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EVALUATION OF GASTROPROTECTIVE POTENTIAL OF LEAVES OF LUFFA ACCUTANGULA (ROXB.) IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Anti-Ulcer therapies are one of the top grossing therapeutic segment in Pharmaceutical Industry globally. **Aims and Objective of the Study**: To evaluate and predict anti-ulcer activity in the leaves extracts of plant Luffa accutangula on experimental Rat models. **Materials and Methods**: The experiment was conducted in ethanol extracts of the leaves of Luffa accutangula on Pylorus ligation induced ulcer model as well as alcohol induced ulcer model. The plant leaves were collected from Seema nursery in Chaudhary Charan Singh University, Meerut. The leaves were shade dried before taking out its extracts. Healthy Rats (Albino) of 250 to 300 grams were selected for the study respectively. Two Extracts of the plant were used for comparative study, which were aqueous extract and ethanol extracts. Phytochemical screening of the extracts was carried out before proceeding to experiments. All experiments were conducted in accordance with the set guidelines of, CPCSEA, Ministry of Forest, Government of India. **Results**: The yield of Aqueous Extract was found to be, 3.0 % and that of Ethanolic Extract was 3.5 %. Upon phytochemical screening, the leaves of the plant was found to have, Cellulose, tannins, Calcium carbonate crystals, alkaloids, triterpenes and proteins. The experiment was conducted using different groups of animals divided on the basis of extracts and treatments used. The group IV and V rats were subjected to the treatment with the test compound i.e. *AELA* at the doses of 200 mg/kg and 400 mg/kg (p.o) respectively showed significant decrease in ulcer index (21.54%, 27.97), gastric volume content (7.67%, 22.48%), total acidity (5.12%, 18.09%), free acidity (5.34%, 19.65%), however the pH (35.39%, 52.11%) of the gastric juice was significant increased and the ulcers were inhibited by 22.75% and 26.7% respectively. The group VI and VII rats were treated with the other test compound (EECD) with its two selected doses i.e. 200 mg/kg and 400 mg/kg (p.o) showed significant decrease in ulcer index (35.05% 52.87%), gastric volume content (33.72% 45.46%), total acidity (20.9% 35.24%), free acidity (28.75% 35.8%), where as pH (40.79%, 56.07%) was significant increase and the ulcers were inhibited by 35.76%, 54.12% respectively. The activity of both extract with their respective doses, when compared with control, the order of potency was found to be Ranitidine > *EELA (Ethanolic Extract)* >*AELA (Aqueous Extract)* in a dose dependant manner. The plant has significant anti-ulcerant potential.

KEYWORDS: Plant Medicines, Herbal Medicines, Luffa accutangula, Phytochemical

INTRODUCTION

Gastric ulcers are one of the leading cause of morbidity and in certain cases of mortality. Gastric ulcers is a broader terms used for peptic ulcer diseases which are mainly segregated in terms of location of ulcers,[1] like duodenal ulcers in duodenal part and oesophageal ulcers in oesophagus part. Ulcers develop when erosion of cells takes place due to presence of pepsin as well as acids. In some cases the defensive mechanisms in gastric part gives up leading to acid attack in normal circumstances.[1]

Ulcers lead to pain, bleeding and utmost discomfort in patients and must be tackled using good and better anti-ulcer therapies. The synthetic drugs and chemicals

available in the market, do have better effects, but are not without side effects. Plant medicines in the other hand have better effects with no or very less side effects. Because of which, the prime focus of the present study was to carryout experiment on a plant medicine.[2]

