INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

OPEN ACCESS

Research Article

Therapeutic Potential of *Hemidesmus Indicus*

Ashna Shiju [1], Dr. Parvathy Prasad*[2]

[1]*Research intern, Bioroot Exploration India Pvt Ltd, Thiruvananthapuram, Kerala, India.*
[2]*Founder/Managing Director, Bioroot Exploration India Pvt Ltd, Thiruvananthapuram, Kerala, India.*

ARTICLE INFO

Published: 01 May 2025
Keywords:
Hemidesmus indicus, phytochemicals, therapeutic activity, soxhlet extraction.
DOI:
10.5281/zenodo.15318543

ABSTRACT

The present study investigates the phytochemical constitution and therapeutic potential of aqueous extract of H.indicus leaves. Hemidesmus indicus, commonly known as Indian sarsaparilla, is an Indian medicinal plant renowned for its medicinal value. It is a twining, semi-erect shrub that grows in mesophilic to semi-dry conditions at an altitude of 600m. The plant contains various bioactive compounds which accounts for its therapeutic properties. Extracts of the plant exhibits significant pharmacological properties like anti-oxidant, anti-diabetic, hepatoprotective effects, wound healing activity, anti-ulcer, anti-inflammatory, anti-arthritic, anti-microbial, and many more. Qualitative and quantitative studies indicate the presence of phytochemical compounds like alkaloids, flavonoids, tannins, saponins, phenols and terpenoids. The leaf extract showed significant anti-oxidant, anti-inflammatory and anti-arthritic activity than that of standard. The anti-diabetic and anti-ulcer activities are maximum at the lowest concentration; however, the anti-microbial activity was not found on the extract. Wound healing activity was most significant at highest concentration (500 µg/ml). The extract also portrays notable hepatoprotective effects, with the lowest concentration exhibiting greater cell viability.

INTRODUCTION

Hemidesmus indicus, commonly referred to as Indian sarsaparilla is a traditional medicinal plant belonging to the family Apocynaceae [1]. The plant is a repository of numerous bioactive compounds like alkaloids, flavonoids, tannins, steroids, glycosides and phenolic compounds that are responsible for its therapeutic potential. The major pharmacological properties of the plant encompass anti-oxidant, anti-diabetic, anti-inflammatory, anti-cancerous, anti-ulcer, anti-arthritic, anti-fungal, hepatoprotectivity, neuroprotection, cardio protection, and wound healing [2,3,4]. The phytochemicals present in the plant can be extracted by soxhlet extraction using water as

***Corresponding Author:** Dr. Parvathy Prasad
Address: Founder/Managing Director, Bioroot Exploration India Pvt Ltd, Thiruvananthapuram, Kerala, India.
Email ✉: paruprasad@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

solvent. Oxidative stress is a major characteristic of chronic diseases that can be combated by improving the endogenous anti-oxidant defenses [2]. Phytochemicals like flavonoids, triterpenoids and phenolics present in the plant performs anti-oxidant property, thereby reduces the oxidative stress [5]. These phytochemicals are also valued for its anti-diabetic property. Diabetes is an intricate chronic metabolic disorder, caused by the impairment of regulation in blood glucose level, that leads to very serious health complications [6,7]. Treatment with the extract effectively reduces lipid peroxidation in liver and kidneys and reverses all the metabolic alterations imposed by diabetes [8]. Another therapeutic potential of the plant extract includes hepatoprotectivity. Compounds that have the potency to reduce inflammation and restore the function of liver are called as hepatoprotectants [9]. Hepatoprotectivity of *H.indicus* is mostly due to the presence of coumarin olignoids, such as hemidesmin-I and hemidesmin-II[10]. The plant extract also showed anti-arthritic activity. Arthritis is a complex disease caused by inflammation in the joints and are characterised by symptoms like pain, stiffness and deformities of the joints [11]. The anti-arthritic property is due to the presence of phytonutrients such as polyphenols, phenols, flavonoids and steroids present in the plant [12]. These compounds also demonstrate anti-inflammatory property. Inflammation is defined as a safeguarding response of the body against pathogens or injury [13]. Saponin, a phytonutrient in *H.indicus* have anti-inflammatory activity against edema induced by formalin [14]. Chronic inflammation can result in the impairment of mucosal lining of stomach that can lead to ulceration [15]. Peptic ulcer is considered as the imbalance of digestive acids and the mucosal protective layer of the stomach [16]. Extract of *H.indicus* have potential antiulcer activity, improving mucous secretion, thereby protecting the gastric mucosa and sub-mucosa from inflammatory reaction [17]. Antiulcer property of the plant is due to the presence of phytochemicals like alkaloids, tannins, phenols and saponins [16]. These compounds also have wound healing activity. Wound healing is a combined and complex sequence of several biochemical and cellular events [18]. The extracts of *H.indicus* have anti-oxidant, anti-inflammatory and anti-fungal properties which can contribute to wound healing by preventing infections, thereby stimulates wound healing activity [4,19]. Anti-fungal activity is another therapeutic application of *H.indicus* extract. A compound that can selectively eliminate fungal pathogens from host organism with minimal toxicity is known as an anti-fungal agent. *H.indicus* contain a phytonutrient called glycoside, that inhibits the adherence of microorganism to the host cell and thereby reduces its pathological effects [20]. The plant extract is potential for inhibiting the growth of fungal pathogens like *Candida albicans, Aspergillus niger* and *Aspergillus fumigatus* [21]. The study is intended to evaluate the therapeutic potential of leaf extract of *H.indicus* by exploring the presence of its phytochemicals, which may exhibit anti-oxidant, anti-diabetic, hepatoprotective, anti-arthritic, anti-inflammatory, anti-ulcer, wound healing properties, as well as antifungal effects.

MATERIALS AND METHODS:

Chemicals and Reagents

3-(4,5- dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide (SRL), Acarbose, Acetaminophen, Aluminium chloride (NICE), Ascorbic acid (HIMEDIA), Bovine Serum Albumin (SRL), Bromocresol green (MEDILISE), Chloroform (MEDILISE), Diclofenac (SIGMAALDRICH), Dinitrosalicylic acid, Diosgenin (SRL), Dulbecco's Modified Eagle Medium (HIMEDIA), Dulbecco's Phosphate Buffer Saline (HIMEDIA), Egg albumin

(MEDILISE), Ethanol, Ferric chloride (Kanton Laboratories), Folin – Ciocalteu (MEDILISE), Gallic acid (NICE), Gelusil, Gentamicin (HIMEDIA), Glacial Acetic acid (NICE), Hydrochloric acid (MEDILISE), Linalool, Methanol (NICE), Perchloric acid (MEDILISE), Phosphate Buffer Saline (HIMEDIA), Potassium ferrous cyanide, Potato Dextrose Agar (HIMEDIA), Quercetin (SRL),Silymarin, Sodium carbonate (NICE), Sodium hydroxide (Kanton Laboratories), Sodium potassium tartrate (MEDILISE), Sodium nitrate (Kanton Laboratories), Starch (SRL), Sulphuric acid (MEDILISE), Tannic acid (MEDILISE), Trichloroacetic acid (MEDILISE), Wagner's reagent (Kanton Laboratories), vanillin (NICE), α-amylase (Kanton Laboratories).

Instruments

Visible spectrophotometer (Thermo SCIENTIFIC), Water bath (ROTEK), Weighing machine (SHIMADZU), Phase contrast microscope, Biosafety cabinet Type - II B II (ROTEK), Water bath (ROTEK), Autoclave (FOURTECH), CO_2 Incubator (FORMA SERIES II WATER JACKET), soxhlet apparatus, Magnetic stirrer (Lab Companion).

Cell lines

L929 and HepG2 cell line (a gift from Bioroot Exploration India Pvt Ltd).

Preparation and extraction of the sample

Fresh leaves of *H.indicus* were collected from Thiruvananthapuram, Kerala, India. The leaves were washed, dried, powdered and transferred to a timble and placed for soxhlet extraction. 50 ml of double distilled water was added as solvent to a round bottom flask and the process was carried out at 80°C for 6 hrs. The extract was collected on the same flask and stored at 4°C for future use [22].

Qualitative screening of phytochemicals

Test for alkaloids

A few drops of Wagner's reagent were added to 1 ml of the sample. A reddish-brown precipitate was formed, which indicates the presence of alkaloids in the test sample [23,24].

Test for phenols

A few drops of $FeCl_3$ were added to 1 ml solution of the sample and mixed well by shaking. A bluish-black colour was formed after shaking which indicates the presence of phenols in the test sample [23,24].

Test for saponins

1ml of sample solution was mixed with 1ml distilled water and shaken vigorously. The foam formation after shaking indicated the presence of saponin in the sample [24].

Tests for flavonoids

2 ml of 2% NaOH were added to the sample and mixed well. A yellow colour was observed. A few drops of Dil.HCL were added to this mixture and the colour disappears. This showed the presence of flavonoid in the test solution [23].

Test for terpenoids

In 1ml of the sample, 2ml chloroform were added. To this mixture a few drops conc. H_2SO_4 were added along the side of the test tube and mixed well. The formation of a reddish-brown colour indicates the presence of terpenoids in the sample taken [24,25].

Test for glycosides

A few drops of aqueous NaOH were mixed with the sample solution. No chemical reaction occurred which indicates the absence of glycoside in the test solution.

Test for tannins

1ml of 10% NaOH were added to the sample and shaken vigorously. A slight emulsion was formed which indicates the presence of small concentration of tannin in the taken sample.

Test for coumarins

To the sample few drops of 10% NaOH and chloroform were added. No colour reaction formed, which indicates the absence of coumarin in the sample.

Test for steroids

10 ml of chloroform and conc.H_2SO_4 were added to the sample along the side of the test tube. No chemical reaction occurred; which confirmed that steroids are absent in the sample solution [24,26].

