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Pharmacognostical and Phytochemical Characterization with Anti-Ulcer Assessment of *Andrographis echioides* (Acanthaceae)

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Abstract

The present study was undertaken to evaluate the anti-ulcer activity of the ethanolic leaf extract of *Andrographis echioides*. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, gums, and phytosteroids. Acute oral toxicity studies were carried out according to the OECD Guideline 423 (Acute Toxic Class Method). The ethanolic extract did not produce any mortality or signs of acute toxicity for up to 3 days at a dose of 2000 mg/kg body weight, indicating its safety profile. Several phytoconstituents identified in the extract, particularly flavonoids, tannins, and terpenoids, have been widely reported in the literature for their gastroprotective and anti-ulcer properties. Among these, flavonoids, tannins, and triterpenes are recognized as important cytoprotective compounds who's anti-ulcerogenic effects have been extensively documented. The ethanolic extract of *Andrographis echioides* was found to contain flavonoids and their glycosides, tannins, and triterpenoids, which may contribute to its protective effect against gastric mucosal damage induced by pylorus ligation. The extract demonstrated a dose-dependent ulcer healing and protective effect when compared with the ulcer control group. At doses of 200 mg/kg and 400 mg/kg, the ethanolic extract produced percentage inhibition of ulceration of 26.50% and 53.06%, respectively. The gastroprotective effect observed at the higher dose of 400 mg/kg was appreciable, although it was lower than that of the standard anti-ulcer drug, Ranitidine, which showed an ulcer inhibition of 77.50%. These findings suggest that the ethanolic leaf extract of *Andrographis echioides* possesses significant anti-ulcer activity, likely due to the synergistic action of its bioactive phytoconstituents.

Keywords: *Andrographis echioides*, anti-ulcer activity, Ranitidine, phytochemical evaluation.

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1. Introduction

Peptic ulcer disease is characterized by the formation of painful sores or ulcers in the mucosal lining of the stomach or the proximal part of the small intestine, known as the duodenum [1]. Although no single factor has been identified as the sole cause of ulcer formation, it is now well established that peptic ulcers develop as a result of an imbalance between the aggressive factors and the protective mechanisms of the gastrointestinal mucosa, particularly the digestive secretions present in the stomach and duodenum [2].

The major causes associated with peptic ulcer disease include infection by *Helicobacter pylori* (*H. pylori*) and the prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, naproxen, and ibuprofen, among others [3]. These medications, including enteric-coated and powdered formulations of aspirin, are known to increase the risk of gastric mucosal injury and ulcer formation [4].

Peptic ulcers may occur with or without noticeable symptoms. When symptoms are present, patients commonly experience a burning or gnawing pain in the upper or middle abdomen, particularly between meals or during the night [5]. Other frequently reported symptoms include abdominal bloating, heartburn, nausea, and vomiting. In more severe cases, ulcers may lead to complications manifested by black

or tarry stools due to gastrointestinal bleeding, vomiting of blood, unexplained weight loss, and severe abdominal pain [6].

Although some ulcers may heal spontaneously, their symptoms should not be ignored. If left untreated, peptic ulcers can result in serious complications such as gastrointestinal hemorrhage, perforation of the stomach or duodenal wall, and gastric outlet obstruction caused by edema or scar tissue formation that interferes with the normal passage of food from the stomach into the small intestine [7].

In the present investigation, an effort was made to explore and enhance the existing knowledge regarding the anti-ulcer potential of the ethanolic leaf extract of *Andrographis echioides*. The study was undertaken to scientifically evaluate its gastroprotective activity and to provide experimental evidence supporting its possible therapeutic application in the management of peptic ulcer disease.

2. Materials and Methods

2.1 Plant Material

The plant of *Andrographis echioides* was collected from Thirumalaisamudram 7 km away from Thanjavur (Tamil Nadu) in the month of January 2025. The plant was identified by local people of that village and authenticated by Dr. N. Ravichandran, Asst. professor, drug testing Laboratory, CARISM, SASTRA University Thanjavur, and the Voucher specimen is preserved in laboratory for future reference.

2.2 Pharmacognostical Screening of Plant

Macroscopic characters, Microscopic characters and Physiochemical Parameters of *Andrographis echioides* and leaf powder: The macroscopic evaluation was carried out for shape, size, colour, odour, taste and fracture of the drug. The microscopic evaluation was performed the transverse section of midrib and lamina region of the leaf. Different physio- chemical values (Table 1) such as Ash values, extractive values, loss on drying, foreign organic matter, crude fibre content, were determined. Crude powder subjected to carried out the chemical test (Table 2) based upon the colour reaction.