Plant Profile: *Luffa accutangula*

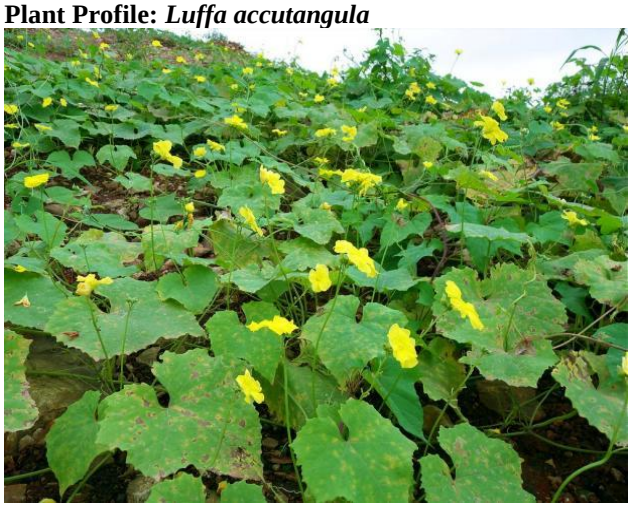


Figure 1: *Luffa accutangula* Leaves.

Biological Classification

It belongs to Kingdom, Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Family: Cucurbitaceae, Genus: Luffa, and species: Accutangula.

It is known by name: Ribbed Sponge Gourd, Jhimani, and Karviturai in hindi.[2]

Morphology

Leaves alternate, simple; stipules absent; petiole up to 15 cm long; blade broadly ovate to kidney-shaped in outline, 10–25 cm × 10–25 cm, shallowly palmately 5–7-lobed with broadly triangular to broadly rounded lobes. Flowers are yellow and large. [3]

Distribution

Luffa acutangula (L) Roxb. Var. *amara* of family Cucurbitaceae considered as indigenous to India. It is found throughout India. In Maharashtra particularly in Melghat and Satpuda ranges it occurs frequently.[4]

Propagation: Propagation of *L.acutangula* is by seeds.

Native range: India and naturalised tropic and subtropics.

Cultivation: *Luffa acutangula* can grow in all type of soils and can be grown in summer or in rainy season. Seeds can accordingly be sown either in february-march or june-july.[5]

Chemical constituents

Chemical constituents of *Luffa acutangula* mainly include carbohydrates, carotene, fat, protein, phytin, aminoacids, alanine, arginine, cystine, glutamicacid, glycine, hydroxyproline, leucine, serine, tryptophan, pipecolic acid, flavonoids, saponins. The fruit contains an amorphous bitter substance luffin.[6]

Traditional Medicinal Uses

The pounded leaves are applied locally to splenitis, haemorrhoides, ringworms and leprosy. It also contains cucurbitacin compounds which have got significant antineoplastic properties. Juice of the fresh leaves is given to children in granular conjunctivitis as a drop, to prevent adhereing of lids at night from excessive meibomian secretion. Fruit is demulcent, diuretic and nutritive. The seeds possess purgative, emetic and anthelmintic properties.[7]

LITERATURE REVIEW

Pal, Ashim, et al. carried out research work on the plant Averrhoa cartambola Linn. And studied Anti-ulcer activity using, petroleum ether, chloroform, ethanol, and water extracts of the leaves of the plant. The results of his studies were that both Aqueous and Ethanolic extracts were tolerable at dose of 2000 mg/kg. Both the extracts had significant gastro-protective activity.[8]

Bhajoni, P.S. et al. studied anti-ulcer activity in the plant Azadiracta indica in experimental animal models. The researcher studied anti-ulcer activity in the aqueous extract of the leaves of the plant Azadiracta indica. He used the models, namely, Pyolrus ligation, Aspirin induced and cold restrained anti-ulcer models. The author found dose dependent anti-ulcer activity in Aqueous extracts of Azadiracta indica plant with significant difference compsared to standard at ($P < 0.05$).[9]

Liu, Wei, et. al. studied anti-ulcerant activity in Modified Xiao Chaihu Decoction and studied different analytical psarameters to test anti-ulcer activity. The models pylorus ligation and acetic-acid induced ulcer models was used. The researcher found, significant anti-inflammatory, anti-ulcerant property in the decoction. Plus the extracts were found to have significant cell proliferation activity.[10]

MATERIALS AND METHODS

The leaves of *Luffa accutangula* was collected from Seema nursery at Dogra Mandir Meerut and positively identified by Dr. Vijay Malik, Head, Department of Taxonomy of Botany, C.C.S. University, Meerut.