Quantitative estimation of phytochemicals

Alkaloids

5 mL of phosphate buffer and 5 mL of bromocresol green solution were added to 1 mg/ml of the sample. The complex was extracted by transferring the mixture into a separating funnel to which 4ml of chloroform was added by shaking vigorously and collected in a test tube. Different concentrations of quercetin from 0.2 to 1 mg/ml were taken as standard. The absorbance was read at 470 nm [27].

Phenolic compounds

To 1mg/ml of sample 2ml of FC reagent and 7.5 % sodium carbonate diluted in 4ml double distilled water were added in the ratio 1:10. The test tube was then kept at dark for incubation for about 30 mins. Different concentrations of gallic acid from 10 to 100 µg/ml were taken as standard. The absorbance was read at 760 nm using a visible spectrophotometer [28].

Saponins

To 1mg/ml of sample 250µl of the reagent containing 0.8%vanillin dissolved in 1ml ethanol were added along with 2.5ml of conc.H_2SO_4. The test tube was incubated for 15 mins at 60°C in a water bath and allowed to cool down at room temperature. Different concentrations of diosgenin from 20 to 100 µg/ml were taken as the standard. The absorbance was read at 544nm using a visible spectrophotometer.

Flavonoids

To 1mg/ml of sample 2ml of 5% sodium nitrite was added and kept at dark for incubation for 5 mins. 3ml of 10% $AlCl_3$ along with 2ml of 1M NaOH was added to the test tube and again the tube was kept for incubation at dark for 5 minutes. After incubation the solution was made upto 10ml by adding distilled water and again the tube was incubated at dark for 10 mins. Different concentrations of quercetin from 200 to 1000 µg/ml were taken as the standard. The absorbance was read at 510 nm using a visible spectrophotometer [29].

Terpenoids

To 100µl of 1mg/ml of sample 150µl of reagent containing 0.055g of vanillin dissolved in 1.1ml of 5% glacial acetic and 500µl of perchloric acid were added. The test tube was then incubated in a water bath for 45 mins at 60°C, and later cooled under running tap water. After cooling 2.25 ml of glacial acetic acid were added to this mixture and the absorbance was read at 548 nm using a visible

spectrophotometer. Different concentrations of linalool from 200 to 1000µg/ml were taken as standard.

Tannins

To 1mg/ml of sample 0.5ml of FC reagent and 35 % Na_2CO_3 diluted in 1ml of double distilled water were added in the ratio 1:1. The test tube is then kept at dark for incubation for 30 mins. Different concentrations of tannic acid from 200 to 1000 µg/ml were taken as the standard. The absorbance was read at 700 nm using a visible spectrophotometer [30].

Anti-oxidant property

Anti-oxidant property of the leaf extract is evaluated using FRAP assay. Different concentrations of the sample (25, 50, 100, 200, 500 µg/ml) were prepared in solvent. 2.5 ml of sodium phosphate buffer (pH = 6.6) and 2.5 ml of ferric cyanide were added to sample and incubated for 20 mins in a boiling water bath at 60°C. After incubation 2.5 ml of TCA was added to the solutions. An aliquot of 2 ml was transferred to a fresh test tube and 2ml of distilled water and 0.5 ml of ferric chloride was added to it. Absorbance was read at 593 nm after incubating for 10 minutes at room temperature. Ascorbic acid was taken as the standard with same concentrations as that of the extract [31,32].

Anti-diabetic property

Anti-diabetic property of the leaf extract is evaluated using α-amylase enzyme. Different concentrations of the sample (25, 50, 100, 200, 500 µg/ml) were prepared in solvent. 100 µl of sodium phosphate buffer and 100 µl of α-amylase were added to each test tubes and incubated for 10 mins at RT. It is followed by adding 1 ml of starch and again incubated for 10 minutes. Subsequently, 1 ml 3,5-Dinitrosalicylic acid (DNS reagent) was added and tubes were incubated in a boiling water bath for 10 mins. After cooling, 100 µl of distilled water was added and the absorbance was read at 540 nm using a visible spectrophotometer. Acarbose were taken as the standards at the same concentrations as that of the sample [33].

% inhibition = [(AC-AS)/AC] x 100, where AC is the absorbance of the control and AS is the absorbance of the sample.

Anti-inflammatory property

Anti-inflammatory property of the leaf extract is evaluated using protein denaturation method. Different concentrations of the sample (25, 50, 100, 200, 500 µg/ml) were prepared in solvent. 200 µl BSA and 1.8 ml PBS buffer (Ph = 6.4) were added to the sample and incubated for 20 mins at RT, followed by 5 mins of incubation in a boiling water bath at 70°C. After incubation, the tubes were cool down and absorbance was measured at 660 nm using visible spectrophotometer. Diclofenac was taken as the standard with the same concentrations as that of the sample [34].

% inhibition = [(ACAS)/AC] x 100, where AC is the absorbance of the control and AS is the absorbance of the sample.

Anti-microbial property

The anti-microbial activity of *H.indicus* is evaluated using agar well diffusion method. Two bacterial species, *Bacillus cohnii* and *Staphylococcus aureus* and two fungal species *Aspergillus niger* and *Candida albicans* were used for the test. The bacterial species were inoculated in a nutrient broth whereas the fungal species are inoculated in potato dextrose agar (PDA), both are incubated in an incubator for 24hrs. Nutrient agar and PDA were prepared and autoclaved as the

media, and poured into sterilized petri plates, on which the respective microbes are plated after incubation. Wells were made using pipette tips and different concentrations of the sample (25, 50, 100, 200, 500 µg/ml) were poured into it. An antibiotic, gentamicin was used as the positive control and autoclaved distilled water was used as the negative control. The plates were then incubated for microbial growth.

Anti-arthritic assay

The anti-arthritic activity of *H.indicus* was assessed using protein denaturation method. Different concentration of the sample (25, 50, 100, 200, 500 µg/ml) were prepared and made-upto 1 ml using the solvent. 200 µl of egg albumin were added to the extract, along with 2.8 ml of PBS buffer (pH = 6.4). After mixing all the reagents, the tubes were incubated for 15 mins in an incubator and again for 5 mins in a boiling water bath at 70°C. After cooling down at room temperature, the absorbance was read at 660 nm using visible spectrophotometer. Diclofenac was taken as standard with the same concentration as that of the sample.

% inhibition = [(ACAS)/AC] x 100, where AC is the absorbance of the control and AS is the absorbance of the sample.

Wound healing property

Wound healing activity of *H.indicus* was determined using scratch assay. 0.3 x 106 cells/ml of L929 cell line were seeded in a 6 well plate and incubated until they reach a confluency of 80 %. The culture media was discarded after incubation and a scratch was made on each well using a sterile micropipette tip. Different concentrations of the sample (25, 50, 100, 200, 500 µg/ml) were added to the wells, after adding 1 ml of fresh media. One of the remains untreated, leaving it as the control. The wound healing property was detected by measuring the area of gap closure after a period of 24 hrs [35].

% of wound closure = (A0h – A24h)/A0h x 100 where A0h and A24h is the area of wound at 0th hour and 24th hours.

Hepatoprotective property

Hepatoprotectivity of leaf extract was detected using MTT assay against HepG2 cell line. The cells were plated on a 24 well plate and incubated until the cells reaches its maximum confluency. After incubation, 1 ml of fresh media was added after discarding the used one. The wells were treated with Acetaminophen, except for two wells, that are selected as vehicle control, and the plated were again incubated for 24 hrs. The media was replaced later with fresh media containing different concentrations of the test sample and standard, and the wells were incubated for another 24 hours. On the next day, 700 µl of MTT and 100 µl of DPBS were added to the wells along with 500 µl of fresh media after removing the old one. The plates were incubated for 3 hrs and subsequently 200µl of DMSO was aspired to the medium for dissolving the formazan crystals formed by the reduction of MTT. After 20mins the absorbance was read at 570 nm using a visible spectrophotometer. Silymarin was taken as the standard drug.

Anti-ulcer property

Anti-ulcer activity of leaf extract of *H.indicus* was determined using acid neutralizing capacity method. 5 ml of different concentrations of the sample (25, 50, 100, 200, 500 µg/ml) were prepared in 5 conical flask and are made upto 70 ml using distilled water. The solution was stirred for 1 min, and later for 15mins after adding 30 ml of dil HCL, using a magnetic stirrer. After stirring,

2 - 3 drops of phenolphthalein was added to the mixture, and then titrated against 0.5N NaOH until a pink colour appeared. 5 ml of Gelusil (2.5g) was taken as the standard - anti-ulcer agent.

Extraction of *H. indicus*

Fresh leaves of *H.indicus* were dried, powdered, and placed for soxhlet extraction, using water as solvent (Figure 1 and 2).

RESULTS

Figure 1: Fresh, Dried and Powdered Forms of *Indicus* Leaves

Figure 2: Extract of *H. indicus* leaf

Phytochemical analysis of aqueous extract of *H.indicus*

Phytochemical analysis of leaf extract of *H.*indicus showed presence and absence of various bioactive compounds (Figure 3 and Table 1).

