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# Preliminary phytochemical screening and *in vitro* antacid activity of *Hemidesmus indicus* leaves extract by modified artificial stomach model

**Dhanalakshmi R, Aisha Afrin J, Akila M, Alnoora F, Dharani R and Insath Parveen S**

**Abstract**
*Hemidesmus indicus* (Asclepiadaceae) is important medicinal plants in traditional Indian medicine. The present study was designed to investigate the phytochemical constituents, fluorescence nature and antacid activity of *Hemidesmus indicus* leaves by using a modified artificial stomach model. Parameters such as effect of temperature on pH, neutralization effect, duration of neutralization effect and capacity were evaluated. Sodium bicarbonate and water were used as reference and control respectively. *Hemidesmus indicus* leaves containing phytochemical constituents such as Tannins, Saponins, Terpenoids, Alkaloids, and phenolic compounds, Steroids, Glycosides, Triterpenoids and Flavonoids etc. Fluorescence analysis signifies their characteristics. *Hemidesmus indicus* leaves produced relatively good responses on effect of temperature on pH, neutralization effect, duration of neutralization effect and capacity. It was found to be higher than that of water but less when compared to sodium bicarbonate. Hence this plant can be an effective alternative for sodium bicarbonate which is reported to have various side effects.

**Keywords:** *Hemidesmus indicus*; phytochemical analysis; fluorescent analysis; antacid; acid neutralizing capacity; artificial stomach

## Introduction
Peptic ulcer is a gastro intestinal disorder due to an imbalance between the aggressive factors like acid, pepsin, *Helicobacter pylori* and defensive factors like bicarbonate secretion, prostaglandins, gastric mucus, and innate resistance of the mucosal cell factors [1]. Normally peptic ulcer develops when aggressive factors overcome the defensive factors [2]. Gastric acid is a digestive fluid formed in the stomach having a pH of 1 to 2. It is a mixture of hydrochloric acid, large quantities of potassium chloride and sodium chloride. The acid in stomach plays a significant role in the digestion of proteins, by activating digestive enzymes and making ingested proteins unravel so that digestive enzymes can break down the long chain amino acids. The hyper secretion of gastric acid (HCl) is closely related to peptic ulcer disease. General symptoms found in children are respiratory problems, inadequate weight, vomiting, coughing and turning down food. The symptoms shown by adults include long heartburn, chest and stomach pain, gas formation in stomach, inflammation in chest; gastro oesophageal reflux, voice change and formation of ulcer in oesophagus, pain during muscular contractions and pain in ears are some of the symptoms of acidity [3-6]. Peptic ulcer disease and its complications remain the cause of significant morbidity worldwide, representing a major burden for health care resources [7]. The conventional drugs used in the treatment of ulcer include histamine receptor antagonists, prostaglandins analogues, proton pump inhibitors, cytoprotective agents, antacids and anticholinergics [8]; among these, antacids heal ulcers through elimination of gastric acid by neutralization and have been used in the treatment of peptic ulcer for many years. Common antacid preparations include sodium bicarbonate (SB), calcium carbonate, and salts of aluminum and magnesium. Antacids that contain Aluminum contribute aluminum to the diet but may cause constipation or lead to phosphorous deficiency where as on long term or inappropriate use can lead to Aluminum toxicity. Calcium containing antacids contribute calcium to diet and may produce constipation. Magnesium containing antacids contribute magnesium to diet and may produce a side effect of diarrhoea on prolonged use may even lead to magnesium toxicity [9]. It is reported that sodium bicarbonate should be avoided even though it is a potent neutralizer of acid as it contains significant amounts of sodium and may alter the systemic pH. In addition, antacid drug interactions have been frequently reported and this is a problem worthy of being noticed. Antacid may even alter biochemical mechanisms of the body upon chronic usage. Considerable side effects and