Healthy adult *Albino rats* of 250-300 gms of either sex were selected for the study. The animals were obtained from animal house facility of Translam Institute of Pharmaceutical Education and Research. The animals were housed in standard cages and kept under standard condition. They were given a standard diet and water. Prior approval was taken for Animal studies from IAEC, T.I.P.E.R., Meerut constituted by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Chemical and Reagents

The chemicals and reagents used to carry out the undertaken study were of analytical grade and were procured from authorised distributor/suppliers. The following chemicals, reagents and equipment's are used

Table 1: Chemical and reagents used during the study.

Name of Chemical/Reagents	Manufacturer
sodium Carboxymethylcellulose salt	SDFCL
Acetic acid	SDFCL
n-butanol	SDFCL
DTNB	SDFCL
Disodium hydrogen phosphate	SISCO
EDTA	SDFCL
Reduced GSH	SDFCL
Hydrogen peroxide	SDFCL
Potassium dihydrogen phosphate	SISCO
Pyridine	SDFCL
Pyrogallol	SDFCL
Sodium citrate	SDFCL
Sodium dodecyl sulfate (SDS)	SDFCL
Sodium hydroxide	SDFCL
2-thiobarbituric acid	SDFCL
Trichloroacetic acid	SDFCL
Tris HCl buffer	SISCO
Albumin bovine fraction V (powder)	SDFCL
Sodium hydroxide pellets purified	SDFCL
Sodium hydrogen carbonate	SDFCL
Potassium sodium L (+) tartarate	SDFCL
Cupric sulphate	SDFCL
Folin Phenol reagent	SDFCL
1,1,3,3-tetra ethoxy propane	Sigma Aldrich
Ethanol absolute 99.9%	SDFCL

Table 2: Instrument/Equipment used.

Equipment's Name	Company Name
Rotary evaporator	Perfit, India
Spectrophotomer	UV-1601, UV-visible spectrometer, Shimadzu
Centrifuge	Remi Scientific Indst., Mumbai
Micropipette	J Mitra& co., Delhi
Oven	WISWD Instrument, Mumbai
Incubator	HICON Incubator
Electrical balance	AG 135 MettlerTaldedo
Water bath	HICON water bath

Equipment's Name	Company Name
pH meter	Control Dynamics
Vortex Mixer	Remi equipment, Mumbai
Syringes	Hindustan syringes & Medical device Ltd., Faridabad
Centrifuge tube	Remi equipment Mumbai
Test tube	Remi equipment Mumbai
Homogeniser	Universal Motor, Remi motors

Procurement of experimental animals

Healthy *Albino rats* (150-200 gm) of either sex were issued from diseased free animal house of Translam Institute of Pharmaceutical Education and Research, Meerut, U.P.

Plan of Work

The experimental work was planned and divided into modules as follows:

Module: Workout for test compound (Leaves of *Luffa accutangula*)

1. Selection of the plant medicine
2. Collection, Identification and authentication of Plant leaves.
3. Drying and size reduction of leaves.
4. Extraction of leaves of *Luffa accutangula* with solvents.
5. Phytochemical screening of Plant leaves.
6. Physicochemical characters of plant leaves.

RESULTS AND DISCUSSION

Authentication: The leaves of *Luffa accutangula* was collected from seema nursery at dogra mandir Meerut and positively identified by Dr. Vijay Malik, Head, Department of Taxonomy in Botony, CCS University, Meerut.

Percentage Yield Of Ethanolic Extract

The shade dried plant material (500 g) was coarsely powdered and subjected to extraction with petroleum ether in a Soxhlet apparatus. The marc was then extracted with ethanol to obtain the ethanolic extract. The yield of the extract was found to be 3.45% (w/w).

Percentage Yield Of Aqueous Extract

The shade dried plant material (500 g) was coarsely powdered and subjected to extraction with petroleum ether in a Soxhlet apparatus. The marc was then extracted with distilled water to obtain the aqueous extract. The yield of the extract was found to be 3.0% (w/w).

3.4 Experimental Animals Approval

The animal studies were conducted as per CPCSEA guideline. The study was conducted after obtaining Ethical Committee Clearance from the Institutional Animal Ethical Committee (IAEC) T.I.P.E.R., Meerut.