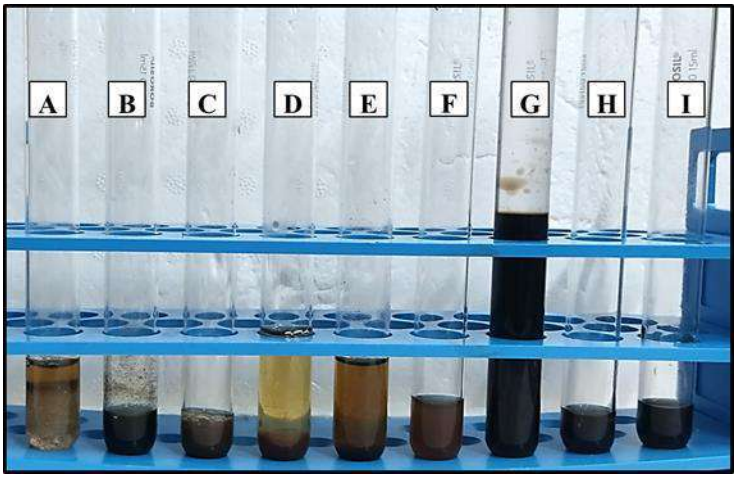

Figure 3: Qualitative Analysis of Phytochemicals (A- Terpenoids, B- Phenols, C- Alkaloids, D - Flavonoids, E- Tannins, F- Saponins, G- Steroids, H- Glucosides, I- Coumarin)

Table 1: Qualitative Analysis of Phytochemicals ('+++' – High Expression, '++' - Moderate Expression, '+' - Low Expression, '-' - Absent)

Phytochemicals	Results		
Alkaloids	+	+	-
Phenols	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Glycosides	-	-	-
Tannins	+	-	-
Coumarins	-	-	-
Steroids	-	-	-

Quantitative phytochemical analysis

Alkaloids

Quantity of alkaloids from the aqueous extract of *H.indicus* leaves is calculated using the equation y = 0.256x + 0.882 from the standard curve of quercetin (Figure 4, Figure 5, Table 2, and Table 3).

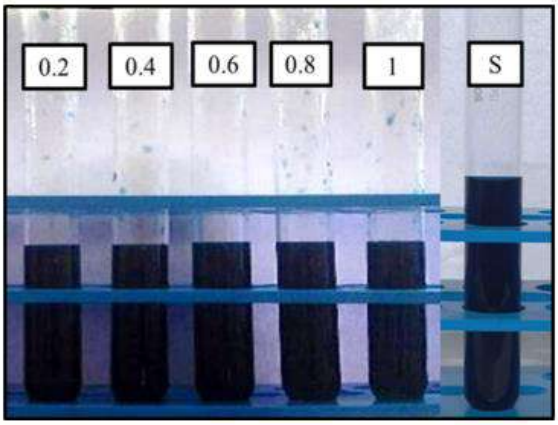

Figure 4: Different concentrations of standard (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, 1mg/ml). S :- Sample

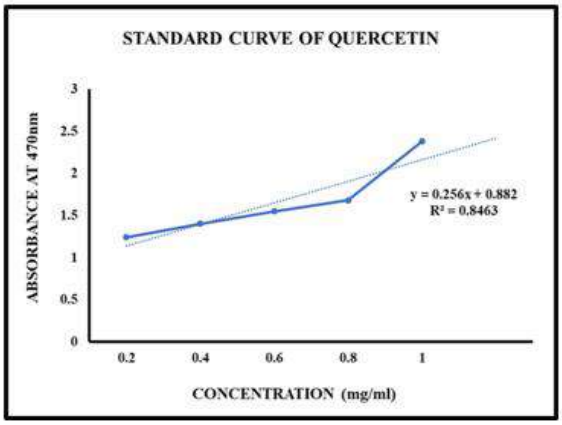

Figure 5: Standard curve of quercetin

Table 2: Different Concentrations and Absorbance of Quercetin

=	Absorbance at 470 nm
0.2	1.24
0.4	1.40
0.6	1.55
0.8	1.68
1	2.38

Table 3: Quantity of alkaloids in leaf extract

Phytochemical	Quantity
Alkaloids	0.998mg/ml

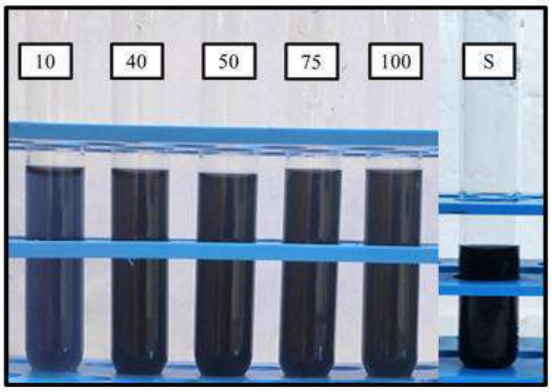

Figure 6: Different concentrations of standard (10µg/ml, 40µg/ml, 50 µg/ml, 75µg/ml, 100µg/ml). S: - Sample

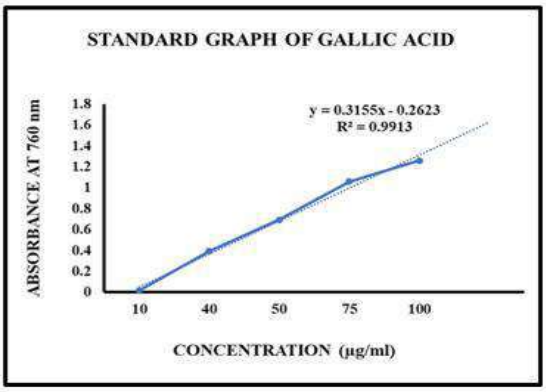

Figure 7: Standard curve of gallic acid

Table 4: Different concentrations and absorbance of gallic acid

Concentrations (µg/ml)	Absorbance at 760 nm
10	0.016
40	0.393
50	0.692
75	1.060

Table 5: Quantity of phenolic compounds in leaf extract

Phytochemical	Quantity

Phenols

The quantity of phenol from aqueous extract of *H.indicus* leaves is calculated using the equation y = 0.3155x - 0.2623 from the standard curve of gallic acid (Figure 6, Figure 7, Table 4, and Table 5).

Phenolic compounds	2.8103µg/ml

Saponins

Quantity of saponins from the aqueous extract of *H.indicus* leaves is calculated using the equation y = 0.1172x + 0.0318 from the standard curve of diosgenin (Figure 8, Figure 9, Table 6, and Table 7).

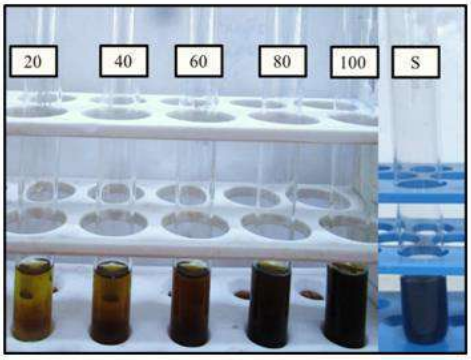

Figure 8: Different concentrations of standard (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml). S :- Sample

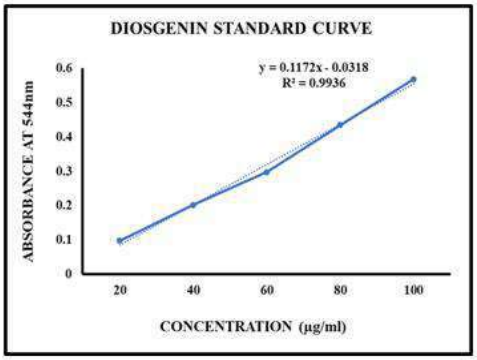

Figure 9: Standard curve of diosgenin

Table 6: Different Concentrations and Absorbance of Diosgenin

Concentrations (µg/ml)	Absorbance at 544 nm
20	0.098
40	0.202
60	0.297
80	0.434
100	0.568

Table 7: Quantity Of Saponins in Leaf Extract

Phytochemical	Quantity
Saponins	3.271µg/ml

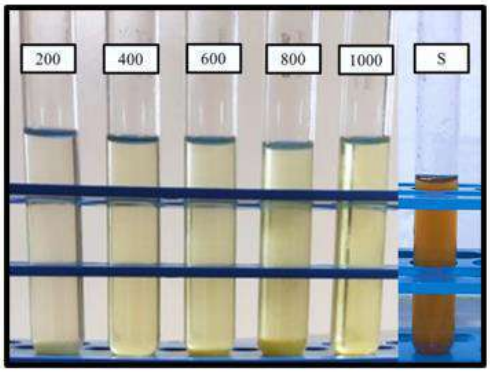

Figure 10: Different concentrations of standard (200µg/ml, 400µg/ml, 600 µg/ml, 800µg/ml, 1000µg/ml). S :- Sample

Table 8: Different Concentrations and Absorbance of Quercetin

Concentrations (µg/ml)	Absorbance at 510 nm
200	0.718
400	1.158
600	1.501
800	1.912

Table 9: Quantity Of Flavonoids in Leaf Extract

Phytochemical	Quantity
Flavonoids	0.534µg/ml

Flavonoids

The quantity of flavonoids from aqueous extract of *H.indicus* leaves is calculated using the equation y = 0.45x + 0.226 from the standard curve of quercetin (Figure 10, Figure 11, Table 8, and Table 9).

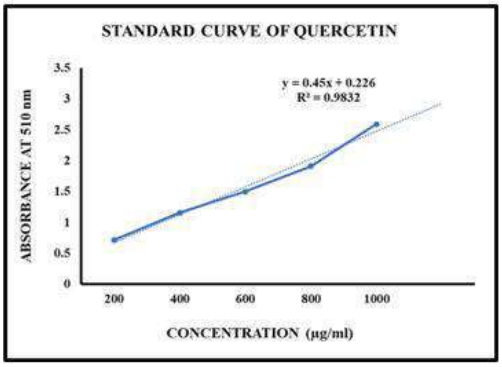

Figure: 11. Standard curve of quercetin

Terpenoids

The quantity of terpenoids from aqueous extract of *H.indicus* leaves is calculated using the equation y = 0.1362x + 0.1094 from the standard curve of linalool (Figure 12, Figure 13, Table 10, and Table 11).

