

Research Article**Cough suppressant and expectorant activities of ethanolic extract of *Pergularia daemia* in mice model**Prathap Balasubramanian^{1*}, Jacob Varghese Powathikunnil¹, Sengottuvelu Singaravel²¹Department of Pharmacology, Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Maduranthagam, Tamilnadu, India²Department of Pharmacology, Nandha College of Pharmacy, Erode, Tamilnadu, India

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Abstract

Objective: The purpose of this study was to investigate the cough suppressant and expectorant activities of ethanolic extract of *Pergularia daemia* (100 and 200mg/kg) in mice. **Materials and Methods:** The cough suppressant activity of *Pergularia daemia* was evaluated against sulfur dioxide induced cough in mice. Codeine sulfate (30mg/kg) was used as reference control. Number of cough and percentage inhibition was measured after test drug administration. The expectorant activity was evaluated by the secretion of phenol red in mice tracheas which was measured by UV spectrophotometer and ammonium chloride was used as reference control. **Results:** In cough suppressant activity, both the doses of *Pergularia daemia* significantly ($P < 0.001$) decrease the cough and the percentage inhibition of cough at 100 and 200mg/kg of *Pergularia daemia* was 50.81% and 64.54% respectively. In expectorant activity, both the doses of *Pergularia daemia* significantly ($P < 0.001$) enhance the phenol red secretion and the percentage increase at 100 and 200mg/kg of *Pergularia daemia* was 53.13% and 65.63% respectively. **Conclusion:** From the result, it was concluded that, ethanolic extract of *Pergularia daemia* exhibited cough suppressant and expectorant activity against experimental animal models.

Keywords: *Pergularia daemia*, expectorant, cough suppressant, phenol red, sulfur dioxide

Introduction

Expectorants are drugs which enhance the secretion of the sputum by the air passages so that it is easier to remove the phlegm through coughing. They are used in cough mixtures for this purpose they act either by increasing the bronchiole secretion or by making it less viscous. Expectorants are defined as medications that improve the ability to expectorate purulent secretions. This term is now taken to mean medications that increase airway water or the volume of airway secretions, including secretagogues that are meant to increase the hydration of luminal secretions (eg, hypertonic saline or mannitol) and adhesives that decrease the adhesivity of secretions and thus unstick them from the airway (eg, surfactants) (Rubin, 2007).

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Cough is a natural protective reflex to remove airway secretions and pathogens from the respiratory tract, it is one of the common symptoms associated with chronic inflammatory disease of the respiratory tract such as asthma, chronic bronchitis, pneumonia and postnasal drip syndrome (Irwin and Madison, 2000). Cough can be regulated by medications such as codeine and dextromethorphan, which are associated with side effects including drug dependency or drowsiness. Sputum tenacity, which results in adhesiveness and cohesiveness of sputum, considerably influences the clearance of sputum (Rubin, 1998). An expectorant is a drug that is thought to increase the hydration of either mucus or the periciliary fluid (Rubin, 2007). Although increasing hydration of the sputum itself will not improve clearance in the case of depleted airway surface liquid, improving the hydration of this surface fluid may detach secretions from the epithelium, thus decreasing tenacity and improving transportability and clearance of sputum (Donaldson et al., 2006; Ratjen, 2008). Therefore, there is a need for herbal based expectorant with fewer adverse effects.

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The plant *Pergularia daemia* (Asclepiadaceae) known as “Veliparuthi” in Tamil. A slender, hispid, fetid-smelling perennial climber (Khare, 2007). A widely distributed in the tropical and sub tropical area. In India it is very commonly found in hedges through cut most of cenfry to an altitude about 1000m in Himalayas and 900m in Southern India.

Ethnomedical survey reveals various uses of *Pergularia daemia*. The aerial parts of *Pergularia daemia* used for snake bite. Entire plant of *Pergularia daemia* used as an anthelmintic, emmenagogue, emetic, antiseptic, expectorant and used to facilitate parturition. In Ayurvedic medicine the *Pergularia daemia* used in amenorrhea, asthma, snakebite, rheumatic swellings, post-partum hemorrhage and to delay childbirth. Latex of this plant used for boils and sores. Dried leaf of *Pergularia daemia* used as an emetic, antirheumatic and used for bronchitis. The fresh leaf juice of *Pergularia daemia* was used for amenorrhea, dysmenorrhea, catarrhal infections, infantile diarrhea and reduce the body pain. Dried root of *Pergularia daemia* was used as an abortifacient, emetic, bronchitis and used to treat cough, asthma and constipation (Bhaskar and Balakrishnan, 2009). Some of its ethnomedical claims were scientifically proved but not all. The present study was conducted with an aim to justify its expectorant property by evaluating the whole plant extract of *Pergularia daemia* on phenol red secretion model in mice.

Materials and Methods

Plant material

The plant *Pergularia daemia* was collected from the outskirts of Pollachi. The plant was identified as *Pergularia daemia* and authenticated by the botanist, Botanical Survey of India, Agricultural University, Coimbatore. The voucher specimen (BSI/SRC/11/72/2017-18/Sci/01298) has been deposited in the herbarium for future reference.

Preparation of extract

The plant *Pergularia daemia* was washed in running water to remove the adhering foreign matter and shade dried. The dried plant materials were coarsely powdered by mechanical blender. The coarse powder of *Pergularia daemia* was soaked in 70% ethanol for 24 h followed by cold maceration for further 48 h with occasional shaking. The mixture was filtered using muslin cloth followed by removal of excess of solvent by rotatory evaporator. The dried extract of *Pergularia daemia* was used for further pharmacological studies.

