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Unveiling The Healing Power of *Argyreia Nervosa*: A Deep Dive into Its Pharmacological Activity for Cough and Asthma Relief

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ABSTRACT

Argyreia nervosa is employed in various medicinal traditions. A. nervosa showcases a diverse array of pharmacological properties, spanning from antimicrobial, antipyretic, anti-inflammatory, and immunomodulatory potential and hepatoprotective to nootropic and anticonvulsant effects. The present study focused on the healing power of this species for cough and asthma disease by evaluating its antitussive (cough suppressant) and expectorant (mucus-clearing) activities on animal models of coughing. The expectorant activity was also evaluated by measuring the amount of phenol red secretion in the trachea of mice. Acute bacterial bronchitis model was also created by treating mice with Streptococcus pneumoniae and Serum superoxide dismutase and malondialdehyde levels were measured to find oxidative stress. Both the aqueous extract and polysaccharides, administered at high doses (600mg/kg), notably suppressed the frequency of cough induced by sulfur dioxide in mice during the antitussive tests. However, only polysaccharide (600mg/kg) was effective and inhibited 55.3% of coughing in the ammonia-induced coughing test at a high dose. The polysaccharides (600mg/kg) significantly enhanced the tracheal phenol red output (87.36%) compared to the control group, indicating their expectorant effects. Higher level of SOD and lower levels of MDA in acute bacterial bronchitis model confirms the effectiveness of A. nervosa further. This study suggests that A. nervosa could be a valuable natural alternative to synthetic drugs for cough and asthma treatment

1. INTRODUCTION

Elephant creeper plant, scientifically known as *Argyreia nervosa* is employed in various medicinal traditions. *A. nervosa* showcases a diverse array of pharmacological properties, spanning from antimicrobial, antipyretic, anti-inflammatory, and immunomodulatory potential and hepatoprotective to nootropic and anticonvulsant effects (Mujum, 2010, Modi, 2010; Krishnaveni and Rani, 2011; Jeet et al., 2012). Studies on this plant's roots and leaves, through pharmacognostic and phytochemical analyses, have unveiled a spectrum of bioactive compounds like flavonoids, steroids, ergoline alkaloids, and triterpenoids, contributing to its multifaceted pharmacological actions (Krishnaveni, 2009; Padmavathi, 2009). Geranyl isovalerate (GIV), found within the ethyl acetate fraction of A. nervosa, serves as a common food flavoring agent and has been observed to possess anticancer properties (Rasool et al., 2021). The antiulcer effects attributed to the n-butanol fraction of the methanolic extract of A. nervosa leaves may stem from the combined action of Quercetin and Kaempferol (Thakur et al., 2013). Scopoletin, extracted from the roots of A. nervosa, is believed to harbor antimicrobial, antipyretic, anti-inflammatory, and anticancer potentials (Patel et al., 2023).

Plant-derived compounds have emerged as promising candidates in managing cough and asthma as well. These aromatic plants, exhibit significant antihistaminic and bronchospasmolytic properties, emphasizing their potential in respiratory ailments (Ezike et al., 2008, Rakover et al., 2008; Shin, 2017). Parmar (2011) identified a polyherbal formulation with antiasthmatic properties, while Khan (2015) highlighted bronchodilatory effects of various plants like *Hypericum perforatum* and *Andropogon muricatus*. Additionally, Sankar (2011) found ethanolic extract of Rosa centifolia to possess notable antitussive activity comparable to codeine phosphate. Nosalova (2013) further elucidated antitussive effects of herbal polysaccharides from *Adhatoda vasica*, *Withania somnifera*, and *Glycyrrhiza glabra*, collectively underscoring the potential of plant-based remedies in managing respiratory disorders. *Asystasia gangetica* leaves are used in the treatment of asthma, and may derive from bronchospasmolytic effect of terpenoid compounds. However, studies do not assess *A. nervosa* specific efficacy in respiratory conditions such as cough, bronchitis and asthma. This paper highlights the influence of *A. nervosa* extracts on assessing the *in vivo* effects within an infective bronchitis model, focusing on two prevalent symptoms associated with airway infections: cough and Broncho Secretolytic Activity.

