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# Pharmacological evaluation of *Symplocos Racemosa* bark extracts on experimentally induced ulceritis in rat model

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**ABSTRACT**

Peptic ulcer disease (PUD) is chronic and appalling. Now-a-days it is the most prevalent disease affecting the world's population. PUD is the main reason for morbidity and mortality in 50% of world's working population. The pathophysiology of peptic ulcer disease is best viewed as an imbalance between mucosal defense factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (acid and pepsin)[3]. Review of Literature suggests that the presence of tannins, flavonoids, Alkaloids, saponins, terpenoids, and steroids is responsible for antiulcerogenic activity. Preliminary Phytochemical investigation of aqueous and ethanolic extracts from bark powder of *symplocos racemosa* (SR) reveals the presence of tannins, carbohydrates, proteins, alkaloids and steroids .Hence the present study aims at pharmacological evaluation of anti ulcer activity of SR (symplocaceae) in experimentally induced (pylorus ligation and Aspirin-induced) ulcers in rat models. $LD_{50}$ was found to be 2000mg/kg body weight, so, the therapeutic doses 250mg/kg and 500mg/kg were selected for both the extracts (alcoholic and ethanolic). Considering the parameters like $p^H$, gastric volume, free acidity, total acidity and ulcer index (percentage protection) we concluded that aqueous extract 500mg/body weight was equipotent to the standard (lansoprazole-8mg/kg body weight). The same results was reflected by statistical analysis (one way ANOVA $p<0.05$) and histopathological study.



## Introduction

Ayurveda is the most ancient medical system practiced in India. Recently there is considerable growth in the field of herbal medicine due to its natural origin and lesser side effects when compared to Allopathy. Selected herbal plant Symplocos racemosa (Symplocaceae) commonly known as Lodhra is most commonly found in the Poorvothara Pradesh of India mainly in planes and lower hills of Bengal, Assam and Burma. Its main useful part is Bark. Its chemical constituents are tri terpenes like betulin, butulinic acid, acetyloleanolic acid, oleanolic acid, flavonoids and anthrasinins like 3-mono gluco furanoside of 7-O-methyl leucopelargonidin glucosites, symposide, tannins like allergic acid, alkaloids like loturine, loturidine and colloturine. Our study investigated the antiulcer activity of alcoholic and aqueous extracts from the bark powder of SR in experimentally induced ulceritis.

## Plant Material and Extraction:

The plants were collected from forest of Gulbarga and are authenticated by Dr.G.S.Chowdary, Reader, M.J College, Jalgaon. They were shade dried and powdered coarsely. The coarse powder was soxhlet extracted using analytical grade solvents (water and alcohol). The extracts obtained were then concentrated under reduced pressure.

## Phytochemical Screening:

Preliminary qualitative phytochemical screening of Symplocos racemosa bark powder shows positive test for carbohydrates (Benedict's test and Barfoed's test), proteins (Millons test), flavonoids (Shinoda test), alkaloids ( Dragendroff's test) and Tannins (color reaction with ferrous chloride).

## Test Animals:

Albino wistar rats of either sex weighing between 150-250 gm and Albino mice of either sex weighing between 20 to 25gms were procured from registered breeders (149/1999/CPCSEA, Mahavir Enterprises, Hyderabad). These animals were housed under standard conditions of temperature ($25\pm2^0$C) and relative humidity 30-70 % with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and the food was withdrawn 18–24 hr before the experiment though water was allowed *ad libitum.*

## Acute Oral Toxicity Study:

Acute toxicity studies for aqueous and ethanolic extract of mixture of *SR* bark was conducted as per OECD guideline 420 (modified, adopted 23rd march 2006) using Albino Wister mice. Each animal was administered ethanolic and aqueous extracts solution by oral route. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD50, confidence intervals. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

## Experimental procedure:

Aqueous and ethanolic extracts of SR in doses of 250mg/kg and 500mg/kg and proton pump inhibitor (lansoprazole-8mg/kg) were prepared as suspension using 2% Acacia and administered orally. Control group of animals received suspension of 2% acacia.

## Pylorus ligatated induced ulcers:

Albino Wistar rats of either sex weighing between 150-200g were divided into six groups each.

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1. Group-I _ Control
2. Group-II (Lans) _ Standard (Lansoprazole 8mg/kg in 2% gum acacia p.o)
3. Group-III (ETH 250) – Ethanolic extract *symplocos racemosa* (250mg/kg p.o..
4. Group-IV (ETH 500) – Ethanolic extract *symplocos* racemosa (500mg/kg p.o.)
5. Group-V (AQ.E 250) – Aqueous extract *symplocos* racemosa (250mg/kg p.o.)
6. Group-VI (AQ.E 500) – Aqueous extract *symplocos* racemosa (500mg/kg p.o.)