3.5 Macroscopical Characters

The leaves of *Luffa accutangula* were observed for macroscopical characteristics like colour, size, odour and taste.

Table 3: Macroscopical character of *Luffa accutangula.*

Sr. no.	Orgnoleptic Parameter	Observation
1	Size	2.5 to 8 cm and 1.3 to 2.5 cm
2	Colour	Mature fruit are dark green to yellowish green
3	Odour	Characteristics
4	Taste	Intense Bitter

Physiochemical Characters of *Luffa Accutangula* Leaves

Physiochemical Characters like total ash, acid insoluble ash and water insoluble ash of *Luffa accutangula* leaves were determined as per the procedure given under section.

Total Ash Value of *Luffa accutangula* Leaves

The total ash value of the plant was calculated by subtracting total weight of crucible and weight of ash from weight of empty crucible. The total ash value of *Luffa accutangula* was found to be 10.5 %.

Table 4: Total ash value of *Luffa accutangula* leaves.

Plant	Weight of crucible (g) A	Weight of drug (g) B	Weight of crucible + weight of ash (g) C	Ash obtained (g) (C – A)	Total ash (%)
Luffa accutangula	28.89	2.10	30.89	0.210	21.0/2 = 10.5

Note: Results were the means of three observations of drug sample.

Acid Insoluble Ash Value of *Luffa accutangula* Leaves

The acid insoluble ash of the plant was calculated by subtracting total ash from weight of crucible and weight of acid insoluble ash. The acid insoluble ash of *Luffa accutangula* was found to be 1.4 %.

Table 5: Acid insoluble ash value of *Luffa accutangula* leaves.

Weight of crucible (g)	Weight of crucible+ drug (g)	Weight of crucible + Total ash (g) A	Weight of crucible + weight of acid insoluble ash (g) B	Acid insoluble ash obtained (g) (A – B)	Acid insoluble ash (%)
28.68	30.68	28.89	28.86	0.028	1.4%

Note: Results were the means of three observations of drug sample.

Water Soluble Ash Value of *Luffa accutangula* Leaves

The water soluble ash of the plant was calculated by subtracting total ash from weight of crucible and weight of water soluble ash. The water soluble ash of *Luffa accutangula was* found to be 2.5%.

Table 6: Water Soluble ash value of *Luffa accutangula* leaves.

Plant	Weight of crucible (g)	Weight of crucible + drug (g)	Weight of crucible+ total ash (g) A	Weight of crucible + weight of water soluble ash (g) B	Water soluble ash obtained (g) (A – B)	Water soluble ash (%)
Luffa accutangula	28.68	30.68	28.89	28.84	0.05	2.5%

Note: Results were the means of three observations of drug sample.

Pharmacological Study

Pylorus Ligation Induced Ulcer Model in Rats

Pylorus ligation induced ulcer models was used to screen the antiulcer activity of test compound.

In Pylorus ligated group II rats (Negative control) the ulcer index (5.064±0.2586), volume of gastic juice (8.77±2.07), free acidity (88.09±1.46) and pH (2.37±0.31) was observed and noted.

In contrast the group III rats treated with ranitidine (standard drug, 50 mg/kg p.o) was significant reduced the ulcer index (77.49%), volume of gastric juice (54.76%), total acidity (59.94%), free acidity (61.62%) but pH (147.57%) of the gastric juice was significant increase and the ulcers were inhibited by 76.6%.

The group IV and V rats were subjected to the treatment with the test compound i.e. *AELA* at the doses of 200 mg/kg and 400 mg/kg (p.o) respectively showed significant decrease in ulcer index (21.54%, 27.97), gastric volume content (7.67%, 22.48%), total acidity (5.12%, 18.09%), free acidity (5.34%, 19.65%), however the pH (35.39%, 52.11%) of the gastric juice was significant increased and the ulcers were inhibited by 22.75% and 26.7% respectively.