interactions of antacids, thus emphasizing need to search for new alternatives. Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since ancient times as reported by [10]. As high as 80% of the world population depends on plant-derived medicines for the first line of primary health care [11] reinforcing the theory that plant extracts can be good sources of new drugs. *Hemidesmus indices* are commonly known as Indian Sarsaparilla belonging to the family Asclepiadaceous. It is a lactiferous shrub, with aromatic and woody roots, slender stems and greenish flowers. This is a common medicinal plant widely used in Indian system of medicine [12] and also an official drug in Indian pharmacopoeia [13] and British pharmacopoeia [14]. The name "Hemidesmus" is derived from Latin word "Hemidesmos" which means 'half bond'. It is so named in allusion to sub connate filaments at their base – joint pods and connected stamens. Word "indicus" stands for 'of India'. *Hemidesmus indicus* belongs to family Asclepiadaceae which is derived from word "Askleplos" – means 'God of Medicine' [15]. Vernacular name "Anantmul" is a Sanskrit word which means 'endless root' [16]. *Hemidesmus indicus* is a renowned component in Ayurvedic and Unani medicines for treating various diseases such as respiratory disease, biliousness, rheumatism, skin disease, diarrhoea, burning sensation, bronchitis, fever, antileukemic activity, eye diseases and gastric disorders [17]. Phytoconstituents of *Hemidesmus indicus* ranges from hydrocarbons, glycosides, oligoglycosides, and terpenoids to steroids [18, 19]. Ethanol extract of *Hemidesmus indicus* root possesses significant antiulcer property which could be either due to cytoprotective action of the drug or by strengthening of gastric mucosa and thus enhancing mucosal defence [20]. However there is no specific report on antiulcer property of *Hemidesmus indicus* leaves. Therefore, the present study was undertaken to evaluate the antacid effect of *Hemidesmus indicus* leaves on gastric acid *in vitro* using the modified model of Vatier's artificial stomach and the titration method of Fordtran's model.

## Materials and Methods

### Collection and Identification of the Plant material

Fresh leaves of the selected plant *Hemidesmus indicus* were collected from Thirunelveli district, Tamil Nadu, India. The plant materials were taxonomically identified and authenticated by Dr. V. Chelladurai, Research officer - Botany (scientist C), Central council for research in Ayurveda and Siddha, Govt. of India; Thirunelveli. The plant was thoroughly washed in running tap water to remove soil particles and adhered debris and finally washed with distilled water. The leaves of the plant alone were segregated and dried under shade, pulverized by a mechanical grinder into fine powder. The powdered materials were stored in air tight polythene bags till use.

### Preparation of extracts

The powdered plant materials of *Hemidesmus indicus* leaves were extracted with ethanol (99.9%). The extraction was done by hot continuous percolation method in Soxhlet apparatus for 24 hrs. The extract was concentrated by using a rotary evaporator till dry powder was obtained.

### Percentage yield for the ethanolic extract of *Hemidesmus indicus* leaves

The percentage yield for the ethanolic extract of *Hemidesmus indicus* [HI] leaves was calculated with reference to air dried powder taken using the formula given below.

$$\text{Percentage yield} = \frac{\text{Weight in grams of extracts obtained}}{\text{Weight in grams of plant materials taken}} \text{ X } 100$$

### Preliminary phytochemical screening:

Preliminary phytochemical screening was conducted on ethanolic extract of *Hemidesmus indicus* leaves to determine the different phytochemical constituents present in the extracts [21].

### Fluorescence Analysis of *Hemidesmus indicus* leaves powder

0.5gms of dried leaf powder of *Hemidesmus indicus* were taken into clean and dried test tubes. To each tube 5ml of different organic solvents like Alcoholic I N NaOH, Aqueous I N NaOH, 50% Hcl, 50% HNO3, 50% H2SO4, Petroleum ether, Chloroform, Picric acid, 5% FeCl3 solution, 5% Iodine, Methanol, HNO3+ NH3 were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The fluorescence nature of *Hemidesmus indicus* leaves was determined under ordinary light and UV light of short (254 nm) and long (365 nm) wave length [22].

### Determination of *in vitro* Antacid activity of *Hemidesmus indicus* leaves extract by modified artificial stomach model

Evaluation of antacid activity of *Hemidesmus indicus* leaves extract were carried out using two different concentrations of 400 mg/mL and 800 mg/mL. The volume of test solution was 90 mL.

### Preparation of artificial gastric acid

2 g of NaCl and 3.2 mg of pepsin enzymes were dissolved in 500 ml distilled water. Hydrochloric acid (7.0 ml) and adequate water were added to make a 1000 ml solution of artificial gastric acid. The pH of the artificial gastric acid solution was adjusted to 1.20.

### Determination of pH of the *Hemidesmus indicus* leaves extracts

The pH of ninety milliliters of each test solution was determined at temperatures ranging from $25^0$C to $37^0$C. The pH values of the sodium bicarbonate (SB) and water was also determined for comparison.