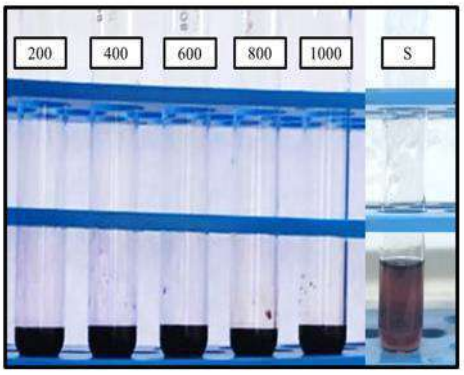

Figure 12: Different concentrations of standard (200µg/ml, 400µg/ml, 600 µg/ml, 800µg/ml, 1000µg/ml). S :- Sample

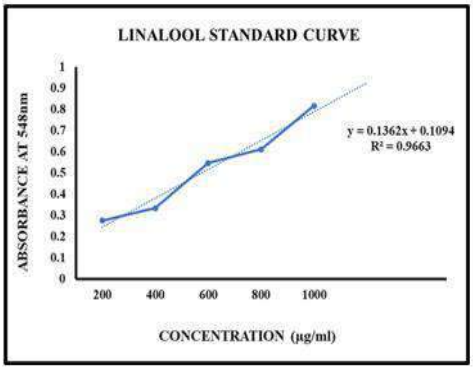

Figure 13: Standard curve of linalool

Table 10: Different concentrations and absorbance of linalool

Concentrations (µg/ml)	Absorbance at 548 nm
200	0.276
400	0.335
600	0.548
800	0.613

Table 11: Quantity Of Terpenoids in Leaf Extract

Phytochemical	Quantity
Terpenoids	0.7898µg/ml

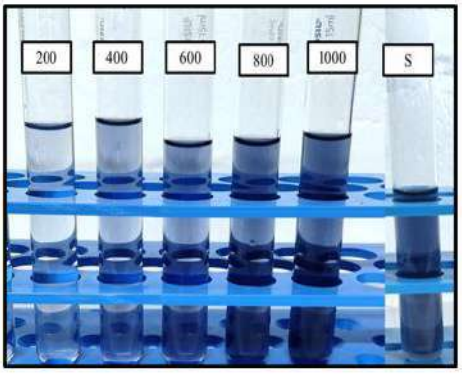

Figure 14: Different concentrations of standard (200µg/ml, 400µg/ml, 600 µg/ml, 800µg/ml, 1000 µg/ml). S :- Sample

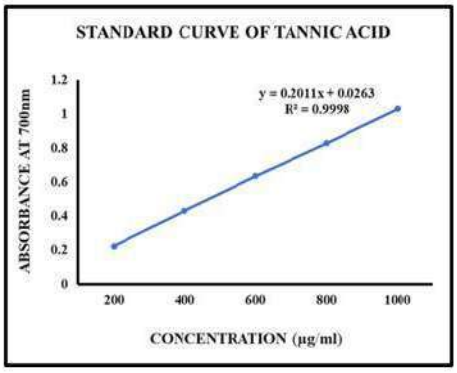

Figure15: Standard curve of tannic acid

Table 12: Different concentrations and absorbance of tannic acid

Concentrations (µg/ml)	**Absorbance at 700 nm**
200	0.223
400	0.431
600	0.636
800	0.828

Tannins

The quantity of tannins from aqueous extract of *H.indicus* leaves is calculated using the equation y = 0.2011x + 0.0263 from the standard curve of tannic acid (Figure 14, Figure 15, Table 12, and Table 13).

Table 13: Quantity Of Tannins in Leaf Extract

Phytochemical	Quantity
Tannins	0.8683µg/ml

Anti-oxidant property

The anti-oxidant activity of leaf extract was assessed using FRAP assay with ascorbic acid as the standard. The result showed that, the extract has more anti-oxidant property than the standard.

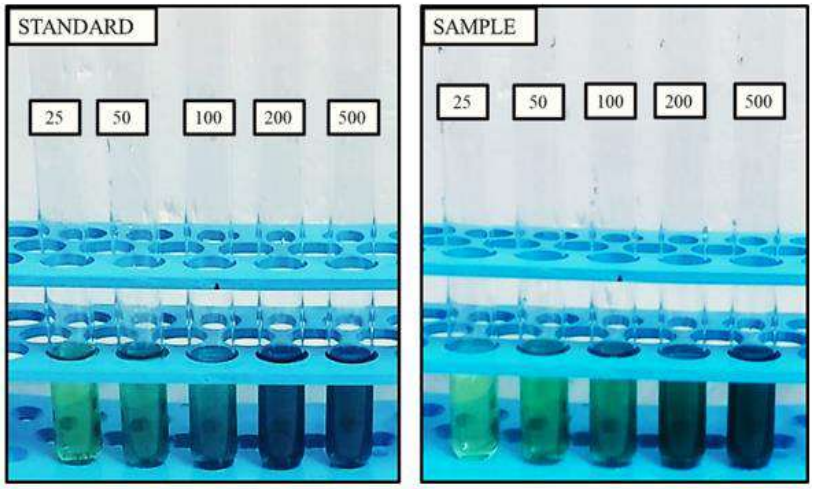

Figure 16: Anti-oxidant activity of ascorbic acid and leaf extract of *H.indicus*

Table 14: Absorbance Value of Ascorbic Acid and Sample

Concentrations (µg/ml)	Absorbance at 593 nm	
	Ascorbic acid	Sample
25	0.477	25
50	0.978	50
100	1.346	100
200	1.829	200
500	2.2	500

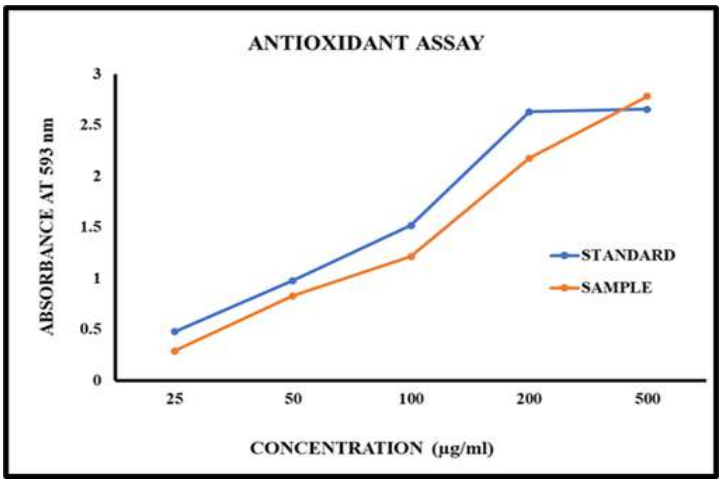

Figure 17: Absorbance of standard and sample at 593 nm

Anti-diabetic property

The anti-diabetic activity of leaf extract was evaluated using α-amylase enzyme, with acarbose as the standard. As the concentration of the standard increases, the percentage inhibition also increases, whereas the sample showed higher inhibition at lower concentrations.

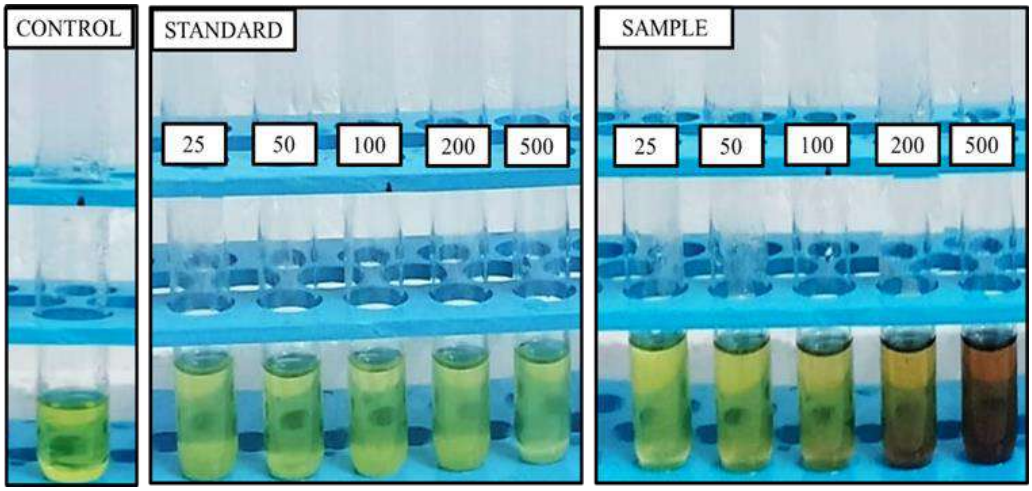

Figure 18: Anti-Diabetic Property of Acarbose and Leaf Extract Of *H. Indicus*

Table 15: Percentage Inhibition of Acarbose and Sample

Concentrations (µg/ml)	% of inhibition	
	Acarbose	
25	18.793	25
50	26.476	50
100	35.619	100
200	33.079	200

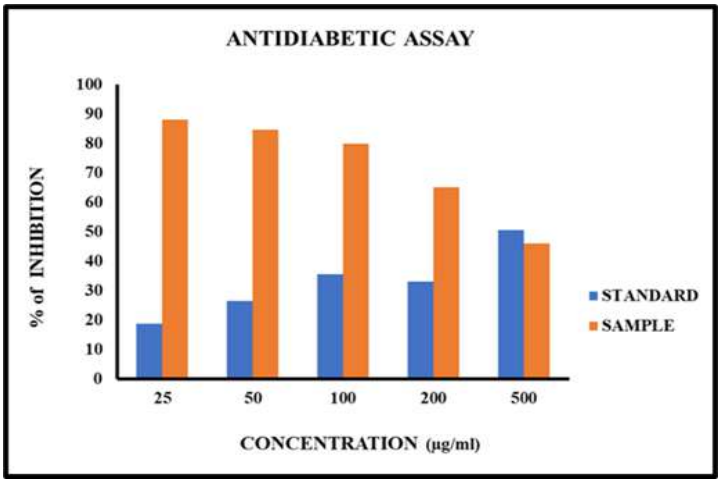

Figure 19: Percentage Inhibition of Standard and Sample

Anti-inflammatory property

The anti-inflammatory activity of leaf extract was determined using protein denaturation method. The results showed that both the standard and sample exhibited higher level of inhibition at the greatest concentration**.**