The group VI and VII rats were treated with the other test compound (EECD) with its two selected doses i.e. 200 mg/kg and 400 mg/kg (p.o) showed significant decrease in ulcer index (35.05% 52.87%), gastric volume content (33.72% 45.46%), total acidity (20.9% 35.24%), free acidity (28.75% 35.8%), where as pH (40.79%, 56.07%) was significant increase and the ulcers were inhibited by 35.76%, 54.12% respectively.

The activity of both extract with their respective doses, when compared with control, the order of potency was found to be Ranitidine > *EELA* >*AELA* in a dose dependant manner.

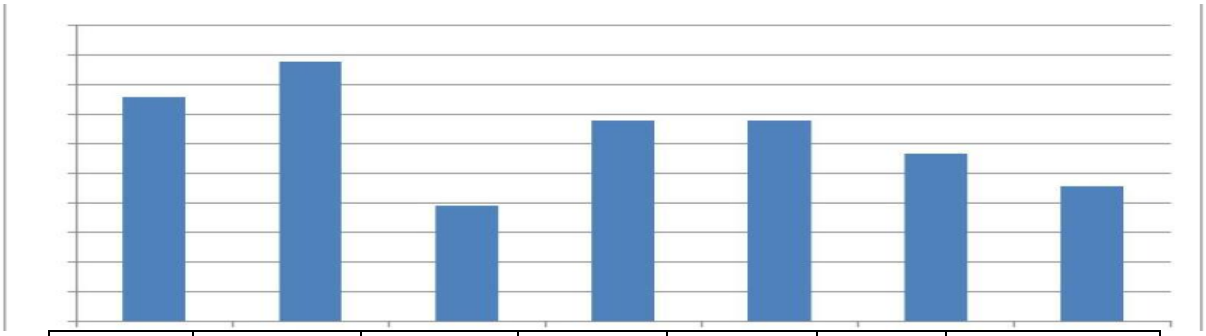
The results are shown in table 3.2, 3.3, and represented graphically in figure 3.1, 3.2, 3.3, 3.4 and 3.5.

Table 7: Effect of *AELA* & *EELA* on gastric content, pH, total and free acidity in pylorus ligation induced ulcer in rats.

Group	dose (mg/kg)	Gastric Content (ml)	pH	Free Acidity (mEq/L)	Total Acidity (mEq/L
I	Normal Control	7.56±1.55	3.44±0.62	62.12±1.32	86.41±2.32
II	Negative Control	8.77±2.07	2.37±0.31	88.09±1.46	115.35±2.43
III	Ranitidine 50	3.89±0.09**	5.07±0.22**	34.60±0.47**	46.01±1.52**
IV	*AELA* 200	6.78±0.23*	3.48±0.14**	86.12±1.40**	112.09±2.32*
V	*AELA* 400	6.79±0.08**	3.38±0.17**	69.75±0.632**	92.80±1.10**
VI	*EELA* 200	5.66±0.23**	3.54±0.28*	65.28±1.47**	93.05±1.65**
VII	*EELA* 400	4.56±0.22**	3.68±0.27**	56.07±0.71**	75.41±1.20**

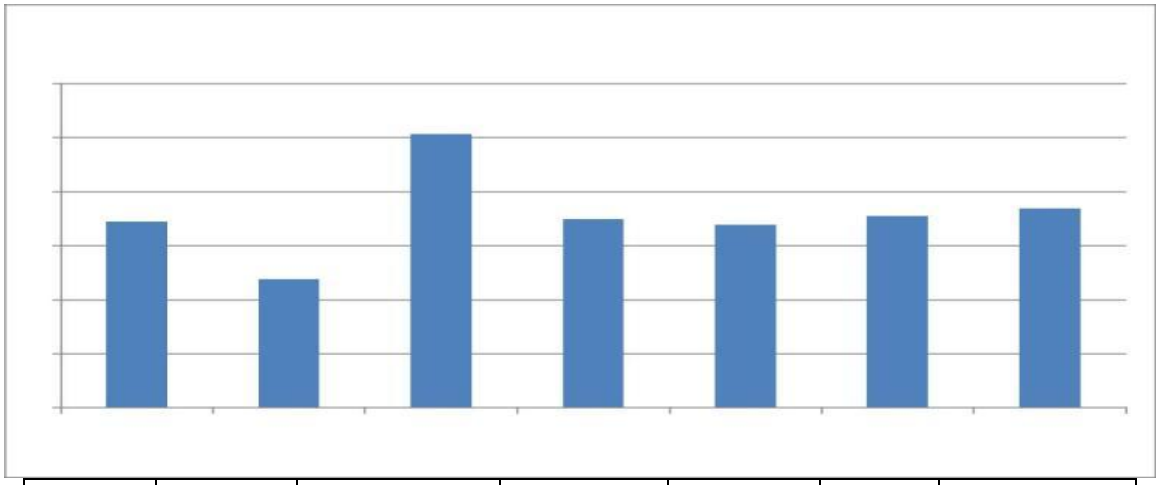
All the values are expressed in mean ± S.E.M, n=6/group, *P<0.01 when compared with control group (NOVA followed by Dunnet's t-tests)