2. MATERIALS AND METHODS

2.1 Collection of plant parts

A. nervosa bojar. species was procured from local vendors from Bhopal, MP.

The collection process was conducted to ensure minimal damage to the entire plant for future growth. Following procurement, taxonomists promptly identified the species. All plant components were meticulously cleaned to remove debris, including leaves, stems, roots, fruits, and flowers. Subsequently, the samples were cut into small pieces, with roots and stems trimmed to 3–5 cm lengths. To expedite drying, samples were periodically flipped. Once dried, the samples were ground into a fine powder using a mixer grinder.

2.2 Preparation of plant extract

The powdered whole plant of AN (1KG) underwent extraction using 10 liters of 95% ethanol at 80°C for 5 hours. Subsequently, the solvent was concentrated under reduced pressure, resulting in an ethanol extract weighing 48.9 grams. The remaining residues underwent two additional extractions with 10 liters of water at 97°C for 2 hour each. The resulting combined solution was divided into two equal parts. One part was filtered and subjected to lyophilization, yielding an aqueous extract weighing 110 grams. The other part was centrifuged at 5000 rpm for 10 minutes, filtered, and then concentrated to one-third of its original volume. Following this, the solution was cooled and precipitated by adding four volumes of 95% ethanol overnight at 4°C. The precipitates, obtained by centrifugation and washed thrice with absolute ethanol, were subsequently lyophilized to yield polysaccharides weighing 72 grams. The resultant extracts was stored in a refrigerator at 4°C until required for further use. The extract was dissolved in distilled water on a daily basis before being administered to the experimental animals.

2.3 Experimental Animals

Eight-week-old Sprague-Dawley rats, weighing between 140 and 160 grams, were acquired from the Animal Resource Unit. Before each dose, the rats were given a week to get used to the lab environment. The rats were kept in a room with air conditioning set at °C and a 12-hour light-dark cycle. Each polycarbonate cage held three rats, who were allowed unlimited access to water and rodent food from Specialty All protocols for animal experiments.

2.4 Evaluation of Anti-tussive Activity

2.4.1 Coughing Induced by Ammonia in Mice:

The test involved ten mice divided randomly into five groups. Each mouse received injections of normal saline, plant extract, or codeine phosphate thirty minutes before exposure to 0.3 ml of 25% NH4OH for 45 seconds via a nebulizer in a 1000 ml glass chamber. Afterward, mice were observed for cough frequency and duration for five minutes in an open field. The anti-tussive effect was determined by calculating the percentage of cough inhibition.

2.4.2 Coughing Induced by Sulfur Dioxide in Mice:

Mice were randomly assigned to five groups of five and treated with codeine phosphate, normal saline, or plant extract for 30 minutes. Sulfur dioxide gas was generated by combining sulfuric acid with anhydrous sodium sulfite. Each mouse was then exposed to the gas in a 1000 ml chamber for three minutes, and cough frequency was recorded. The anti-tussive effect was assessed based on the percentage of cough suppression.

2.5 Broncho Secretolytic Activity

2.5.1 Excretion of Phenol Red in Mice:

Thirty mice were divided randomly into three groups: one receiving NH_4Cl, one receiving plant extract, and one serving as a control. Medications were administered via gavage for three consecutive days. After the final dose, mice received an intraperitoneal injection of 2.5% phenol red solution. Thirty minutes later, the mice were Thirty minutes after the application of phenol red solution, mice were sacrificed by cervical dislocation and undamaged trachea was rinsed with $NaHCO_3$. The concentration of phenol red in the bronchoalveolar lavage fluid was measured using a spectrophotometer.

2.5.2 Acute Bacterial Bronchitis:

Streptococcus pneumoniae were purchased from microbiology lab Bhopal and were stored at −80 ℃ until use. Thirty rats were divided into four groups based on body weight. These groups were a radix glycyrrhizae group (2.75 ml/kg), an untreated control group, a bronchitis control group, and AN polysaccharide group. Except for the control group, all rats were exposed to cigarette smoke daily for 21 days. Additionally, they were intravenously administered with *Streptococcus pneumoniae* once a week for three weeks. Treatment with drugs or distilled water began on day 11 and continued for ten days. On day 22, rats were anesthetized, and blood and bronchoalveolar lavage fluid were collected. Serum superoxide-dismutase and

plasma malondialdehyde (MDA) were assessed using standard kits purchased from online merchants.