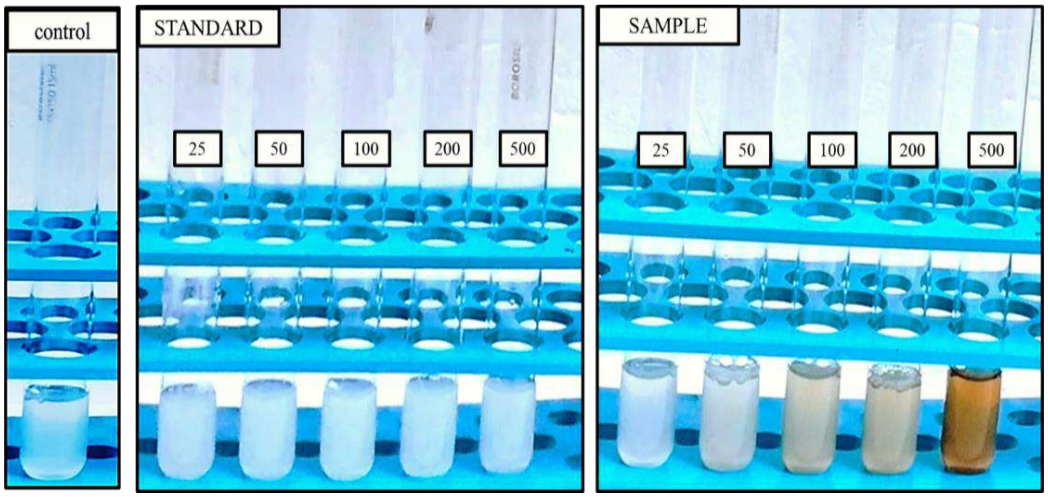

Figure 20: Anti-Inflammatory Activity of Diclofenac And Leaf Extract Of *H. Indicus*

Table 16: Percentage Inhibition of Diclofenac and Sample

Concentrations (µg/ml)	% of inhibition	
	Diclofenac	Sample
25	9.305	25
50	20.425	50
100	23.659	100
200	29.179	200

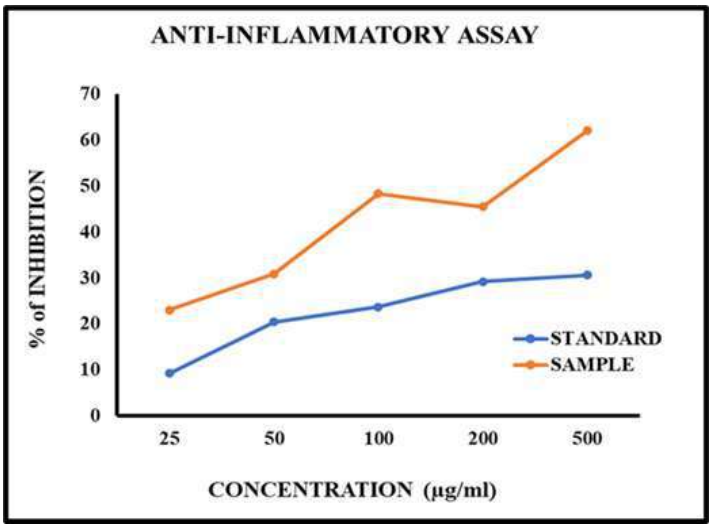

Figure 21: Percentage Inhibition of Standard and Sample

Anti-Microbial Property

Anti-microbial activity of leaf extract was evaluated using agar well diffusion method. The result indicates that the extract did not exhibit any anti-microbial activity towards the tested organisms. Only the positive control showed zone of inhibition against pathogenic microbes.

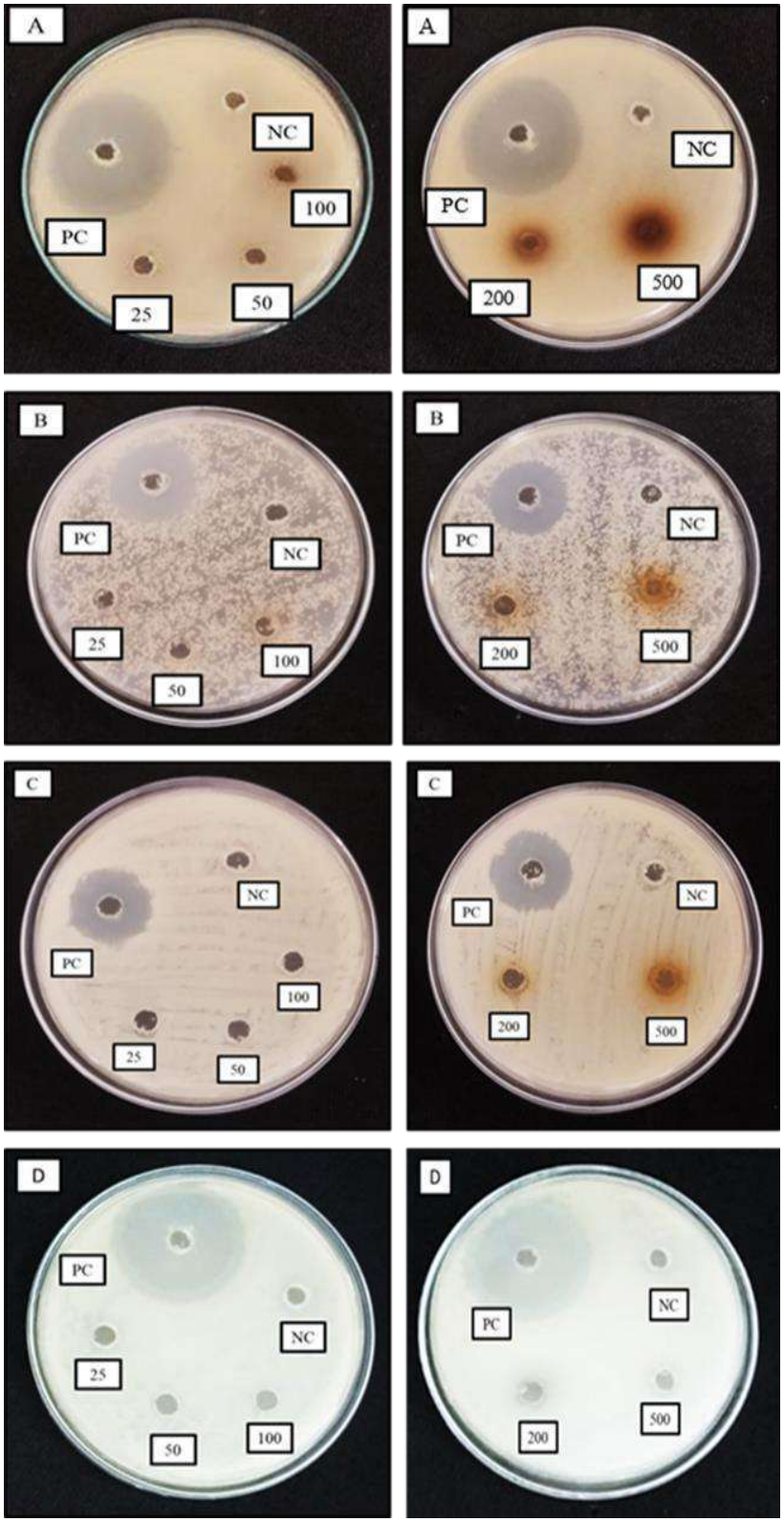

Figure 22: A - *S. aureus*, B - *Angier*, C - *C. albicans*, D - *B. cohnii*. PC - Gentamicin, NC - autoclaved distilled water

Anti-arthritic property

The anti-arthritic activity of leaf extract was determined using the protein denaturation method.

The results showed that both the standard and sample exhibited higher level of inhibition at the greatest concentration.

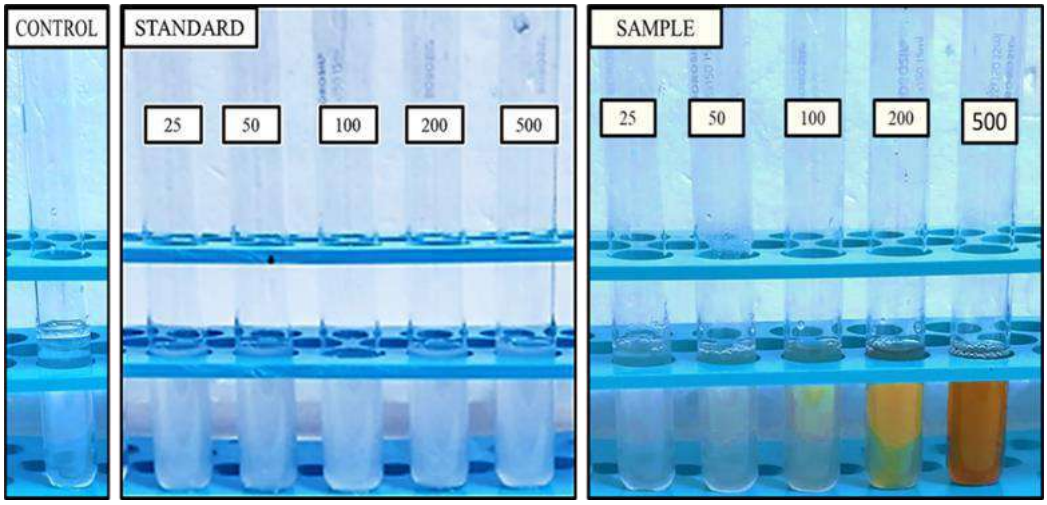

Figure 23: Anti-Arthritic Property of Diclofenac and Leaf Extract Of *H. Indicus*