Gastric Content



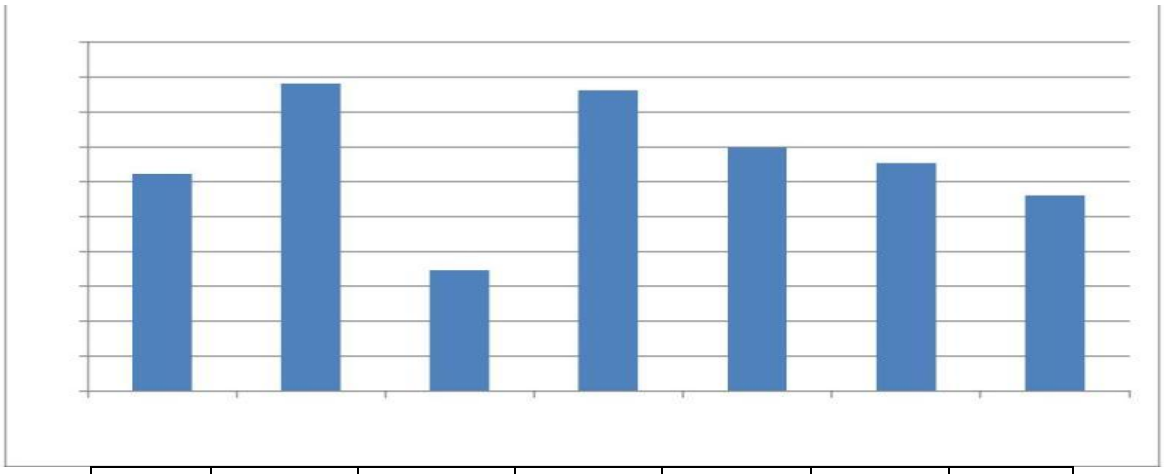
Normal Control	Negative Control	Ranitidine (50 mg/kg)	*AELA* (200 Mg/kg)	*AELA* (400 mg/kg)	*EELA* (200 mg/kg)	*EELA* (400 mg/kg)

Figure 2: Effect of *AELA* and *EELA* on gastric content in pylorus ligation induced ulcer in experimental rats. (Bar graph represents Mean ± S.E.M. of n = 6/group)