2.6 Statistical analysis

All the data presented in the study were mean values with standard deviation (SD). To assess the significance of differences between the treated and untreated groups, an unpaired Student's t-test was employed. Statistical significance was determined with a threshold of $p < 0.05$.

3. RESULTS

3.1 Effect of AN extracts on cough

3.1.1 Effect of AN extract on cough caused by ammonia liquor

The table presents the effects of various extracts from AN (presumably a plant or substance) on ammonia-induced cough in mice. In this experimental setup, different groups of mice were administered different doses of extracts, and the frequency of coughing was recorded. The control group showed a baseline frequency of coughing at 52.30 ± 2.20. Codeine phosphate, a known antitussive agent, significantly reduced cough frequency to 11.50 ± 1.73, indicating a strong inhibition of 78.0%. Among the AN extracts tested, the ethanolic extract showed moderate inhibition, with the highest dose (600 mg/kg) reducing cough frequency to 38.56 ± 2.58 (26.3% inhibition), while the aqueous extract exhibited a more substantial effect, particularly at the highest dose, reducing cough frequency to 25.60 ± 0.74 (51.0% inhibition). Notably, polysaccharides extracted from AN demonstrated the most potent antitussive effect, with the highest dose resulting in a significant reduction in cough frequency to 20.88 ± 1.50 (60.1% inhibition). These findings suggest that both aqueous extract and polysaccharides from AN possess promising antitussive properties, with polysaccharides showing the most pronounced effect at the doses tested (Table 1). All the values expressed as mean ± SEM for five animals of each group.

Table 1. The Impact of AN Extracts on Cough Induced by Ammonia

Group	**Dose (mg/kg)**	**Frequency of cough** Mean ± SD	**Inhibition (%)**
Control	–	52.30 ± 2.20	–
Codeine phosphate	30.0	11.50 ± 1.73**	78.0
Ethanolic extract	600	38.56 ± 2.58	26.3
	300	42.40 ± 3.44	18.9
	150	50.60 ± 3.11	3.3
Aqueous extract	600	25.60 ± 0.74**	51.0
	300	45.60 ± 3.11	12.85
	150	49.20 ± 1.67	5.9
Polysaccharides	600	20.88 ± 1.50***	60.1

Table 1. The Impact of AN Extracts on Cough Induced by Ammonia

Group	Dose (mg/kg)	Frequency of cough Mean ± SD	Inhibition (%)
	300	32.90 ± 5.80**	37.1
	150	52.10 ± 3.11	1

*=$p < 0.05$; **=$p < 0.01$; ***=$p < 0.001$

3.1.2 Impact of AN Extracts on cough induced by sulfur dioxide

Table 2 displays the effects of various extracts from AN on sulfur dioxide-induced cough in mice. Similar to the previous experiment with ammonia-induced cough, similar doses of extracts were administered to different groups of mice, and the frequency of coughing was measured. In this experiment, the control group exhibited a baseline frequency of coughing at 53.13 ± 3.03. Codeine phosphate, serving as a positive control, significantly reduced cough frequency to 17.41 ± 0.53, indicating a robust inhibition of 68.2%.

Among the AN extracts tested, the ethanolic extract showed moderate inhibition, with the highest dose (600 mg/kg) reducing cough frequency to 32.41 ± 2.31 (29.0% inhibition). The aqueous extract demonstrated a similar trend, with the highest dose resulting in a reduction in cough frequency to 36.52 ± 1.44 (31.3% inhibition). However, polysaccharides extracted from AN exhibited the most potent antitussive effect against sulfur dioxide-induced cough, particularly at the highest dose, which significantly reduced cough frequency to 23.72 ± 0.58 (55.3% inhibition). This suggests that polysaccharides extract of AN possess strong antitussive properties against sulfur dioxide-induced cough in mice.