In this method albino rats were fasted in individual cages for 24 hrs. Care was taken to avoid coprophagy. *symplocos* racemosa bark extracts or standard drug or control vehicle was administered 30 min prior to pyloric ligation, under light Ether anesthesia, given an incision of 1cm long in the abdomen just below sternum. Exposed the stomachs and a thread was passed around the pyloric sphincter and a tight knot was applied. Care was taken so that no blood vessel was tied. The stomach was replaced carefully. The animals were deprived of water during the post–operative period. After 4hrs stomachs were dissected out and cut along the greater curvature, washed with running water and ulcers were scored with the help of hand lens. 0=normal stomach, 0.5= red coloration, 1.0= spot ulcers, 1.5= hemorrhagic streaks, 2.0 =ulcer>3 but <5, 3.0= ulcer>5. Mean ulcer score for each animal is expressed as Ulcer index. The Percentage protection was calculated by the formula:

% protection = (1-ulcer index in test/ulcer index in control) x 100

**Estimation of $P^H$ ,Volume, Free acidity and Total acidity:**

The gastric content that was transferred into centrifuge tubes was used for estimation of gastric volume, *p*H and total acidity. The tubes were centrifuged at 1000 rpm for 10 min and the gastric volume was directly read from the graduation on the tubes. The supernatant was then collected and *p*H was determined by using a digital *p*H meter (Nirmala) .Free acidity was determined by adding 1.0 ml of gastric juice, 2 to 3 drops of Topfers reagent and titrated with 0.01N NaOH until all traces of red color disappears and turns to yellowish orange. The volume of alkali added was noted which corresponds to free acidity. Then 2 to 3 drops of Phenolphthalein solution was added and titration was continued until a define red tinge reappears. Again the total volume of alkali added was noted. The volume corresponds to total acidity. The acidity was calculated by using formula,

Acidity =volume of NaOH × Normality of NaOH × 100

**Aspirin induced ulcers:**

Albino Wistar rats of either sex weighing between 150-200g were divided into six groups each as under the section ( ). In this method albino rats were fasted in individual cages for 24 hrs.care was taken to avoid coprophagy. Aspirin in doses of 200mg/kg body weight was administered to the (groups I to VI) animals on the day of experiment. After 4hrs.administration the animals were scarified and the stomach was then excised, cut along with greater curvature, washed with running water and ulcers were scored and calculated as under section ()and sample was send to histopathological study.

**Histopathological evaluation:**

The stomachs were immersed in 10% formalin solution on the day of experiment itself. These tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue was cut on half along the long diameter. If the stomach was protected from basal part using microtone, sections of thickness of about 5µm were cut and stained with haemotoxylin and eosin. These were examined under the microscope for histopathological changes such as hemorrhage, necrosis, inflammation, erosion, ulcer and photographs were taken.

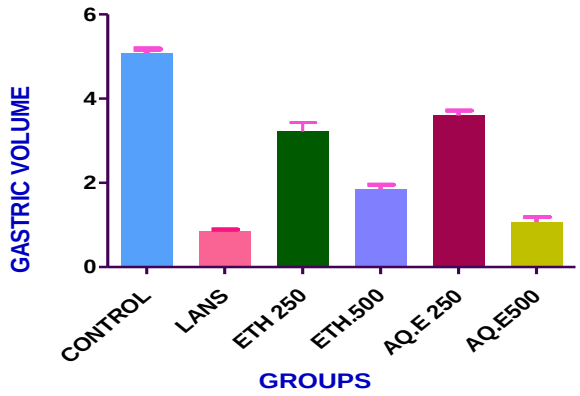
**Statistical analysis:**

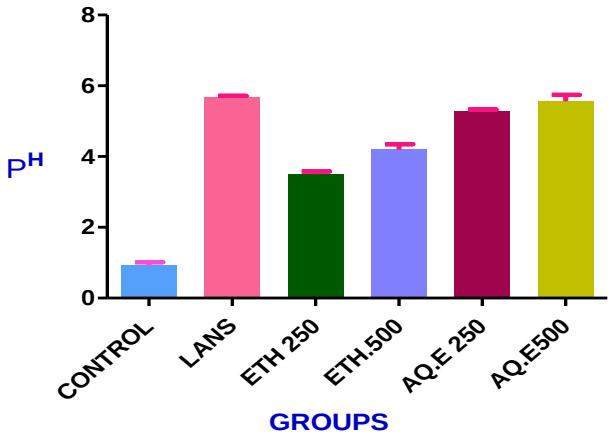
All the data was presented as mean ± S.E.M and was analysed by one-way ANOVA was followed by post tests like tukey's multiple comparision test (Anwar R. Shaikh et al.,2006). A value of P< 0.05 was considered statistically significant.

