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PHYTOCHEMICAL AND ANTI-ULCER ACTIVITY OF PETROLEUM ETHER AND CHLOROFORM EXTRACTS OF LEAVES OF ALANGIUM SALVIFOLIUM LINN. (FAMILY-ALANGIACEAE)

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Abstract: Alangium salvifolium Linn. (family-Alangiaceae), is found in different parts of India. It is widely used indigenously for different purposes. The present study is an attempt foe preliminary investigation of phytochmical constituents and to explore the anti-ulcer activity of petroleum ether and chloroform extracts of leaves of Alangium salvifolium by developing gastric ulcer by pyloric ligation and aspirin + pyloric ligation model. The extracts were administered to experimental animals orally at the doses of 100mg and 200mg per kg with ulcer developed animals. Ranitidine (5mg/kg) was administered as standard drug. All the test doses were administered orally by making suspension with 0.5% carboxy methyl cellulose. The result is that the extracts have protective activity over ulceration because the parameters which were observed, total gastric volume, total acidity, free acidity, ulcer score index and gastric pH. The author compared the result at the standard drug and leaf extracts. It has been observed that as the dose increases the protective capacity also increases.

Keywords: *Alangium salvifolium,* Phytochemical tests, pyloric ligation, petroleum ether and chloroform extracts, anti-ulcer activity.

INTRODUCTION

The roots of Alangium salvifolium are considered to be acrid, astringent, emollient, anthelmeintic, and effects on gastro-intestinal tract. Root bark acts as an antidote for poisoning, useful in external application in case of acute rheumatism, leprosy and inflammatory patches¹. In ayurveda it is considered to be kattu (bitter), teekshna (pungent), snigdha (cooling), ushna (astringent), laghu (relieving) and purgative with uronicidal action, neutralising colic, ama (dysentery) oedema (inflammation), mental disorders, poisoning etc, also reported antidote to rat and snake bite². Leaf paste used in rheumatic pain and also in the treatment of jaundice, gastric disorders.

Extraction

The dried powdered leaves of Alangium salvifolium was extracted successively with petroleum ether (60-80°c) and chloroform in soxhlet apparatus. The extracts were dried and stored in refrigerator for further use of various chemical group identification and pharmacological investigations.

Phytochemical tests of leaf extracts of Alangium salvifolium^{3, 4, 5}

The preliminary phytochemical studies were performed by testing various chemical groups present in two fractions obtained by soxhlet extraction.

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Toxicity study of a new compound must be done accurately during its screening. The purpose of an acute toxicity test is to determine the nature and extent of the untoward reactions that might follow the administration of a single dose.

The LD_{50} is determined as OECD-425 guidelines. It has been determined that at 1000 mg/kg there is no death. So author took 100 mg & 200 mg as therapeutic doses.

Animals used

All the animals were used in experiments approved by Institutional Animal Ethics Committee (CPCSEA approval)

Anti-ulcer study

Pyloric ligation method: it is one model of stress induced ulcer⁷. In this model the animals used are albino rats (180-220gm). Rats were divided into 6 groups and each group consists of six animals. Food was

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withdrawn for 24 h with free access to water and under light ether anaesthesia pyloric ligation was made^{8, 9}. It is the most commonly used technique for ulcer formation. The procedure is simple and produces reliable number of gastric ulcers¹⁰. The leaf extracts of Alangium salvifolium at dose levels of 100 mg and 200 mg/kg were taken as test drugs and ranitidine (5 mg/kg) as a standard drug, 0.5% carboxy methyl cellulose as a control administered orally. After fasting, under light ether anaesthesia abdomen is opened and the stomach was isolated and after suturing the pyloric ligation was made. After 4 h again the abdomen was opened after anaesthesia and the stomach was isolated after suturing the oesophageal end. After opening the stomach along with greater curvature the gastric juice collected in a measuring cylinder. The mucosa of stomach washed with 1ml distilled water and the washings were added to the gastric juice. 11 the gastric contents were centrifuged at 2000 rpm for 10 min. 1ml of supernatant was diluted to 10 ml with distilled water. The solution was titrated against 0.01N sodium hydroxide using topfer's reagent as indicator. The end point is solution turns to orange colour. Note the volume of NaOH which corresponds to the free acidity. Titrate further using phenolphthalein indicator till colour changes to pink colour. Note the total volume of NaOH which corresponds to total acidity.