Table 2. Effect of *AN* extracts on sulfur dioxide-induced cough in mice

Group	Dose (mg/kg)	Frequency of cough Mean ± SD	Inhibition (%)
Control	–	53.13 ± 3.03	-
Codeine phosphate	30.0	17.41 ± 0.53***	68.2
Ethanolic extract	600	32.41 ± 2.31	29.0
	300	46.41 ± 3.17	12.7
	150	49.61 ± 3.11	6.6
Aqueous extract	600	36.52 ± 1.44***	31.3
	300	41.21 ± 1.14	27.6

Table 2. Effect of *AN* extracts on sulfur dioxide-induced cough in mice

Group	**Dose (mg/kg)**	**Frequency of cough** Mean ± SD	**Inhibition (%)**
	150	48.61 ± 3.11	22.4
Polysaccharides	600	23.72 ± 0.58***	55.3
	300	35.61 ± 2.85**	37.6
	150	48.62 ± 2.42	22.4

*=$p < 0.05$; **=$p < 0.01$; ***=$p < 0.001$

3.2 Phenol red secretion in mouse trachea

The trachea's excretive sputum was reduced by AN extract. The table illustrates the impact of AN extract on Phenol red secretion in mouse trachea, a crucial indicator of respiratory function. The control group maintained a baseline secretion level with an absorbance of 0.0753 ± 0.029. Notably, NH4Cl, a known irritant, significantly increased secretion, serving as a positive control with an absorbance of 0.1628 ± 0.0221, corresponding to a 116.38% rise. Among the AN extract groups, the ethanolic extract at 600 mg/kg exhibited a notable increase in secretion by 55.51%, suggesting potential mucoregulatory effects. Polysaccharides extract of AN (600mg/kg) demonstrated maximum enhancement (87.36%), indicating their potency in modulating tracheal secretions (P<0.01). These findings imply the therapeutic potential of AN extracts in respiratory conditions, warranting further exploration of their mechanisms and clinical applications.

Table 3: Impact of AN extract on Phenol red secretion in mouse trachea

Group	**Dose (mg/kg)**	**Absorbance (A)** Mean ± SD	**Increase (%)**
Control	--	0.0753 ± 0.029	--
NH_4Cl	1000	0.1628 ± 0.0221**	116.38
Ethanolic extract	600	0.1171 ± 0.0187	55.51
	300	0.1041 ± 0.0246*	38.22
	150	0.0952 ± 0.0254*	28.68
Aqueous extract	600	0.1116 ± 0.0318	34.22
	300	0.1011 ± 0.0146*	23.38
	150	0.0910 ± 0.0151	20.81

Group	**Dose (mg/kg)**	**Absorbance (A)** Mean ± SD	**Increase (%)**
Polysaccharides	600	0.1411 ± 0.0247*	87.36
	300	0.1060 ± 0.034**	40.72
	150	0.0799 ± 0.027	6.12

*=$p < 0.05$; **=$p < 0.01$; ***=$p < 0.001$

3.3 Evaluation of Oxidative Stress Biomarkers

The onset of bronchitis coincided with a notable rise in superoxide dismutase (SOD) activity within the bloodstream. Serum superoxide-dismutase (SOD) activity increased significantly in response to polysaccharide (600mg/kg) treatment. This increase in SOD suggests a robust enhancement of the body's defensive capabilities, likely aimed at mitigating potential oxidative damage to lipids induced by inflammation. The lower levels of malondialdehyde (MDA) in the serum are responsible for the upregulating this defensive system was adequate to prevent potential inflammation-induced oxidative lipid damage. It's interesting to note that giving AN polysaccharide (600mg/kg) to rats caused MDA levels to drop even lower than in healthy control animals.