Table 17: Percentage Inhibition of Diclofenac and Sample

Concentrations (µg/ml)	% of inhibition

	Diclofenac	Sample
25	31.851	25
50	36.298	50
100	38.341	100

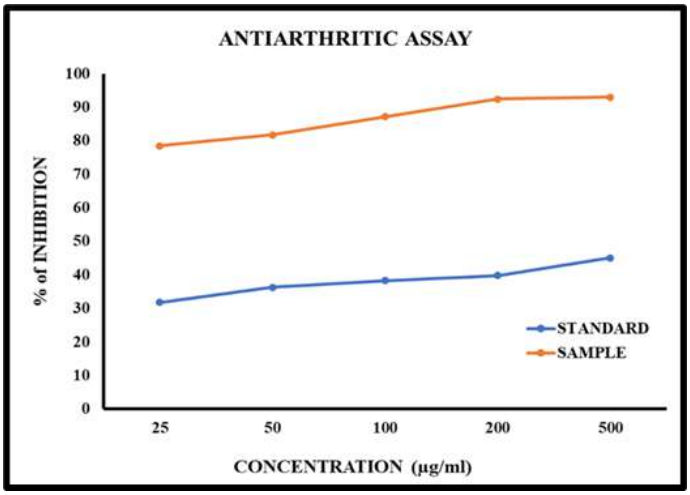

Figure 24: Percentage Inhibition of Standard and Sample

Wound healing property

Wound healing activity of leaf extract was determined using scratch assay by analyzing and comparing the area of gap closure between treated and untreated cells within a time period of 24 hrs.

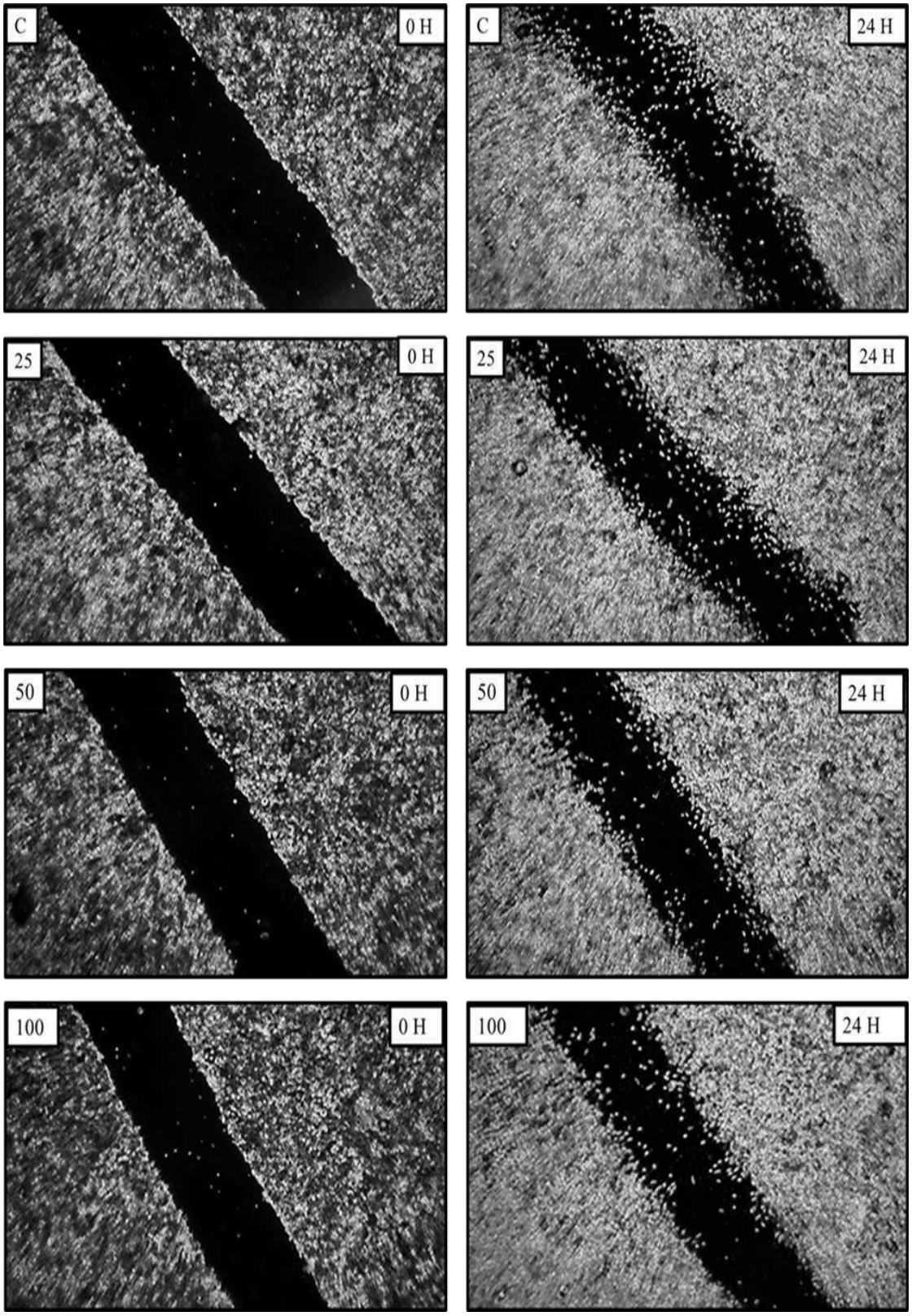

Figure 25: Area Of Gap Closure Before and After Incubation Of 24 Hrs.

Table 18: Percentage Of Wound Closure at Different Concentrations

Concentrations (µg/ml)	Percentage of wound closure
0	14.959

25	10.485
50	7.896
100	8.399

200	9.310
500	17.597

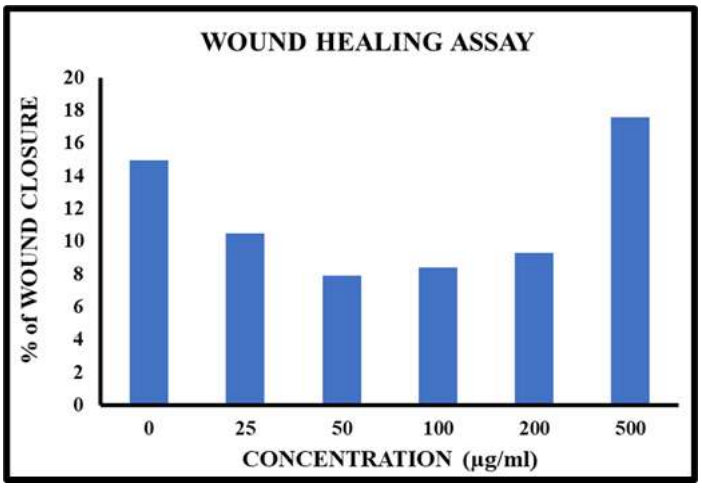

Figure 26: Percentage Of Wound Closure

Hepatoprotective property

Hepatoprotectives effect of aqueous leaf extract was assessed using MTT assay against HepG2 cell line. The result showed that, the extract has more hepatoprotective effect compared to standard. Silymarin was used as the standard drug.

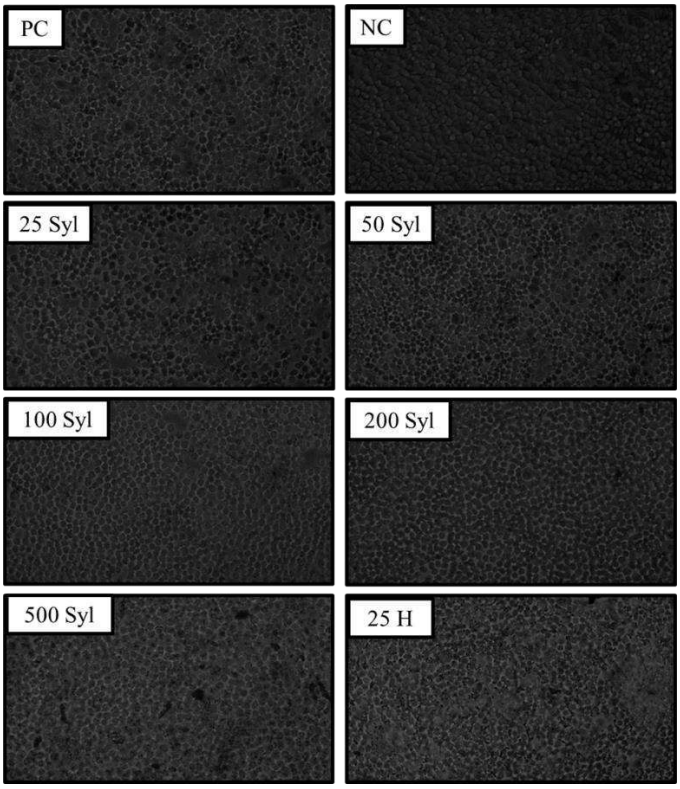

Figure 27: HepG2 cell line after treated with different concentrations of standard and sample

Table 19: Percentage Inhibition of Standard and Sample

Concentrations (µg/ml)	% of cell viability	
	Standard	Sample
25	80.222	25
50	43.175	50
100	75.766	100
200	62.674	200

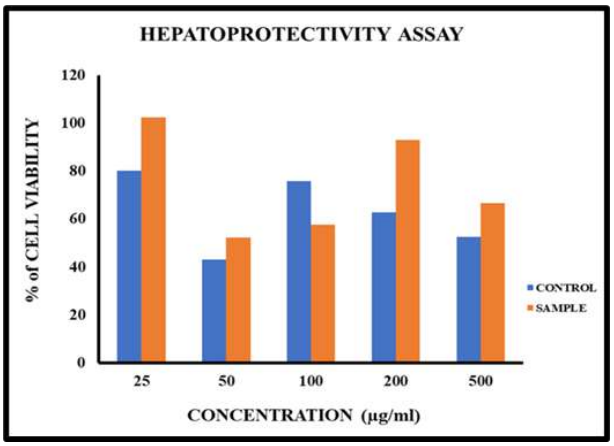

Figure 28: Percentage cell viability of standard and sample

Anti-ulcer property

Anti-ulcer activity of leaf extract was evaluated using acid neutralizing capacity method. Gelusil was used as standard. The result showed significant anti-ulcer property for the sample.