2.2.1 Florescence Analysis Study of *Andrographis echioides* Leaves Powder

Florescence analysis study (Table 3) of powdered drug material with different reagents was carried out observe the reactions.

2.2.3 Preparation of Extracts from *Andrographis echioides* Leaves Powder

Both the leaves were dried under shade, powdered and passed through 40 meshes and stored in closed vessel for further use. The dried powder material (20 gm) was subjected to soxhlet extraction with ethanol for continuous hot extraction for 6hrs. The extracts were concentrated under reduced pressure to obtain the extracts solid residues. The percentage value of the extracts was 9.35%w/w.

2.2.4 Phytochemical Evaluation of Ethanolic Leaf Extract of *Andrographis echioides*

The Ethanolic extract of *echioides* (leaf) was subjected to preliminary phytochemical test followed by6 the methods of Harbome (1998)[2], and Trease and Evans (1983)[3] and the phytoconstituents reported in table no 4.

2.3 Screening of Thin Chromatography

The dried ethanolic leaf extract of *Andrographis echioides* was used for chromatographic analysis. Approximately 100 mg of the dried extract was dissolved in 10 mL of analytical-grade ethanol and sonicated for 10 minutes to ensure complete dissolution. The solution was filtered through Whatman No. 1 filter paper to remove any insoluble particles. The clear filtrate obtained was used as the test sample for TLC analysis. Thin layer chromatography reported in the table no 5 based upon determining the R_f value using the difference chemical mobile phase and detecting reagents.

2.4 Evaluation of Anti-Ulcer Activity

2.4.1 Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 180–220 g was used for the study. The animals were housed under standard laboratory conditions with free access to food and water. Prior to the experiment, the animals were acclimatized to the laboratory environment for at least one week.

2.4.2 Experimental Design

The animals were randomly divided into five groups containing six animals each.

Group I – Normal Control

Animals received the vehicle only and were not subjected to ulcer induction.

Group II – Ulcer Control

Animals received the vehicle and were subjected to pylorus ligation.

Group III – Standard Drug

Animals received the standard anti-ulcer drug, such as Ranitidine (20 mg/kg, p.o.), prior to pylorus ligation.

Group IV – Test Group Low Dose

Animals received ethanolic leaf extract of *Andrographis echioides* at a low dose (e.g., 200 mg/kg, p.o).

Group V – Test Group High Dose

Animals received ethanolic leaf extract of *Andrographis echioides* at a high dose (e.g., 400 mg/kg, p.o.). The treatments were administered orally once daily for the designated study period or as per the experimental protocol. Under light ether anesthesia, the abdomen was opened and the pylorus was ligated. The abdomen was then sutured. After 4 hrs of pyloric ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out. The gastric juice thus collected was centrifuged and the volume of gastric juice, pH of gastric juice was noted. The stomach was opened along the greater curvature and the severity of hemorrhagic erosions in the acid secreting mucosa was assessed on a scale (table 6) of 0 to 3 as given below.

2.4.3 Biochemical Parameters

The stomach was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The gastric contents were analyzed for gastric juice volume, pH.

2.4.4 Measurement of Gastric Juice Volume and pH:

Gastric juice was collected from pylorus lygated rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100 g body weight. The pH of the supernatant was measured using digital pH meter

2.4.5 Ulcer Index (UI):

The mucosa was flushed with saline and stomach was pinned to frog board. The lesion in glandular portion was examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the values and their mean values were determined.

Ulcer Scoring System

Table 1: Ulcer Scoring Score

Score	Observation
0	Normal stomach
0.5	Red coloration
1	Spot ulcers
1.5	Hemorrhagic streaks
2	Deep ulcers
3	Perforated ulcers

2.4.6 Percentage inhibition

Percentage inhibition was calculated using the following formula.

% inhibition =

UI ulcer control — UI ulcer treated X 100 / UI ulcer control

2.4.7 Statistical Analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one-way ANOVA and compared by using Tukey- Kramer multiple comparison test. The values are statistically significant at three levels, ***p 0.05.

3. Results

3.1 Macroscopic Characters of *Andrographis echioides*

Annual dense herb grows up to 50 cm, stems are angular, densely hairy, leaves oblong to oblanceolate 3-5 x 0.7 -1 cm in size hairy on both the sides. Racemes are 3-5 cm long; the length of the raceme does not exceed length of leaves. Racemes are scarcely branched. Calyx lobes are 5, linear, hairy 6 mm long. Corolla white with brown tinged, 2 lips, upper lib is oblong 5-5.5x2 mm, upper one is with two lobes, lower lib 7 mm long oblong to lanceolate with three lobes. Filaments are flattened. Capsule 1-2 x 0.5 cm in size.