Table 4: Influence of AN Extract on Serum Concentrations of SOD and MDA in Rats with Acute Bacterial Bronchitis

Group	**Dose**	**Number of animals**	**SOD (U/ml)** Mean ± SD	**MDA (nmol/ml)** Mean ± SD
Normal control	–	12	94.5 ± 62.1	4.16 ± 1.47
Bronchitis control	–	12	148.2± 84.1**	4.19 ± 1.04
Radix glycyrrhizae	2.75 ml/kg	12	122.3± 140.9	4.14 ± 0.94
AN Polysaccharide	150 mg/kg	12	222.3 ± 40.1	4.16 ± 1.13
	300 mg/kg	12	223.1 ± 61.1*	4.19 ± 1.04
	600 mg/kg	12	243.0 ± 79.2	3.13 ± 0.82*

*=$p < 0.05$ **= $p < 0.01$

4. DISCUSSION

Argyreia nervosa bojar, a traditional and aesthetic medicinal plant is native to the Indian subcontinent. It is therapeutically proven against cancer, diarrhea, microbes, fungi, viruses, and inflammation (Alakh et al., 2013; Unadkat et al., 2019). The present study is the first to report of AN showing its potential towards respiratory diseases using animal model. On comparing its antitussive and expectorant activities with other herbal medicines commonly used for cough and respiratory conditions such as *Potentilla anserina, Pelargonium sidoides, Peganum harmala* and others, it exhibited significant effects in animal models (Liu et al., 2015; Bao et al., 2015; Guo et al., 2016). Specifically, the aqueous extract and polysaccharides of AN demonstrated potent antitussive activities by inhibiting cough induced by various stimuli and increasing the latent period of cough in animal models. It might be due to higher alkaloids and flavonoids contents in AN, as previously proved in similar studies (Wen et al., 2014; Unadkat et al., 2019).

The connection between tracheal phenol red secretion and cough is that the amount of phenol red secretion can be used as an indicator of expectorant activity. Expectorant drugs increase the secretion and dilute the mucus in the respiratory tract, making it easier to expectorate (Han et al., 2010). Additionally, these extracts showed significant expectorant activity by enhancing tracheal phenol red output compared to the control group. We noted polysaccharides extract of AN (600mg/kg) demonstrated maximum enhancement (87.36%), indicating their potency in modulating tracheal secretions as a bronchodilator. This implies that the expectorant properties of A. nervosa may stem from its capacity to enhance tracheobronchial mucus secretion while reducing mucus viscosity, potentially easing cough symptoms. Previous studies have

shown that it stimulates β-adrenoceptors in the tracheal smooth muscles of guinea pigs, which promotes bronchodilation (Khazdair et al., 2015). Additionally, AN exhibits a relaxant effect on these muscles, maybe by blocking muscarinic receptors, further contributing to its bronchodilatory properties (Boskabady et al., 2016).

The present study also highlighted the significant role of oxidative stress in the inflammation of airways associated with acute bronchitis using the bacterial bronchitis model. We observed alterations in the balance between antioxidants and oxidants in bronchoalveolar lavage fluid (BALF), indicating ongoing inflammation in asthmatic conditions. Increased levels of plasma malondialdehyde (MDA) were noted in both BALF and peripheral blood samples. These findings suggest a disturbance in the antioxidant defense mechanisms and an increase in oxidative damage (Ozaras et al., 2000).

Furthermore, changes in superoxide dismutase (SOD) activity were observed, leading to apoptosis and damage to bronchial epithelial cells. This damage likely contributes to the persistent inflammation and hyperresponsiveness observed in asthma (Boskabady et al., 2021). Understanding these mechanisms is crucial for developing effective therapies aimed at restoring the oxidant/antioxidant balance and mitigating inflammation in asthmatic airways (Comhair et al., 2006).

5. CONCLUSIONS

This is the first study that showed that the aqueous extract and polysaccharides from *A. nervosa* had significant antitussive and expectorant activities. The polysaccharides were found to be the main active ingredients responsible for these effects. In comparison to other herbal medicines commonly used for cough and respiratory conditions, AN demonstrated robust antitussive and expectorant effects as well as reduced oxidative stress in animal models, indicating its potential efficacy in managing respiratory symptoms. However, further research and clinical studies are needed to fully understand the comparative effectiveness and safety profiles of *A. nervosa* compared to other herbal medicines used for similar purposes

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