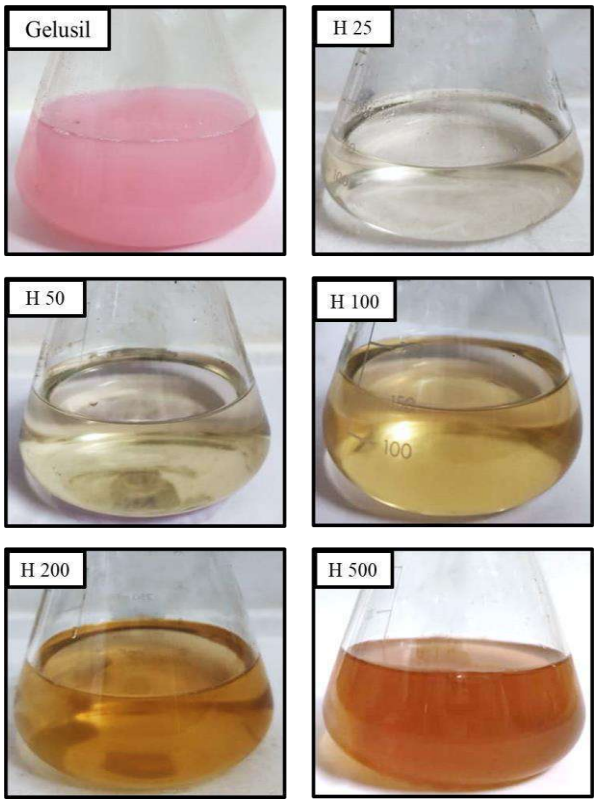

Figure 29: Acid Neutralizing Capacity of Different Concentrations of The Sample Against Gelusil

Concentrations (µg/ml)	Volume of NaOH	Acid neutralizing capacity of antacid
25	48.00	0.96
50	46.50	0.46
100	44.30	0.22
200	45.60	0.11
500	45.00	0.04

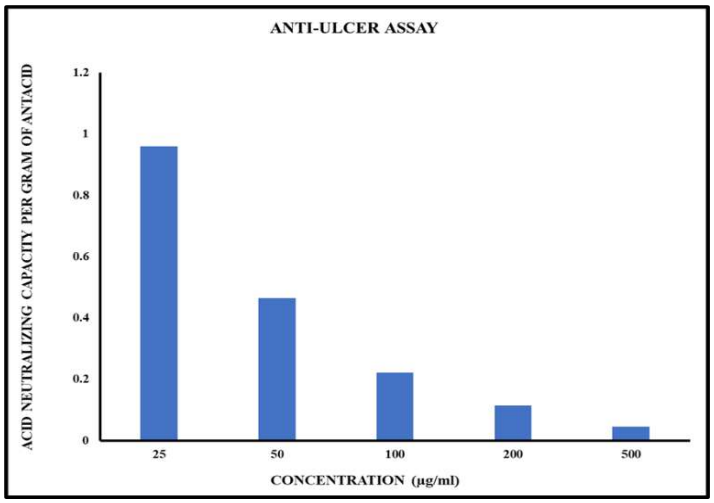

Figure 30: Acid Neutralizing Capacity of The Sample

DISCUSSION

This study aspires to examine the therapeutic potential of *H.indicus*, evaluating the presence of various phytochemicals, along with anti-oxidant, anti-diabetic, anti-arthritic, anti-inflammatory, hepatoprotectivity, wound healing, anti-ulcer, and anti-microbial properties of the plant. Phytochemicals present on the aqueous extract of *H.indicus* leaves were evaluated using various qualitative and quantitative assays. The results of the assays unveiled the presence of bioactive compounds like alkaloids, flavonoids, tannins, phenols, saponins and terpenoids, whereas compounds like glycosides, coumarin and steroids were found to be absent in the extract. (Shalini, 2021) conducted similar experiment on aqueous and alcoholic root extract of *H.indicus*, and the result showed the presence of steroids, flavonoids, phenolic compounds, tannins and lignins. Quantitative analytic assays were carried out for detecting the quantity of aforementioned phytochemicals present in the extract. Similar result was reported by (Balaji *et al*., 2017) from their experiment conducted on screening phytochemicals present on the ethanolic root extract of *H.indicus*. The anti-oxidant activity of *H.indicus* was detected using FRAP assay. The result showed that, the sample has more anti-oxidant potential compared to standard. The highest anti-oxidant activity was observed at the highest concentration (500µg/ml) of the extract. Studies conducted by (Ravikiran *et al*., 2016) and (Mary *et al*., 2003) showed similar anti-oxidant activity of root and leaf extract of *H.indicus*. From their experimental observations, they suggest that, *H.indicus* have notable free radicle scavenging activity, by neutralizing the highly charged molecules that cause oxidative stress, and thereby protecting the cells from cellular damage caused by oxidative stress. The anti-diabetic activity of *H.indicus* was evaluated using α-amylase inhibition assay. The leaf extract showed significant α-amylase inhibition property at

variable concentrations, with the highest range of inhibition was obtained at the lowest concentration (25µg/ml) of the sample. It demonstrates that, as the concentration increases, anti-diabetic property of the extract decreases. (Taidala *et al*., 2024) reported that methanolic root extract of *H.indicus* also exhibited similar inhibition against α-amylase enzyme activity. The extract contains anti-diabetic agents that can regulate blood glucose level, regain enzymatic and metabolic processes and minimize lipid peroxidation (Gayathri and Kannabiran, 2008). Anti-inflammatory activity of *H.indicus* leaf extract was evaluated using protein denaturation method. The result showed up remarkable anti-inflammatory potential of the sample, compared to standard used, signifying its capability in reducing inflammation. The extract showed variable inhibition pattern, with the lowest concentration exhibiting minimal protein inhibition. Study by (Dutta *et al*., 1982) showed similar anti-inflammatory activity of *H.indicus* extract. Saponin, a phytochemical present on the extract of *H.indicus* attributes to its anti-inflammatory potency (Lalrinpuia *et al*., 2017). Anti-arthritic activity of *H.indicus* leaf extract was also assessed by protein denaturation method. The result suggests that, the extract have significant anti-arthritic property that increases steadily with increase in its concentration, indicating that it is beneficial for pharmacological applications, demanding persistent protein inhibition. (Abiraamasri and Lakshmi, 2016) reported similar findings from their study. (Mehta *et al*., 2012) described that, anti-arthritic property of *H.indicus* is *attributed* to compounds like terpenes, sterols, and phenols and polyphenols present on the extract. The anti-microbial activity of *H.indicus* leaf extract was examined using agar well diffusion method. Bacterial species like *Bacillus cohnii* and *Staphylococcus aureus* and fungal species such as *Aspergillus niger* and *Candida albicans* were taken for detecting the anti-microbial potential of the leaf extract. From the result it is observed that, the leaf extract of *H.indicus* does not exhibit any anti-microbial activity. The zone of inhibition appeared only on the positive control. The absence of anti-microbial activity by the extract may attribute to various factors such as chemical structure of the compounds, the particular strain of the organisms used or even the experimental conditions applied. Wound healing property of *H.indicus* leaf extract was estimated using scratch assay against L929 cell line. The outcome of the assay indicates that, greater stimulation for cell migration was performed by the extract at its higher concentration (500µg/ml). This demonstrates that, at this level of concentration, the extract can stimulate wound healing by accelerating the repair of damaged tissues, and thus, the extract can be used for the treatment of cuts, burns and other skin injuries (Ganesan *et al*., 2012). Experiment made by (Kurapati *et al*., 2012) endorsed that *H.indicus* carries significant wound healing property. The anti-oxidant and anti-inflammatory properties of the extract can enhance its wound healing ability by preventing infections. Hepatoprotective effect of *H.indicus* leaf extract was detected using MTT assay on HepG2 cell line. The result showed that, the extract has more hepatoprotective effect compared to standard. The extract at different concentrations exhibits variable hepatoprotective effects, in which the lowest concentration (25µg/ml) of the sample performs maximum hepatoprotective effect. (Baheti *et al*., 2017) conducted similar experiment on animal model. The study states that methanolic root extract of *H.indicus express* significant hepatoprotective effect against CCl_4 paracetamol induced hepatic cell damage. Anti-ulcer activity of *H.indicus* leaf extract was determined using acid neutralizing capacity method. The result showed significant anti-ulcer property of the extract at its lower concentration (25µg/ml). The anti-ulcer activity of

the extract decreases steadily with increase in its concentration. This indicates the dose depending acid neutralizing potency of *H.indicus* leaf extract. Study conducted by (Manonmani *et al*., 1995) using ethanolic extract of *H.indicus* showed significant hepatoprotective effect in rat models. The presence of phytochemicals like saponins, alkaloids, phenols and tannins attributes to the hepatoprotective effects of *H.indicus* extract (Anoop and Jegadeesan, 2002).