Table No. 2: Macroscopic characters of *Andrographis echioides*

Parameter	Observation
Colour	Green to dark green
Odour	Characteristic
Taste	Bitter
Shape	Elliptic to ovate
Surface	Pubescent, rough
Apex	Acute
Margin	Entire
Venation	Reticulate
Texture	Soft, hairy
Fracture	Short and brittle

Figure 1: *Andrographis echioides*

3.2 Powder Microscopy

Powder microscopy shows the presence of fibres with blunt end, fibre cells with tapering ends, fibres with peg like outgrowths and also fibres with simple pits. Tracheids are seen with pitted thickening. Xylem vessels are with bordered pitted thickening and spiral thickenings. And also the powder microscopic studies show the presence of Prismatic and acicular calcium oxalate crystals. Simple and compound round and oval shaped starch grains are present.

Figure 2: Powder microscopy of Andrographis echioides

3.3 Preparation of Extracts from Andrographis echioides Leaf

The leaves were dried under shade, powdered and passed through 40 meshes and stored in a closed vessel for further use. The dried powder material (100gram) was subjected to Soxhlet extraction with Ethanol for continuous hot extraction for 6 hours. The extracts were concentrated under reduced pressure to obtain the extracts solid residues. The percentage value of the extracts was 23.64%w/w.

Table No. 3: Phytochemical Evaluation of Ethanolic extract of *Andrographis echioides*

Phytoconstituents	*Andrographis echioides*
Alkaloids	-
Protein	+
Flavonoids	+
Saponin glycoside	+
Volatile oils	-
Amino acid	+
Phenolic compounds	+
Steroids	-
Carbohydrate	+
Bitter glycoside	-
Fats and oils	-

\+ Present, - Absent

3.4 Phytochemical Evaluation of Ethanolic extract of *Andrographis echioides*

The ethanolic leaf extract of *Andrographis echioides* was subjected to qualitative phytochemical screening using standard chemical tests for the detection of various classes of secondary metabolites. Approximately 100 mg of ethanolic extract was dissolved in 10 mL of ethanol and filtered. The filtrate was used for various phytochemical tests.

3.5 Thin layer chromatography of ethanol extract of Andrographis *echioides:*

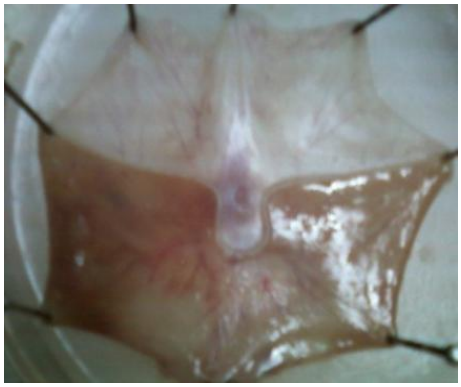
The developed TLC plate was first examined under normal daylight. Any coloured spots visible to the naked eye were observed and marked. The dried chromatogram was examined under a UV chamber at 254 nm (short-wave UV) and 365 nm (long-wave UV). The plate was uniformly sprayed with vanillin–sulphuric acid reagent and heated at 105°C for 5–10 minutes. Characteristic coloured spots corresponding to terpenoids, steroids, and other phytoconstituents were developed.

Table No. 4: Thin Layer Chromatography of Ethanol Extract of *Andrographis echioides*

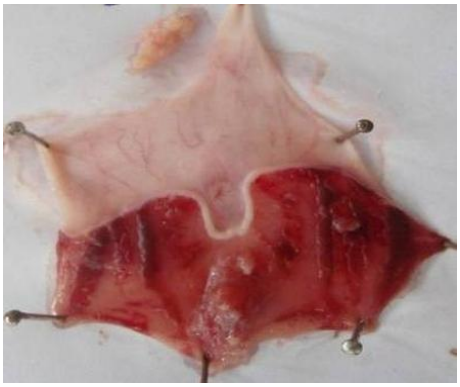
Constituents	R_f Value
Amino acids	0.65
Flavanol glycosides	0.46
Flavonoids	0.45
Saponins	0.42, 0.61
Phenolic compounds	0.44, 0.67
Terpenes	0.28, 0.66