### Determination of the neutralizing effects on artificial gastric acid

Freshly prepared ninety milliliters of each test solution; water (90 ml) and the active control SB (90 ml) were added separately to the artificial gastric juice (100 ml) at pH 1.2. The pH values were determined to examine the neutralizing effects on artificial gastric juice.

### Determination of the duration of consistent neutralization effect on artificial gastric acids using the modified model of Vatier's artificial stomach model [23, 24]

The apparatus of the modified model of Vatier's artificial stomach was made up of three elements: a pH recording system (R), a stomach (S) and a peristaltic pump (P). The stomach was made up of three portions, S1, S2 and S3. S1 was a reservoir (container), S2 modeled the secretory flux (F-IN), and S3 modeled the gastric emptying flux (F-OUT). Freshly prepared ninety milliliters of each test solution was

added to 100 ml of artificial gastric juice at pH 1.2 in the container of the artificial stomach at 37$^0$C and continuously stirred (30 rpm) with a 2.5-cm magnetic stirring apparatus. Aeration was given at 136 air bubbles per minute. Artificial gastric juice at pH 1.2 was pumped at 3 ml/min into the container of the artificial stomach, and pumped out at 3 ml/min at the same time. A pH meter was connected to continuously monitor the changes of pH in the container of the artificial stomach. The duration of the neutralization effect was determined when the pH value returned to its initial value (pH 1.2).

**Determination of the neutralization capacity *in vitro* using the titration method of Fordtran's model** [25, 26]

Freshly prepared ninety milliliters of each test solution was placed in a 250 ml beaker and warmed to 37$^0$C. Aeration was given at 136 air bubbles per minute. A magnetic stirrer was continuously run at 30 rpm to imitate the stomach movements. The test samples were titrated with artificial gastric juice to the end point of pH 3. The consumed volume (V) of the artificial gastric juice was measured. The total consumed H+ (mmol) was measured as 0.063096 (mmol/ml) × V (ml).

**Statistical analysis**

The statistical analysis was performed using the software Graph Pad Prism version 7.04. The experimental data obtained were expressed as mean ± SEM; Comparison between the groups was analyzed by One-way Analysis of Variance (ANOVA) using Dunnett Multiple Comparisons Test by considering Test Vs control. The differences were considered to be statistically significant when *P<0.05 where n=6.

**Result and discussion**

**Percentage yield**

The percentage yield for the *Hemidesmus indicus* leaves extract was calculated. Percentage yield was found to be 14.46%w/w

**Preliminary phytochemical screening**

Preliminary phytochemical screening for the ethanolic extract of *Hemidesmus indicus* leaves was carried out and the results are tabulated in Table. 1 The ethanolic extract of *Hemidesmus indicus* leaves containing Tannins, Saponins, Terpenoids, Alkaloids, Phenolic compounds, Steroids, Glycosides, Triterpenoids, and Flavonoids.

**Fluorescence Analysis of *Hemidesmus indicus* leaves powder**

Fluorescence is the phenomenon exhibited by various drugs and will reveal the various chromophores of chemical constituents present in the plant material. Some constituents showed fluorescence in the visible range daylight. UV light produces fluorescence indicative of many natural products (Eg: Alkaloids like Berberine), which do not visibly fluoresce in daylight. If the substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence some crude drugs are often assessed qualitatively by this method and also it is an important parameter of pharmacognostical evaluation. The data of the fluorescence features of *Hemidesmus indicus* leaf powder revealed various shades of brown and yellow which were presented in Table 2

**Table 1**: Phytochemical constituents of ethanolic extract of *Hemidesmus indicus* leaves

| S. No | Phytochemical constituents | Results |
|---|---|---|
| 1 | Tannins | (+) |
| 2 | Saponins | (+) |
| 3 | Terpenoids | (+) |
| 4 | Alkaloids | (+) |
| 5 | Phenol | (+) |
| 6 | Steroids | (+) |
| 7 | Glycosides | (+) |
| 8 | Triterpenoids | (+) |
| 9 | Sterols | (+) |
| 10 | Vitamin C | (-) |
| 11 | Flavonoids | (+) |
| 12 | Emodin | (-) |
| 13 | Quinone | (-) |
| 14 | Anthocyanin | (-) |
| 15 | Amino acid and protein | (-) |