CONCLUSION

The present study focus on analyzing the therapeutic potential of leaf extract of *H.indicus* and screening of phytochemicals present in it. Qualitative phytochemical analysis authenticates the presence of bioactive compounds like alkaloids, flavonoids, saponins, phenolics, tannins and terpenoids. The study showed significant anti-oxidant, anti-diabetic, anti-inflammatory, anti-arthritic, anti-ulcer, hepatoprotective and wound healing activity. Our study also focused on *in-vitro* tests, that provide strong evidence for therapeutic effectiveness of *H.indicus* leaf extract in curing various ailments such as diabetes, inflammation, ulcers and wounds. Further studies using in-vitro models can assist in better understanding of translational potential and therapeutic mechanism of the plant. This additional research will provide important insights of biological activity of the extract and its sustainability for applying in present medical treatments.

REFERENCES

1. Purohit P. A review of important medicinal plant Hemidesmus indicus L.R. Br. (Anantamool). World Journal of Pharmaceutical Research. 2019; 8: 476 - 492.
2. Darshini MD, Sreelakshmi MS, Adithya J, Aryaputhri NS, Lakshmi PK, Nath LR. A systematic analysis of the ethnopharmacological relevance of an Indian traditional plant, Hemidesmus indicus (L.) R. Br. for the past 10 years. J Appl Pharm Sci. 2024; 14: 037 - 044.
3. Mehta A, Sethiya NK, Mehta C, et al. Anti-arthritis activity of roots of Hemidesmus indicus in rats. Asian Pac J Trop Med. 2012; 5: 130 - 135.
4. Ganesan S, Parasuraman S, Maheswaran S, Gnanasekar N. Wound healing activity of Hemidesmus indicus formulation. J Pharmacol Pharmacother. 2012; 3: 66.
5. Jayalakshmi B, Kruthika L, Amruthesh KN. Phytochemical study and antioxidant property of Hemidesmus indicus (L.) R. Br. roots. Asian J Pharm Pharmacol. 2018; 4: 719 - 723.
6. Soumya D, Srilatha B. Late-stage complications of diabetes and insulin resistance. J Diabetes Metab. 2011; 2: 1000167.
7. Salehi A, Kumar VA, Sharopov F, Ramírez-Alarcón K, Ruiz-Ortega J, Sharifi-Rad J. Antidiabetic potential of medicinal plants and their active components. Biomolecules. 2019; 9: 551.
8. Gayathri M, Kannabiran K. Hypoglycemic activity of Hemidesmus indicus R. Br. on streptozotocin-induced diabetic rats. Int J Diabetes Dev Ctries. 2008; 28: 6 - 10.
9. Ilyas U, Katare DP, Aeri V, Naseef PP. A review on hepatoprotective and immunomodulatory herbal plants. Pharmacogn Rev. 2016; 10: 66 - 70.
10. Mookan P, Rangasamy A, Thiruvengadam D. Protective effect of Hemidesmus indicus against rifampicin and isoniazid-induced hepatotoxicity in rats. Fitoterapia. 2000; 71: 55 - 59.
11. Harth M, Nielson WR. Pain and affective distress in arthritis: Relationship to immunity and inflammation. Expert Rev Clin Immunol. 2019; 15: 541 - 552.

12. Swathi S, Amareshwari P, Venkatesh K, Roja Rani A. Phytochemical and pharmacological benefits of Hemidesmus indicus: An updated review. J Pharmacogn Phytochem. 2019; 8: 256 - 62.
13. Ghasemian M, Owlia S, Owlia MB. Review of anti-inflammatory herbal medicines. Adv Pharmacol Pharm Sci. 2016; 2016: 1 - 8.
14. Lalrinpuia B, Upadhyay SN, Mukherjee K, Hazra J. Pharmacological and therapeutic profile of Anantamula (Hemidesmus indicus (L.) R. Br.): A comprehensive review. Int J Ayurveda Pharma Res. 2017; 5: 49 - 57.
15. Dixon MF. Patterns of inflammation linked to ulcer disease. Best Pract Res Clin Gastroenterol. 2000; 14: 27 - 40.
16. Anoop A, Jegadeesan M. Biochemical studies on the anti-ulcerogenic potential of Hemidesmus indicus R.Br. var. indicus. J Ethnopharmacol. 2003; 84: 149 - 156.
17. Zaidi SH, Mukerji B. Experimental peptic ulceration, Part I. The significance of the "mucous barrier". Indian J Med Res. 1958; 46: 27 - 37.
18. Vitorino P, Meyer T. Modular control of endothelial sheet migration. Genes Dev. 2008; 22: 3268 - 3281.
19. Dev SK, Choudhury PK, Srivastava R, Sharma M. Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. Biomed Pharmacother. 2019; 111: 555 - 567.
20. Hiremath SP, Rudresh K, Badami S. Antimicrobial activity of various extracts of Striga sulphurea and Hemidesmus indicus. Indian J Pharm Sci. 1997; 59: 145 - 147.
21. Taidala S, Ramakrishna M, Rokkala SC, Galla R, Matha VS. Extraction of phytochemicals from Hemidesmus indicus leaves: Evaluation of in vitro antioxidant, antidiabetic, and antifungal properties. REDVET - Rev Electrón Veterinaria. 2024; 25: 1695 - 7504.
22. Maheshwari M, Vijayarengan P. Preliminary phytochemical and FT-IR analysis of methanolic leaves extracts of Hemidesmus indicus and Tylophora indica. Plant Arch. 2021; 21: 50 - 54.
23. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 2020; 8: 603 - 608.
24. Balaji VK, Muthuswamy S, Easow JM. Qualitative and quantitative analysis of phytochemicals in Hemidesmus indicus (L.) R. Br. World J Pharm Pharm Sci. 2017; 6: 1083 - 1092.
25. Devasia JV, Cherian P. Phytochemical and antibacterial effect of dry fruits of Garcinia gummi-gutta (L.) Roxb. Int J Pharma Bio Sci. 2020; 11: 121 - 128.
26. Madappa MB, Bopaiah AK. Preliminary phytochemical analysis of leaf of Garcinia gummigutta from Western Ghats. IOSR J Pharm Biol Sci. 2012; 4: 17 - 27.
27. Patel RK, Patel JB, Trivedi PD. Spectrophotometric method for the estimation of total alkaloids in Tinospora cordifolia M. and its herbal formulations. Int J Pharm Pharm Sci. 2015; 7: 249 - 251.
28. Siddiqui N, Rauf A, Latif A, Mahmood Z. Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth). J Taibah Univ Med Sci. 2017; 12: 360 - 363.
29. Pękal A, Pyrzyńska K. Evaluation of aluminium complexation reaction for flavonoid content assay. Food Anal Methods. 2014; 7: 1776 - 1782.
30. Ci KC, Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of Strobilanthes kunthiana (Neelakurinji). J Med Plants Stud. 2016; 4: 282 - 286.

31. Madhuranga HDT, Samarakoon DNAW. Advancing in vitro antioxidant activity assessment: a comprehensive methodological review and improved approaches for DPPH, FRAP, and H_2O_2 assays. Nat Ayurvedic Med. Medwin Publishers; 7: ISSN: 2578 - 4986.
32. Chaves N, Santiago A, Alias JC. Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and hierarchization based on the method used. Antioxidants. 2020; 9: 76.
33. Wickramaratne MN, Punchihewa JC, Wickramaratne DBM. In-vitro alpha-amylase inhibitory activity of the leaf extracts of Adenanthera pavonina. BMC Complement Altern Med. 2016; 16: 466.
34. Anyasor GN, Okanlawon AA, Ogunbiyi B. Evaluation of anti-inflammatory activity of Justicia secunda Vahl leaf extract using in vitro and in vivo inflammation models. Int J Phytomed Phytother. 2019; 5: 49.
35. Varankar SS, Bapat SA. Migratory metrics of wound healing: a quantification approach for in vitro scratch assays. Front Oncol. 2018; 8: 633.
36. Shalini R. Phytochemical screening of Hemidesmus indicus root. Int J Bot Stud. 2021; 6:1398 - 400.
37. Ravikiran T, Shilpa S, Praveen Kumar N, Sowbhagya R, Santosh Anand, Anupama SK, et al. Antioxidant activity of Hemidesmus indicus (L.) R.Br. encapsulated poly (lactide-co-glycolide) (PLGA) nanoparticles. IOSR J Pharm Biol Sci. 2016; 11:9 - 17.
38. Mary NK, Achuthan CR, Babu BH, Padikkala J. In vitro antioxidant and antithrombotic activity of Hemidesmus indicus (L.) R.Br. J Ethnopharmacol. 2003; 87:187 - 91.
39. Dutta MK, Sen TK, Sikda. Some preliminary observations on the anti-inflammatory properties of Hemidesmus indicus in rats. Indian J Pharmacol. 1982; 14:78.
40. Abiraamasri BL, Lakshmi T. In vitro anti-arthritic activity of Hemidesmus indicus root extract. Int J Pharm Sci Rev Res. 2016; 41:15 - 7.
41. Kurapati VK, Nishteswar K, N.T.R. University of Health Sciences. Phytochemical and clinical evaluation of Sariba (Hemidesmus indicus) on wound healing. Int Res J Pharm. 2012; 3:277.
42. Manonmani S, Viswanathan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of Cauvery 100, an Ayurvedic formulation in experimental ulcers. Indian J Pharmacol. 1995; 27:101 - 5.
43. Baheti JR, Goyal RK, Shah GB. Hepatoprotective activity of Hemidesmus indicus R. Br. in rats. Indian J Exp Biol. 2006; 44:399 - 402.
44. Manonmani S, Viswanathan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of Cauvery 100, an Ayurvedic formulation in experimental ulcers. Indian J Pharmacol. 1995; 27:101 - 5.

HOW TO CITE: Ashna Shiju, Dr. Parvathy Prasad*, Therapeutic Potential of Hemidesmus Indicus, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 4, 55-79 https://doi.org/10.5281/zenodo.15318543