Acidity = $\underline{\text{vol. of NaOH} \times \text{Normality} \times 100}$ mEq/l/100gm

0.1

The results were shown in **Table no 2** and **Figure no1, 2, 3, 4 & 5**

PEAS- Pet. Ether Extract of Alangium salvifolium .CEAS- Chloroform Extract of Alangium salvifolium

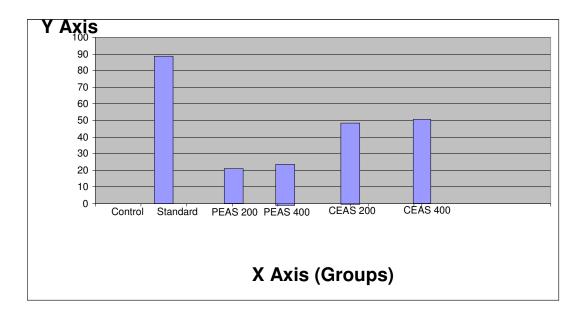


Figure 1. Anti ulcer activity of Alangium salvifolium leaf extracts (pyloric ligation model)

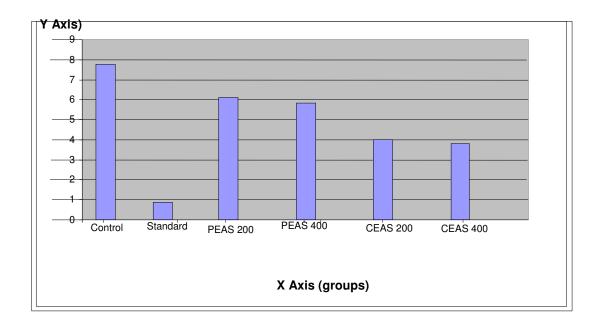


Figure 2. Ulcer Index

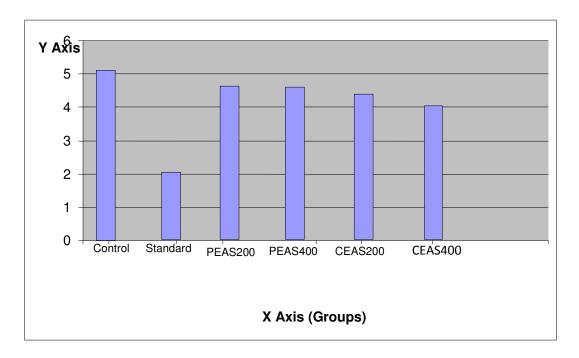


Figure 3. Gastric Volume

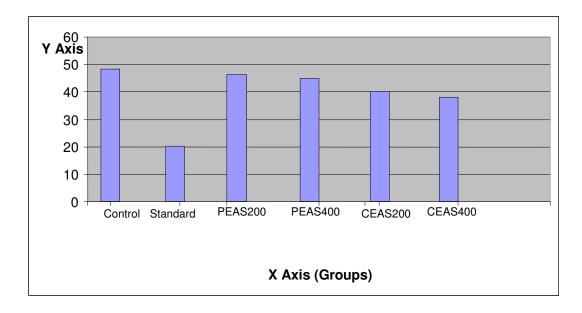


Figure 4. Free Acidity

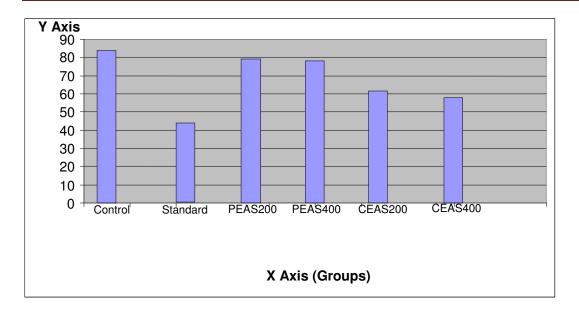


Figure 5. Total Acidity

Evaluation of anti-ulcer activity of extracts of *Alangium salvifolium* leaves by Aspirin+ pylorus ligation model in rats

