

Original Research Article

Invitro and Invivo Antiasthmatic Activity of Ethanolic Bark Extract of *Symplocos Racemosa*

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Abstract: The aim of the present study was to evaluate invitro and invivo antiasthmatic activity of ethanolic bark extract of *Symplocos racemosa*. For invitro study, isolated guinea pig ileum was used and dose response curve of histamine in plain Tyrode solution and in Tyrode solution containing 25 µg/ml ethanolic barks extract of *Symplocos racemosa* were performed. For invivo study histamine induced bronchospasm was performed using histamine chamber. 250 and 500 mg/kg of ethanolic bark extract of *Symplocos racemosa* were used and chlorpheniramine was used as reference control. The mean exposition time and percentage protection were calculated to assess the antiasthmatic potential of *Symplocos racemosa*. In isolated guinea pig ileum preparation, *Symplocos racemosa* relaxed the muscle against histamine and in histamine induced bronchospasm, both the doses of *Symplocos racemosa* bark extract significantly increased the mean exposition time and the percentage protection compared to control. From the result it was concluded that, *Symplocos racemosa* bark extract exhibits antiasthmatic activity.

Keywords: *Symplocos racemosa*, Antiasthmatic activity, Histamine, Chlorpheniramine.

INTRODUCTION

Asthma is a chronic disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. Symptoms may occur several times in a day or week in affected individuals. For some people the symptoms become worse during physical activity or at night. Failure to recognize and avoid triggers that lead to a tightened airway can be life threatening and may result in an asthma attack, respiratory distress and even death. WHO estimates show that 235 million people currently suffer from asthma. Asthma deaths will increase in the next 10 years if urgent action is not taken. Asthma cannot be cured, but proper diagnosis, treatment and patient education can result in good asthma control and management. Asthma occurs in all countries regardless of level of development. Over 80% of asthma deaths occur in low and lower-middle income countries. For effective control, it is essential to make medications affordable and available, especially for low-income families [1].

The use of medicinal plants in the management of illnesses has been since time antiquity, and has continuously grown over time. Though western medicine has influenced the use of herbal remedies, most rural communities still practice complementary medicine as they are readily and cheaply available healthcare alternatives. Medicinal plants are of great importance in providing healthcare to a large portion of the population in India. Indian system of medicine, Ayurveda has described several drugs from indigenous plant sources for use in the treatment of bronchial asthma, and has been successful in controlling the disease as well.

Symplocos racemosa Roxb. is an evergreen Ayurvedic plant widely distributed in the tropics and subtropics of Asia, Australia and America? *Symplocos racemosa* is a small, evergreen tree, upto 6-8.5 m tall found in the plains and lower hills throughout North and East India, ascending in the Himalayas up to an elevation of 1400 m, Bengal, Assam and Chota Nagpur. Traditionally the bark of *Symplocos racemosa* was used

in various ailments like inflammation, constipation, eye diseases, bleeding gums, asthma, arthritis, ulcer, tumours, leprosy, filariasis, gonorrhoea, hepatic damage, haemorrhoids and skin diseases [2, 3].

Symplocos racemosa bark reported to have analgesic and anti-inflammatory activity [4], Antioxidant activity, Antibacterial [5], Anthelmintic activity [6], Anti-angiogenic activity [7], Anticancer activity [8] and Hepatoprotective activity [9, 10]. It also used in the treatment of Alzheimer's disease [11] and Peptic ulcer disease [12]. Current study was conducted to document the traditional claim of *Symplocos racemosa* bark for its antiasthmatic activity.

MATERIALS AND METHODS

Plant Material

The barks of *Symplocos racemosa* Sathuragiri hills. It was identified and authenticated as *Symplocos racemosa*'s bark by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, Coimbatore. The Voucher specimen (BSI/SRC/5/49/14-15/Tech - 656) has been deposited in department for further references.

Preparation of Extract

The collected barks were, shade dried and then ground into coarse powder. The powder was then subjected to exhaustive extraction by a maceration process using 90% methanol as a solvent at room temperature for 7 days. The ethanolic extract was concentrated by vacuum distillation to dry. The collected extract was stored in desiccators and used for further pharmacological study.

Animals

Guinea pigs of either sex, weighing between 280 - 350 Gms were used for this study. The animals were obtained from animal house, of Nandha College of Pharmacy, Erode. The animals were placed at random and allocated to treatment groups stainless steel cages. Animals were housed at a temperature of $24 \pm 2^\circ\text{C}$ and relative humidity of 30 - 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/02/C/CPCSEA).