(+) = Present; (-) = absent

**Table 2:** Fluorescence analysis of *Hemidesmus indicus* leaves powder

| S.No | Particulars of the treatment | Under ordinary light | Under short wave length 254 nm | Under long wave length 365 nm |
|---|---|---|---|---|
| 1 | Plant powder + Alcoholic I N NaOH | Green | Green | Black |
| 2 | Plant powder + Aqueous I N NaOH | Dark Brown | Dark green | Dark black |
| 3 | Plant powder + 50% Hcl | Light Brown | Pale Green | Black |
| 4 | Plant powder + 50% $HNO_3$ | Light Brown | Pale Green | Dark Black |
| 5 | Plant powder + 50% $H_2SO_4$ | Light Brown | Green | Black |
| 6 | Plant powder + Petroleum ether | Dark Green | Dark Green | Dark Brown |
| 7 | Plant powder + Chloroform | Green | Green | Black |
| 8 | Plant powder + Picric acid | Yellowish Green | Green | Dark Green |
| 9 | Plant powder + 5% $FeCl_3$ solution | Dark Green | Dark Green | Dark Black |
| 10 | Plant powder + 5% Iodine | Blackish Green | Dark green | Dark brown |
| 11 | Plant powder + Methanol | Green | Dark Green | Dark Brown |
| 12 | Plant powder + $HNO_3$+ $NH_3$ | Light Brown | Dark Green | Dark Brown |

**Determination of *in vitro* antacid activity of *Hemidesmus indicus* leaves extract by modified artificial stomach model**

**pH values of the *Hemidesmus indicus* (HI) leaves extracts:**

The pH values of each test solution (400 mg/90ml and 800 mg/90ml) at temperatures from 25$^0$C to 37$^0$C ranged from 2.86 to 2.87 and 3.08 to 3.16, respectively. The pH values of water and SB solutions at temperatures from 25$^0$C to 37$^0$C ranged from 6.99 to 7.01 and 8.61 to 8.46, respectively (Figure 1). The results indicate that temperature did not affect pH significantly.

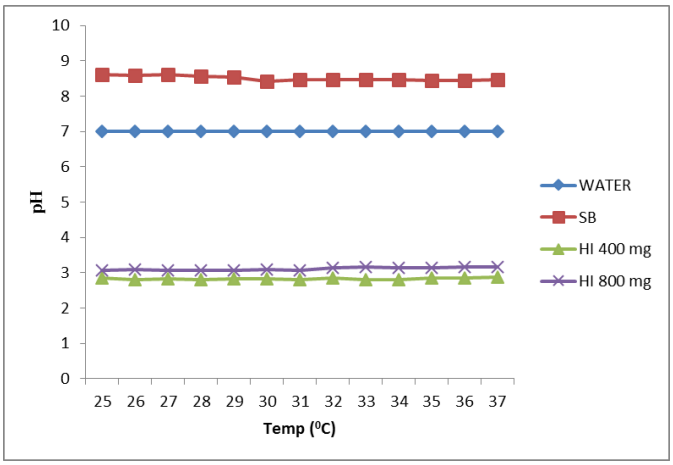


**Fig 1:** pH values of the *Hemidesmus indicus* leaves extracts

**Determination of the neutralizing effects on artificial gastric acids**

When test solution *Hemidesmus indicus* (HI) leaves extracts 400 mg and 800 mg (90 ml) was added to 100 ml of the artificial gastric juice (pH 1.2), the pH values of HI extracts were found to be 1.53 ± 0.01 and 1.54 ± 0.01, respectively. The pH values of water and Sodium bicarbonate solutions were 1.39 ± 0.00 and 1.72 ± 0.00, respectively (Table 3).

**Table 3:** Determination of the neutralizing effects on artificial gastric acid

| S. No | Drug | pH value |
|---|---|---|
| 1 | Water | 1.39±0.00 |
| 2 | Standard (SB) | 1.72±0.00* |
| 3 | HI 400 mg | 1.53±0.01* |
| 4 | HI 800 mg | 1.54±0.01* |

Data are presented as mean ± SEM (n = 6) P*<0.05 when compared with water

This result shows that the neutralizing effect of HI 400 mg and HI 800 mg was significantly better than that of water (Figure 2).