**Results:**

Effect of aqueous and ethanolic extracts of *Symplocos racemosa* on gastric secretion following pyloric ligation in rats.

**Effect of aqueous and ethanolic extracts of *Symplocos racemosa* on gastric volume and $p^H$**




**Effect of aqueous and ethanolic extracts of *Symplocos racemosa* on free acidity following pyloric ligation in rats.**

**Conclusion:**

The phytochemical studies of *Symplocos racemosa* (symplocaceae) bark show the presence of carbohydrate, proteins, flavanoids, alkaloids and tannins. The study was taken up to evaluate aqueous and ethanolic extracts of *Symplocos racemosa* for anti-ulcer activity in pylorus ligation and aspirin induced models.The acute toxicity study conducted for aqueous and ethanolic extracts indicates that they are safe upto 2000mg/kg body weight and was selected $1/8^{th}$ and $1/4^{th}$ of 2000mg/kg i.e.250mg/kg and 500mg/kg respectively as per fixed dose procedure. At 500mg/kg aqueous and ethanolic extracts has reduced ulcer index more significantly than 250mg/kg when compared with the control as evident by decrease in ulcer score in both the models(pylorus ligation and aspirin induced).Anti-secretory activity(decrease in gastric volume) and reduction in free and total acidity of the extracts at 500mg/kg was noticed in pylorus ligation induced ulcer model.

| Group No. | Treatment | Dose (mg/kg b.w) | Gastric volume (ml) | Free acidity (mEq/L)100gms | Totalacidity (Meq/L)100gms | $p^H$ |
|---|---|---|---|---|---|---|
| 1 | Control | 2% Acacia | 5.08±0.086 | 84.3±0.51 | 98.6±1.93 | 0.97 ± 0.09 |
| | Lansoprazole | 8mg/kg | 0.85±0.039 | 82.8±2.22 | 38±1.43 | 5.67 ± 0.045 |
| 3 | Ethanolic extract of SR | 250mg/kg | 3.21±0.197 | 80±1.05 | 83.8±1.40 | 3.5 ± 0.078 |
| 4 | Ethanolic extract of SR | 500mg/kg | 1.83±0.115 | 33±0.74 | 39.3±0.74 | 4.2 ± 0.14 |
| 5 | Aqueous extract of SR | 250mg/kg | 3.6±0.108 | 71.5±1.02 | 80.1±1.04 | 5.3±0.061 |
| 6 | Aqueous extract of SR | 500mg/kg | 1.06±0.111 | 31.8±1.04 | 38.1±0.72 | 5.6±0.16 |

**Effect of aqueous and ethanolic extracts of *Symplocos racemosa* on pylorus ligation induced ulceration in rats**

| Group No. | Treatment | Dose (mg/kg b.w) | Ulcer index | Percentage protection |
|---|---|---|---|---|
| 1 | Control | 2% Acacia | 4.66±0.15 | 0 |
| 2 | Lansoprazole | 8mg/kg | 1.16±0.22 | 71.74 |
| 3 | Ethanolic extract of SR | 250mg/kg | 2.41±0.14 | 47.71 |
| 4 | Ethanolic extract of SR | 500mg/kg | 1.75±0.30 | 62.0 |
| 5 | Aqueous extract of SR | 250mg/kg | 2.16±0.15 | 53.11 |
| 6 | Aqueous extract of SR | 500mg/kg | 1.5±0.20 | 67.44 |

**Effect of aqueous and ethanolic extracts of *Symplocos racemosa* on Aspirin-induced ulceration in rats**

| Group No. | Treatment | Dose (mg/kg b.w) | Ulcer index | Percentage protection |
|---|---|---|---|---|
| 1 | Control | 2% Acacia | 5.08±0.29 | 0 |
| 2 | Lansoprazole | 8mg/kg | 1.33±0.25 | 74.51 |
| 3 | Ethanolic extract of SR | 250mg/kg | 2.33±0.25 | 54.82 |
| 4 | Ethanolic extract of SR | 500mg/kg | 1.66±0.19 | 68.51 |
| 5 | Aqueous extract of SR | 250mg/kg | 2.16±0.22 | 58.67 |
| 6 | Aqueous extract of SR | 500mg/kg | 1.58±0.21 | 70.41 |

Hence, the present study support the folkloric use of the plant in treatment of gastro intestinal disorders and demonstrates that the presence of principle constituents like tannins, alkaloids and flavanoids is responsible for the observed anti-ulcerogenic activity.

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