This model is the combination of chemical with stress induced ulcer.¹²

36 wistar rats of both the sexes of 180-220 gms were taken and divided into six groups and each group contains 6 animals. The test group received only vehicle i.e. 0.5% carboxy methyl cellulose (10ml/kg). Group II & III receives petroleum ether extract at the dose of 100mg & 200mg/kg. Group IV & V receive chloroform extract at the dose level of 100mg & 200mg/kg. Group VI receive ranitidine 5mg/kg as standard drug. Treatment with aspirin 200mg/kg daily once for five days to all the animals. On the 6th day the rats were

kept fasting for 24 h and after that under ether anaesthesia rats were anaesthetized and pyloric ligation was made. After 4 hours the animals were again anaesthetized and after oesophageal ligation stomach were removed and animals were sacrificed. The stomach's were taken and opened at greater curvature. The volume of gastric contents was taken and stomach was again washed with 1ml distilled water. The volume of gastric juice measured by measuring cylinder and then centrifuged at 200 rpm for 10 min. Total acidity and free acidity were determined by titrating with 0.01M NaOH, using topfer's reagent and phenoptheline indicators. The stomachs were examined for ulcer lesions by a 10X magnification and ulcer screening is ulcer index and carried out. The percentage of protection calculated and the

results were depicted in Table no 3 and

Figure no- 6, 7, 8, 9 & 10

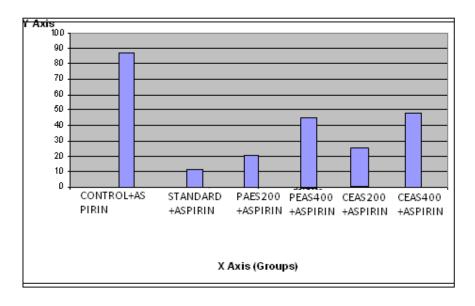


Figure 6. % Protection

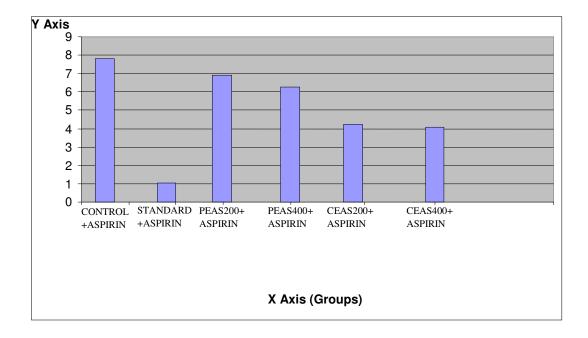


Figure 7. Ulcer Index

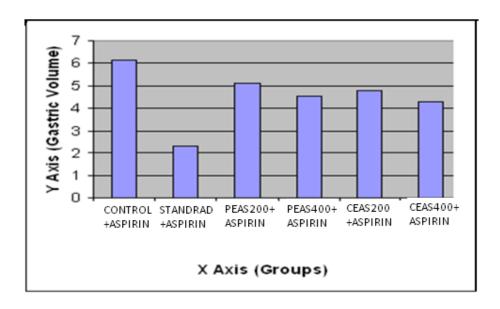


Figure 8. Gastric Volume

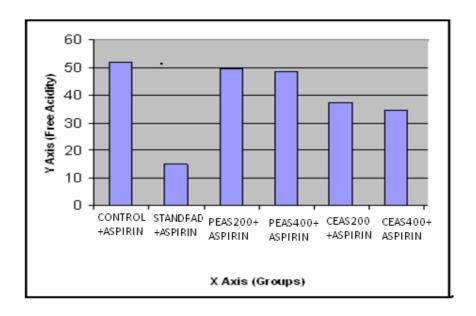


Figure 9. Free acidity

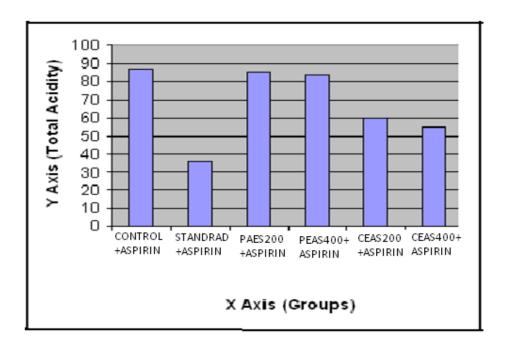


Figure 10. Total acidity

Ulcer score^{7, 10}

- 0 Normal
- 1 Scattered haemorrhagic spots.
- 2 Dense heamorraghage.
- 3 Dense heamorraghage spots and small Ulcers.
- 4 Large ulcers.
- 5 Perforations.