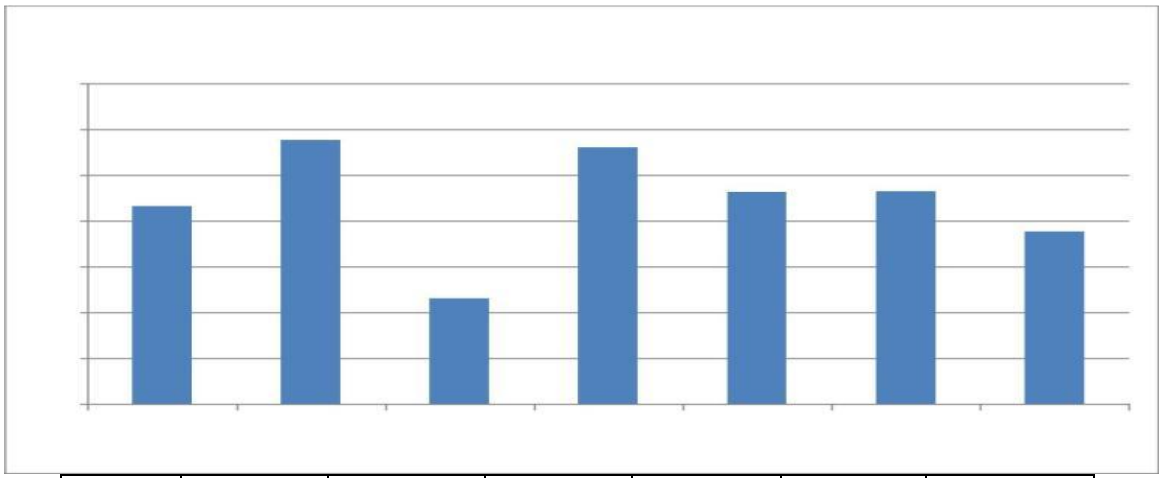
Normal Control	Negative Control	Ranitidine (50 mg/kg)	*AELA* (200 Mg/kg)	*AELA* (400 mg/kg)	*EELA* (200 mg/kg)	*EELA* (400 mg/kg)

Figure 3: Effect of *AELA* and *EELA* on pH in pylorus ligation induced ulcer in experimental rats. (Bar graph represents Mean ± S.E.M. of n = 6/group.



Normal Control	Negative Control	Ranitidine (50 mg/kg)	*AELA* (200 Mg/kg)	*AELA* (400 mg/kg)	*EELA* (200 mg/kg)	*EELA* (400 mg/kg)

Figure 4: Effect of *AELA* and *EELA* on Free acidity in pylorus ligation induced ulcer in experimental rats. (Bar graph represents Mean ± S.E.M. of n = 6/group).



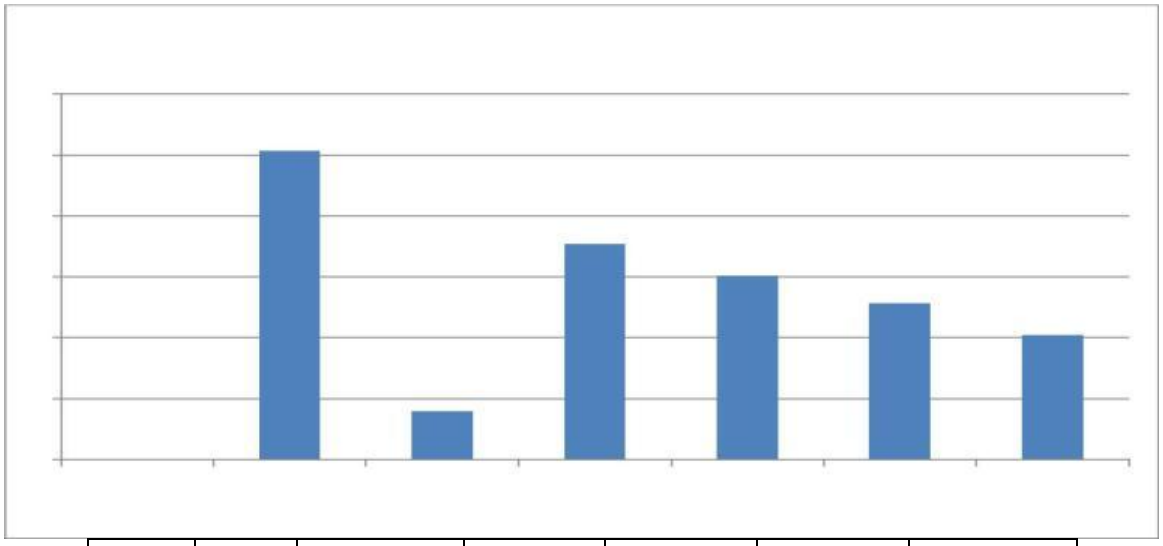
Normal Control	Negative Control	Ranitidine (50 mg/kg)	*AELA* (200 Mg/kg)	*AELA* (400 mg/kg)	*EELA* (200 mg/kg)	*EELA* (400 mg/kg)

Figure 5: Effect of *AELA* and *EELA* on Total acidity in pylorus ligation induced ulcer in experimental rats. (Bar graph represents Mean ± S.E.M. of n = 6/group).

