SK, Singh AK. Chemistry and pharmacology of genus Sida (Malvaceae) - a review. Journal of Medicinal and Aromatic Plant Science. 2002;24:430- 440.
8. Deepak Sharma, Richa Mishra and Manmeet Singh Saluja,A brief overview of the ethno medical, pharmacological, and phytochemical uses of Sida spinosa, International Journal of Medical, Pharmacy and Drug Research (IJMPD), 2023 October 7(5):15-19
9. Whitemore, T. C. 1979. Malvaceae. In An Enumeration of the Flowering Plants of Nepal Volume II. (eds.) Hara, H. and Williams I.H.J. British Museum (Natural History), London, U. K., 66-68.
10. MayoClinic: Peptic Ulcer- Symptoms and Causes.
11. Naik N, Krishna KM, Jayasri P and Elumalai A: Evaluation of diuretic activity of Sida spinosa Linn leaves extract. Journal of Chemistry 2011; 3(6): 1004-8.
12. Narendra Naik D, Kalugonda Murali Krishna, Jayasri P and A. Elumalai, Evaluation of diuretic

activity of Sida spinosa Linn., leaves extract, Journal of Chemical and Pharmaceutical Research, 2011, 3(6):1004-1008.

13. Alamgir,A.N.M.(2017).Therapeutic Use of Medicinal Plants and Their Extracts: Volume1: Pharmacognosy. Springer.
14. SelvaduraiS , Phytochemical Screening of Sida spinosa Linn.(malvaceae), International Journal of ChemTech Research, 2017,10(7): 825-835.
15. Harborne,J.B.(1998).Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis (3rd ed.). Chapman & Hall.