Invitro Antiasthmatic Activity

Isolated Guinea Pig Ileum Preparation [13]

The guinea pigs (overnight fasted) were sacrificed and the ileum was separated and mounted in an organ

bath containing Tyrode solution, which was continuously aerated at $37 \pm 0.5^\circ$. Dose response curve of histamine in plain Tyrode solution and in Tyrode solution containing 25 $\mu\text{g/ml}$ ethanolic barks extract of *Symplocos racemosa* were performed. Percentage maximum contractile response was plotted to generate dose response curve of histamine, in the absence and presence of the plant extract.

In vivo Antiasthmatic Activity

Histamine Induced Bronchospasm in Guinea Pigs [14]

Twenty four guinea pigs of either sex were exposed to aerosol of 1.0 % Histamine diphosphate using nebulizer with constant pressure 40mm/Hg in histamine chamber and time for preconvulsion dyspnoea (PCD) was recorded from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of asphyctic convulsions i.e. Pre-convulsion time (PCT). As soon as PCD commenced, animals were removed from the chamber and placed in fresh air to recover. All the animals were randomly divided into 4 groups each containing six animals. Two and a half hours later, the animals of groups I received 0.1% Carboxy Methyl Cellulose (vehicle) and served as a control. Group II received Chlorpheniramine maleate 2 mg/kg p.o and served as standard. Group III and IV received 250 and 500mg/kg of ethanolic bark extract of *Symplocos racemosa* respectively. The test drugs were administered orally using oral tubes by suspending the extract in 0.1 % CMC. One and half hour after the treatment, animals were exposed to 0.2% Histamine diphosphate aerosol and time taken for onset of PCD was noted. The % protection offered by the extracts was calculated for each dose and positive control.

STATISTICAL ANALYSIS

The values were expressed as mean \pm SEM. The statistical analysis was carried out by paired 't' test for invitro studies and one way analysis of variance (ANOVA) followed by Dunnet's 't' - test for invivo studies using graph pad version I. P values < 0.05 were considered significant.

RESULT

Invitro Antiasthmatic Activity

The invitro antiasthmatic activity of ethanolic bark extract of *Symplocos racemosa* was studied by its effect on histamine induced contraction in isolated guinea pig ileum preparation (Table 1). 25 $\mu\text{g/ml}$ of *Symplocos racemosa* bark extract significantly ($P < 0.01$) relaxed the ileum smooth muscle against the contraction produced by the histamine.

Table 1: Effect of ethanolic bark extract of *Symplocos racemosa* on histamine induced contraction in isolated guinea pig ileum

S.NO	Dose of Histamine	Isolated Guinea Pig Ileum (% Contraction)	
	2.5µg/ml ml	Control	<i>Symplocos racemosa</i> Extract (25 µg/ml)
1	0.1	18.32±0.12	11.92±0.96*
2	0.2	32.56±1.27	19.61±1.03*
3	0.4	59.51±1.63	31.33±2.65*
4	0.8	90.62±2.55	58.62±1.62**
5	1.6	91.72±1.94	62.13±1.83**

Values are in mean ± SEM (n=6),

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Invivo Antiasthmatic Activity

The invivo antiasthmatic activity of ethanolic bark extract of *Symplocos racemosa* was studied by its effect on histamine induced bronchus-constriction in guinea pig (Table 2). Pre-convulsive time was recorded before and after extract administration. Chlorpheniramine maleate was used for reference. Mean exposition time

and the percentage protection was calculated from the pre convulsive time. Both doses of the extract of *Symplocos racemosa* bark extract significantly (P<0.001) increased the mean exposition time and the percentage protection was 48.15% and 79.61% of 250 and 500mg/kg of *Symplocos racemosa* bark extract respectively.

Table 2: Effect of ethanolic bark extract of *Symplocos racemosa* on histamine induced bronchus-constriction in guinea pig

S.NO	Drug Treatment	Pre Convulsive Time (Sec)		Mean Exposition Time (Sec)	% Protection
		Before	After		
1	Control 0.1 % CMC	118.05±5.72	121.17±4.96	3.28±0.21	0.49
2	Chlorpheniramine Maleate (2mg/kg)	109.16±4.97	780.28±6.32	674.27±5.38***	100
3	<i>Symplocos racemosa</i> (250mg/kg)	103.22±2.29	431.83±9.33	324.66±6.62***	48.15
4	<i>Symplocos racemosa</i> (500mg/kg)	98.36±6.51	627.61±4.79	536.78±7.15***	79.61

Values are in mean ± SEM (n=6),

*P<0.05, **P<0.01, ***P<0.001 Vs Control

CONCLUSION

Current study resolved that ethanolic bark extract of *Symplocos racemosa* possess antiasthmatic activity in both invitro and invivo models. *Symplocos racemosa* significantly inhibited the histamine induced contraction in both ileum and bronchus smooth muscle. The effect may be represented by its antihistaminic property. Further investigation on other models may explore its exact mechanism of action.

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