3.6 Anti-ulcer screening - Pylorus ligation induced ulcer in Rats:

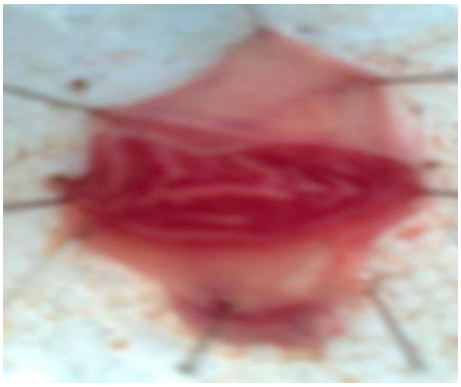
Effects of ethanol extract of *Andrographis echioides* on ulcer index by using pylorus ligation induced ulcer method in rats are shown in Table. Pylorus ligation induced gastric damage showed gross mucosal lesion, including long haemorrhage bands and petechial lesion. Animals pretreated with ethanol extract of *Andrographis echioides* and standard drug Ranitidine showed very mild lesions and sometimes no lesion at all, when compared to ulcer control group. *Andrographis echioides* showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 26.53 and 53.06 at doses of 200 and 400 mg/kg doses respectively. The ulcer protective action of extracts at different doses was better as that of standard drugs, Ranitidine, which exhibited an inhibition percentage of 77.5. Pylorus ligated rats showed severe gastric haemorrhagic lesions. The pathogenesis of pylorus ligation induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leukotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage. So the cytoprotective mechanism of the *Andrographis echioides* extract may therefore include mechanisms other than simple acid neutralization. ulcer, so it can be thought that the antisecretory activity might not be the main mechanism of action of these extracts.



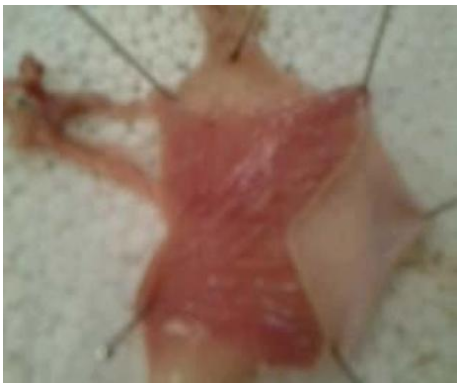
Normal Control



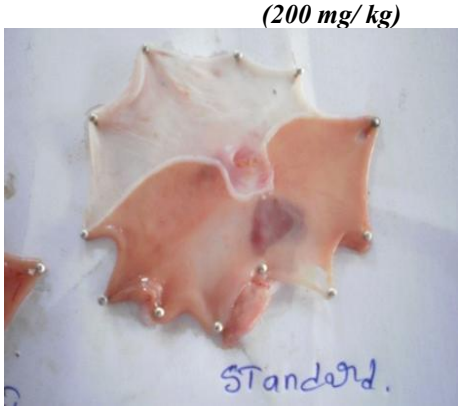
Ulcer Control



Ethanol extract of *Andrographis Echioides* (200 mg/ kg)



Ethanol extract of *Andrographis Echioides* (400 mg/ kg)



Standard drug Ranitidine (20mg/kg)

Figure 3: Effect of ethanol extract of *Andrographis Echioides (EEAE)*on Ulcer Index in pylorus ligated rats.

Table 5: Effect of *EEAE* on Gastric secretion, pH in pylorus

Group	Gastric volume (ml/100 g)	pH of gastric juice
Normal Control	1.025±0.29	1.575±0.22
Ulcer control	3.075 ± 0.206	1.175±0.095
EEPS (200 mg/kg) P.O.	2.65±0.208***	2.25±0.129**
EEPS (400 mg/kg) P.O.	1.85 ± 0.129***	2.575 ±0.125***
Ranitidine (30 mg/kg) I.P.	1.35 ± 0.2***	3.32 ±0.309***

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. *Significant as compared to control P*** < 0.001, P** < 0.05.*