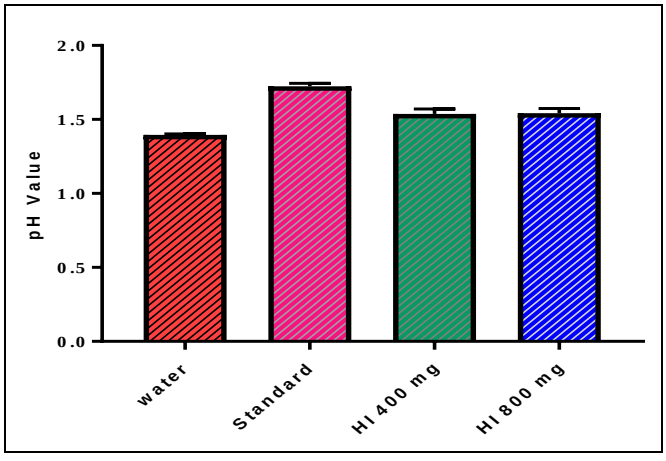


**Fig 2:** Neutralizing effect on gastric acid

**Determination of the duration of consistent neutralization on artificial gastric acids**

The duration of consistent neutralization effect of test solution *Hemidesmus indicus* (HI) leaves extracts 400 mg and 800 mg (90 ml) were found to be 89.83 ± 0.54 min and 96.5 ± 0.71 min respectively. Those of water and Sodium bicarbonate solutions were 48.83 ± 1.81 and 149 ± 0.51 min, respectively (Table 4).

**Table 4:** Duration of consistent neutralization effect on artificial gastric acid

| S. No | Drug | Time (min) |
|---|---|---|
| 1 | Water | 48.83±1.81 |
| 2 | Standard | 149.00±0.51* |
| 3 | HI 400 mg | 89.83±0.54* |
| 4 | HI 800 mg | 96.5±0.71* |

Data are presented as mean ± SEM (n = 6) P*<0.05 when compared with water

The duration of antacid action of Sodium bicarbonate was the longest, followed by the HI 800 mg and 400 mg, which were significantly higher than that for water (Figure 3)

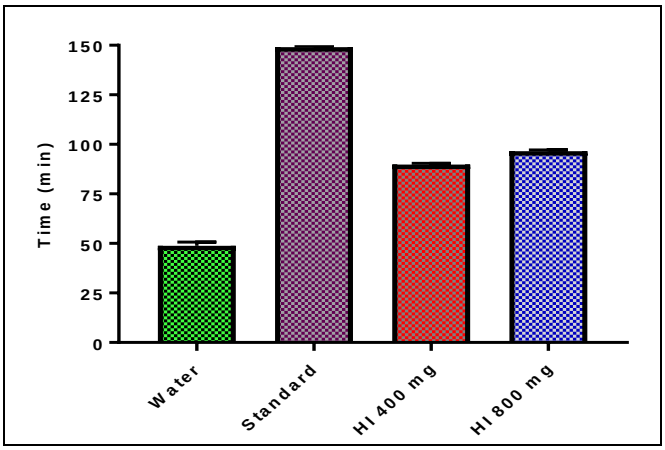


**Fig 3:** Duration of consistent neutralization effect

**Determination of the neutralization capacity *in vitro***

The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water, Sodium bicarbonate, HI 400 mg, HI 800 mg solutions were found to be 1.2±0.02, 34.22±0.59, 8.46±0.07 and 10.5±0.09 respectively The consumed H+ were 0.07±0.00, 2.15±0.03, 0.5±0.00 and 0.6±0.00 mmol, respectively (Table 5). The neutralization capacities of HI 400 mg and 800 mg were lesser than that of Sodium bicarbonate but significantly better than that of water. Both test solution HI 400 mg and 800 mg, exhibited significant antacid potency.

**Table 5:** Consumed volume of artificial gastric juice

| S. No | Drug | Consumed volume of artificial gastric juice (ml) | mmol of H+ |
|---|---|---|---|
| 1 | Water | 1.2±0.02 | 0.07±0.00 |
| 2 | Standard (SB) | 34.22±0.59* | 2.15±0.03* |
| 3 | HI 400 mg | 8.46±0.07* | 0.5±0.00* |
| 4 | HI 800 mg | 10.5±0.09* | 0.6±0.00* |

Data are presented as mean ± SEM (n = 6) P*<0.05 when compared with water

**Conclusion**

In conclusion, the results obtained show the presence of many important phytoconstituents in the ethanolic extract of *Hemidesmus indicus* leaves. One of the phytoconstituents such as flavonoids may be responsible for significant antacid activity. *Hemidesmus indicus* leaves are consistently active in the artificial stomach model. Taking into consideration the side effects and interactions of antacids, the natural and edible products such as *Hemidesmus indicus* leaves should be looked to as an alternative for the treatment of peptic ulcer disease. The mechanism of antacid activity by *Hemidesmus indicus* leaves is not proven from the present results and requires for further investigations.

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