Table 8: Effect of AECD & EECD on % inhibition of ulcers in pylorus ligation induced ulcer model.

Group	Dose	Ulcer index	% inhibition
I	Normal Control	-	-
II	Negative Control	5.064±0.258	.....
III	Ranitidine 50 mg/kg	0.79±0.24**	77.49
IV	*AELA* 200 mg/kg	3.54±0.156*	21.54
V	*AELA* 400 mg/kg	3.01±0.33**	27.97
VI	*EELA* 200 mg/kg	2.56±0.15**	35.05
VII	*EELA* 400 mg/kg	2.04±0.16**	52.87

All the values are expressed in Mean ± S.E.M. of n=6/group, *P<0.05, **P<0.01 when compared with control group (ANOVA followed by Dunnet's t-tests)



Normal Control	Negative Control	Ranitidine (50 mg/kg)	*AELA* (200 Mg/kg)	*AELA* (400 mg/kg)	*EELA* (200 mg/kg)	*EELA* (400 mg/kg)

Figure 6: Effect of *AELA* and *EELA* on Ulcer index in pylorus ligation induced ulcer in experimental rats. (Bar graph represents Mean ± S.E.M. of n = 6/group).

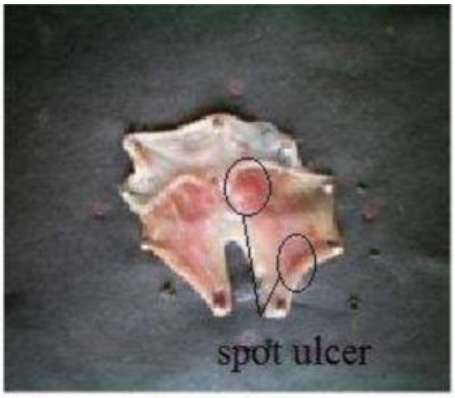
A

B

C

D

E



F

Figure 7: Photograph showing effects of various treatments on pylorus ligation induced ulcer.

(A)Negative Control	**(B) Standard(Ranitidine, 50 mg/kg)**	**(C) *AELA*(200 mg/kg)**
(D) *AELA*(400 mg/kg)	**(E) *EELA*(200 mg/kg)**	**(F) *EELA*(400 mg/kg)**

CONCLUSION

A synthetic as well as herbal drug possesses different integrity in the pharmaceutical market. The selection and rejection of the both option depends upon some obvious reasons like, forthcoming side effects due to long term treatment with synthetic drugs, but herbal or plant products always proved for its safety and efficacy. Herbal therapy provides rational means for the treatment of the many internal diseases. In the present scenario many researcher and manufacturer shows interest in isolation, separation and characterization of the actively plants metabolites because herbal formulations have reached widespread acceptability as therapeutic agents in India and abroad.

Uses of plants metabolites for various ailments become a traditional pattern and thousands of plants successfully highlighted for the various diseases from which some of the indigenous plants available in India found to show significant antiulcer activity. The selected plants and their extracts after detailed investigation have shown significant antiulcer activity in various fractions.

In the present study, preliminary phytochemical evaluation of both extract *AELA* and *EELA* revealed the presence of Alkaloids, xanthone, triterpenes in both the extract and on the basis of that antiulcer activity of aqueous and ethanolic extract of *L. accutangula* was screened by pylorus ligation and ethanol induced gastric ulcer in *Albino rats*.

Finally, the experimental studies on animal model demonstrated the protective and curative activities of the *L. accutangula* against gastric ulceration when compared with control.

DIRECTION FOR FUTURE RESEARCH

Further study is warranted to isolate, characterize and screen the active principles phytoconstituents from the *L. accutangula* that possess gastroprotective, effects and also there is a need to find out the exact mechanism by which the above effects are produced.

Presence of certain active constituents can be effectively formulated into one of the most convenient and promising dosage form for example suspensions and syrup. Further study needs elaborate technique to develop dosage form from these extracts.

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