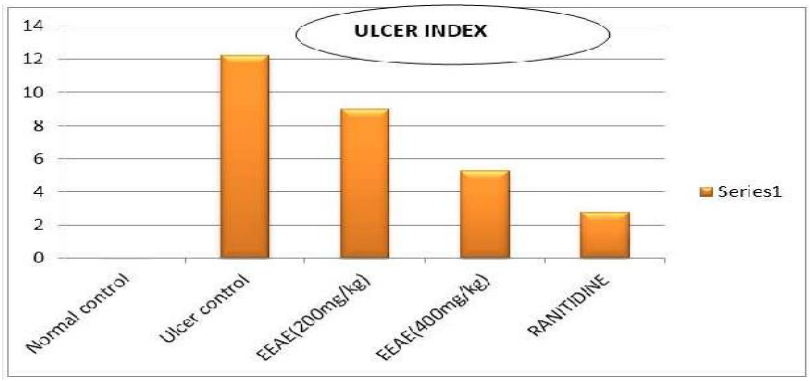


Figure 4: Effect of *EEAE* on Ulcer Index in pylorus ligated rats

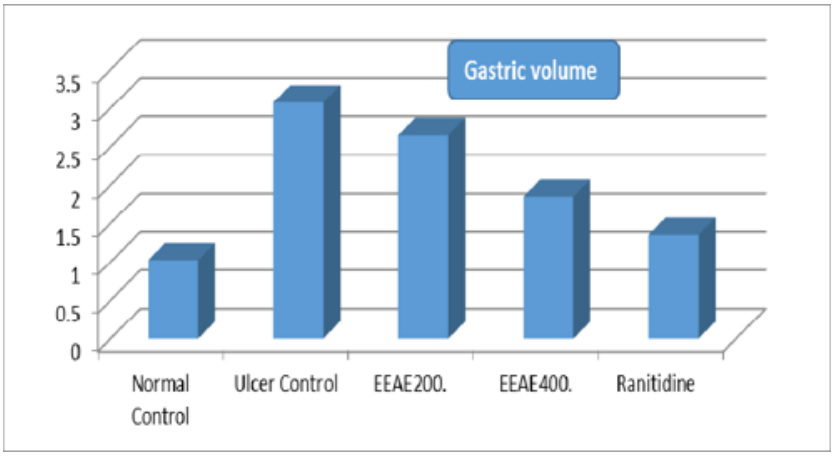


Figure 5: Effect of EEAE on Gastric secretion in pylorus ligated rats

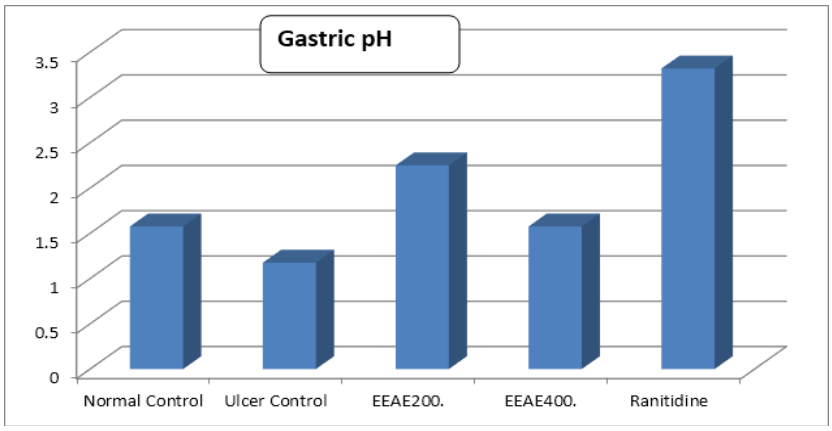


Figure 6: Effect of *EEAE* on Gastric secretion in pylorus ligated rats

4. Discussions

The present study was undertaken to determine the antiulcer activity of the ethanol extract from the leaves of *Andrographis echioides.* The preliminary phytochemical investigation showed the presence of alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, gums and phytosteroids. The pharmacological and acute toxicity studies of ethanol extract were performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed (3 days) up to 2000 mg/kg of body weight. The phytoconstituents like flavonoids, tannins and terpenoids, have been reported in several anti-ulcer literatures as possible gastroprotective agents. Flavonoids, tannins and triterpenes are among the cytoprotective active materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that these compounds will be able to stimulate mucus, bicarbonate and prostaglandin secretion, and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen. Tannins may prevent ulcer development due to their protein precipitating and vasoconstriction effects. Their astringent action can help precipitating micro proteins on the ulcer site, thereby forming an impervious layer over the lining that hinders gut secretions and protects the underlying mucosa from toxins and other irritants.

Similarly, the ethanol extract of *Andrographis echioides* showed the presence flavonoids and their glycosides, tannins and triterpenoids. These phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by pylorus ligation. *Andrographis echioides* showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 26.50 and 53.06 at doses of 200 and 400 mg/kg doses respectively. The ulcer protective action of extracts at 400 mg/kg was good to that of standard drugs, Ranitidine, which exhibited an inhibition percentage of 77.50.

5. Conclusion

The ethanol Leaf extract of *Andrographis echioides* showed the presence flavonoids and their glycosides, tannins and triterpenoids. These phytoconstituents present in the extract could be the possible agents involved in the prevention of gastric lesions induced by pylorus ligation. *Andrographis echioides* showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 26.50 and 53.06 at doses of 200 and 400 mg/kg doses respectively. The ulcer protective action of extracts at 400 mg/kg was good to that of standard drugs, Ranitidine, which exhibited an inhibition percentage of 77.50.

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