The percentage of protection is calculated as follows

(1-t/c)X100

Statistical Analysis¹³

The results were presented as mean <u>+</u>SEM and statistical significance between treated and control group was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t test.

RESULTS AND DISCUSSION

The two extracts of *Alangium salvifolium* at dose levels of 100mg and 200mg/kg showed statistically significant (p<0.001) anti-ulcer activity in pylorus ligated and in aspirin + pylorus ligated rat model. The

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petroleum ether extracts showed better results than the chloroform extracts.

Both the extracts of *Alangium salvifolium* showed significant reduction in gastric acid secretion and gastric ulcer formation. Prostaglandins are known to play a very important role in gastric ulcer formation. Aspirin induces ulcer by inhibiting

prostaglandins synthesis. Thus the extracts have reduction in gastric ulcer and have also cytoprotective action. Thus the activities could be attributed to fixed oils present in the plant which block the cyclooxygenase & lipo-oxygenase.

Table-1.

Phytochemical test for the presence of active constituents in *Alangium salvifolium* leaf extracts:

Active constituents	Petroleum ether extract	Chloroform extract
1. Acidic compounds.	+	+
2. Steroids and terpenoids.	+	+
3. Glycosides.	+	+
4. Carbohydrates and reducing sugars.	+	+
5. Alkaloids.	+	+
6. Flavonoids.	-	-
7. Tannins.	-	-
8. Saponins.	-	-

Note: + = Positive or present.

- = Negative or absent

Table-2.

Antiulcer results of extracts of *Alangium salvifolium* leaves by pyloric ligation model

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Group	Gastric volume	Free acidity	Total acidity	РН	Ulcer index	Ulcer Score	% protectio
		·					n
Control	5.11±0.27	48.31±1.2	83.66±2.0	2.13±0.16	7.75±0.17	5.81	
Standard	2.01±0.13	20.33±1.2	43.61±2.1	4.98±0.16**	0.87±0.14	1	88.70%
PEAS200	4.61±0.20	46.16±1.2	79.33±1.2	2.53±0.15	6.13±0.17	4.5	20.90%
PEAS400	4.58±0.12	45.02±2.0	78.05±2.4	2.95±0.21	5.82±0.21	4.1	24.9%
CEAS200	4.36±0.18	40.01±3.2	61.80±2.9	3.13±0.14	3.96±0.24	3.84	48.93%
CEAS400	4.02±0.26	38.16±1.9	58.02±1.5	3.37±0.15	3.82±0.19*	3.5	50.74%

Values are expressed in MEAN \pm SEM. One way ANOVA followed by Dunnets 't' test, n=6 in each group (**P value <0.01). (*P value <0.05)

Table 3.

Antiulcer results of extracts of *Alangium salvifolium* leaves by Aspirin+ Pyloric ligation induced model

Group No.	Gastric	Free acidity	Total	PH	Ulcer index	Ulcer	%
	volume		acidity			Score	protection
Control	6.16±0.19	51.81±3.75	86.82±3.23	2.53±0.15	7.82±0.24	6.15	
Standard	2.33±0.14	15.03±2.36	36.15±2.91	5.13±0.15	1.02±0.32**	1.5	86.90%
PEAS200	5.12±0.18	49.51±2.90	85.04±2.68	2.88±.014	6.91±0.21	4.8	11.60%
PEAS400	4.54±0.13*	48.51±2.85	83.51±2.05	3.13±0.16	6.25±0.33	4.3	20.63%
CEAS200	4.78±0.33	37.16±2.53	60.16±2.92	3.35±0.18	4.25±0.22	3.95	45.50%
CEAS400	4.31±0.27	34.66±2.91*	55.08±2.97	3.57±0.20	4.10±0.17	3.62	47.82%

Values are expressed in MEAN \pm SEM. One way ANOVA followed by Dunnets 't' test n=6 in each group, (**P value <0.01). (*P value <0.05).

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