Animals

Swiss albino male mice (18 – 20 g) were used in this study. The animals were obtained from animal house, of Kerala Veterinary and Animal Science University, Mannuthy. On arrival, the animals were placed at animal house, Karpaga Vinayaga

Institute of Medical Sciences & Research Institute and randomly allocated to treatment groups in polypropylene cages with paddy husk as bedding. 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 (1818/GO/Ere/S/15/CPCSEA) and were in accordance with the Institutional ethical guidelines.

Sulfur dioxide (SO₂) induced cough

Animals were divided into four groups of six each (n=6). Group I served as control, received 0.1% Carboxy Methyl Cellulose solution (CMC). Group II served as reference control, received Codeine Sulfate (30mg/kg). Group III and IV animals were received 100 and 200mg of ethanolic extract of *Pergularia daemia* respectively. All the test drugs were administered through oral route by suspending in CMC once daily for 5 days. NaHSO₃ solution in water (500 mg/kg, 2.0 ml) was placed at the base of a specially designed desiccators and 0.2 ml of sulfuric acid was added using a pipette which resulted in the formation of sulfur dioxide. After 15 seconds, each mouse was placed on the stage in the desiccator and exposed to SO₂ for 45 seconds. The mice were then removed and placed in a clear glass chamber for counting of bouts of cough for 5 min and percentage inhibition was calculated (Gupta et al., 2009).

Expectorant activity

Animals were divided into four groups of six each (n=6). Group I served as control, received 0.1% Carboxy Methyl Cellulose solution (CMC). Group II served as reference control, received 0.5% Ammonium Chloride (1g/kg). Group III and IV animals were received 100 and 200mg of ethanolic extract of *Pergularia daemia* respectively. All the test drugs were administered through oral route by suspending in CMC once daily for 5 days. After 30 minutes of last administration, 2.5% phenol red solution (0.2 mL) was injected intraperitoneally. Then, 30 minutes after the application of phenol red, the mice were sacrificed. Trachea was dissected and immediately placed into 1 mL of normal saline. After the trachea was washed, 0.1 mL of 1 M NaOH was added to the saline and the optical density was measured at 546 nm. The expectorant activities were assessed by the increase of the optical density in terms of that in control groups by using the following equation. The percentage of increase = $[(Dt-Do)/Do \times 100]$ (Do: The Optical density of control, Dt: the Optical density of the treatment group) (Han et al., 2010).

Table 1. Cough Suppressant effect of ethanolic extract of *Pergularia daemia* against Sulfar Dioxide induced cough in mice

S.No	Drug treatment	Number of cough	Percentage inhibition
1	Control (0.1% CMC)	35.62±1.22	-
2	Codeine Sulfate (30mg/kg)	10.52±0.95***	70.45%
3	<i>Pergularia daemia</i> (100mg/kg)	17.53±1.05***	50.81%
4	<i>Pergularia daemia</i> (200mg/kg)	12.63±0.85***	64.54%

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Control

Table 2. Expectorant effect of ethanolic extract of *Pergularia daemia* on Phenol Red secretion in mice trachea

S. No	Drug treatment	Concentration of Phenol red (mg/ml)	Percentage increase
1	Control (0.1% CMC)	0.32±0.02	-
2	Ammonium Chloride (1g/kg)	0.58±0.03***	81.25%
3	<i>Pergularia daemia</i> (100mg/kg)	0.49±0.02***	53.13%
4	<i>Pergularia daemia</i> (200mg/kg)	0.53±0.05***	65.63%

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Control

Statistical analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test. P values < 0.05 were considered as significant.

Results and discussion

Sulfur dioxide (SO₂) induced cough

Cough suppressant activity of ethanolic extract of *Pergularia daemia* against sulfur dioxide induced cough in mice was studied and the results were given in table 1. In control groups and reference control, the number of cough was 35.62±1.22 and 10.52±0.95 respectively. In the groups treated with 100 and 200mg/kg of *Pergularia daemia*, cough was 17.53±1.05 and 12.63±0.85 respectively. Codeine sulfate and *Pergularia daemia* showed significant (P<0.001) decrease in cough as compared to control. The percentage inhibition in the animals treated with Codeine sulfate was 70.45%. The percentage decrease in cough with 100 and 200mg/kg of *Pergularia daemia* were 50.81% and 64.54% respectively.

Expectorant activity

Expectorant effect of ethanolic extract of *Pergularia daemia* on Phenol Red secretion in mice trachea was studied and the results were given in table 2. Ammonium chloride the known expectorant significantly (P<0.001) enhance the phenol red secretion in trachea up to 0.58±0.03 mg/ml and the percentage increase in secretion was 81.25% compared to control. The phenol red secretion was significantly (P<0.001) increased by both the doses of *Pergularia daemia* (100mg and 200mg/kg) to 0.49±0.02 and 0.53±0.05mg/ml respectively. The percentage

increase in phenol red secretion with 100 and 200mg/kg of *Pergularia daemia* were 53.13% and 65.63% respectively.

Conclusion

From the results, it was concluded that, ethanolic extract of *Pergularia daemia* exhibited significant cough suppressant and expectorant activity in mice. The results also substantiate the traditional claim of *Pergularia daemia* in treating cough and used as expectorant. Further study may be concentrated in isolating the active principals from *Pergularia daemia* which is responsible for its activity.

Conflict of Interest: None declared

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