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PHARMACOLOGICAL ASSESSMENT OF THE ANTI-ASTHMATIC POTENTIAL OF ETHANOLIC EXTRACT FROM ALPINIA CALCARATA RHIZOME

Chandaka Madhu*, Jadi Rajesh Kumar Varma, Mekala Dilip Kumar, Mohammad Saniya Sulthana, Thotakura Harshitha, Kalyanikar Prasanna Bai, Padamatinti Nagasheela

Pratishta Institute of Pharmaceutical Sciences, Durajpally, Suriapet, Chivvemla, Telangana 508214

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Abstract

Alpinia calcarata, commonly known as Dwarf Cardamom or Snap Ginger, is a perennial herb in the Zingiberaceae family. Various doses of the extract be administer including a dose of 2000 mg/kg and from which the low doses of 100 mg/kg and high dose was 200 mg/kg. At the high dose, no fatalities or adverse health effects were observed, indicating that the extract is well-tolerated. The positive safety profile at this dosage supports the selection of lower doses for further therapeutic evaluation. These findings suggest that the ethanolic extract possesses a favorable safety margin, positioning it as a promising candidate for subsequent pharmacological studies.

Keywords: Alpinia calcarata, OECD guidelines, ethanolic extract.

***Corresponding Author**
Dr.Chandaka Madhu

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INTRODUCTION:

A complicated respiratory disorder characterized by airway inflammation and constriction, chronic asthma causes symptoms including wheezing, coughing, chest tightness, and dyspnea. Its pathogenesis involves intricate interactions among genetic, environmental, and immunological factors. Central to asthma's pathophysiology is airway inflammation, triggered by stimuli like allergens and pollutants, leading to the activation of inflammatory cells. Eosinophils release pro-inflammatory mediators that promote tissue remodeling and mucus overproduction, contributing to airflow obstruction. Mast cells, prevalent in bronchial mucosa, release histamine, inducing bronchoconstriction and increasing vascular permeability. T helper 2 (Th2) cells orchestrate the allergic retort by secreting cytokines that stimulate eosinophil recruitment and mucus production. Persistent inflammation causes subepithelial fibrosis, which exacerbate airway hyperresponsiveness (AHR)—an increased bronchoconstrictive reaction to various stimuli. Dysregulated inflammatory signaling and epithelial dysfunction further enhance AHR, impairing airway barrier function and facilitating allergen penetration. Effective asthma management necessitates a thorough understanding of its pathophysiology to develop targeted therapies that can mitigate inflammation, restore airway function, and improve patient outcomes. Identification of asthma involves recognizing symptoms, assessing medical history, conducting physical examinations, and performing diagnostic tests like spirometry and peak expiratory flow monitoring. Through comprehensive evaluation and tailored treatment strategies, the burden of asthma can be alleviated, enhancing the quality of life for those affected.

Alpinia calcarata, commonly known as Dwarf Cardamom or Snap Ginger, is a perennial herb in the Zingiberaceae family, reaching heights of up to 1.5 meters. It is extensively distributed in all the regions, including India, Sri Lanka, Malaysia, and Thailand, thriving in well-drained, shaded soils. The fleshy, aromatic rhizomes are valued in traditional medicine for their carminative, stimulant, and anti-inflammatory properties, effectively treating conditions like bronchitis, asthma, and digestive issues. It affluent in bioactive compounds for instance flavonoids and terpenoids, A. calcarata exhibits significant pharmacological potential, including anti-inflammatory and antioxidant activities, positioning it as a promising candidate for asthma treatment.

With an increasing interest in herbal remedies for their safety and effectiveness compared to synthetic drugs, this

study will evaluate the anti-asthmatic properties of the ethanolic extract from Alpinia calcarata rhizomes, a plant known for its traditional medicinal uses.

Method and Methodology:

Collection of Plant Material

The rhizomes of Alpinia calcarata were sourced carefully to ensure their quality and authenticity, which are vital for the study's reliability. Mature, dried rhizomes were collected from trusted local suppliers specializing in medicinal plants. After gathering, the rhizomes were cleaned thoroughly to remove any contaminants. To protect their natural properties, they were dried in the shade, as direct sunlight can harm important compounds. Once adequately dried, the rhizomes were ground into a coarse powder using a mechanical grinder to facilitate the extraction process.

To confirm the identity of the plant material, an expert botanist authenticated the dried rhizomes. This process involved examining the material both macroscopically and microscopically, as well as comparing it with herbarium specimens. This step ensured that the collected rhizomes were indeed Alpinia calcarata, validating the scientific basis for the subsequent experiments.

Plant Extract Preparation

The powdered rhizomes underwent extraction using ethanol as the solvent. The Soxhlet extraction method was employed to maximize the retrieval of phytochemicals. Approximately 200 grams of the powdered rhizomes were placed in the extractor, and ethanol was used due to its effectiveness in dissolving a wide range of bioactive compounds. The extraction took several hours, allowing the solvent to permeate the material and extract its active ingredients.

After extraction, the ethanol solution containing the phytochemicals was collected and evaporated to concentrate the extract. This crucial step removed the solvent while preserving the bioactive compounds by using a rotary evaporator under reduced pressure to prevent heat degradation. The concentrated extract be then store in a refrigerator at 4°C to maintain stability and stop contamination or degradation.

Phytochemical Screening and Acute Toxicity Studies

Acute toxicity study were conduct following OECD guidelines (Test Guideline 423) to ensure animal welfare and scientific rigor. Healthy adult mice were used for the study, and they were fasted overnight with access only to water to clear their digestive systems. The ethanolic extract of Alpinia calcarata was administer orally in altering doses (5, 50, 300, and 2000 mg/kg bwt) to different groups of mice to assess potential toxic effects.

The mice were monitored closely for immediate signs of toxicity during the first three hours after administration, noting any abnormal behaviors, physical symptoms (like convulsions or lethargy), and mortality. Observations continued for up to 14 days, tracking body weight, food intake, and any delayed toxicity signs, providing a comprehensive safety assessment of the extract.

Histamine Aerosol-Induced Bronchoconstriction

Preparation

A histamine aerosol-induced bronchoconstriction model was use to assess the anti-asthmatic potential of the Alpinia calcarata ethanolic extract. To simulate bronchial asthma in guinea pigs, a 0.2% w/v solution of histamine was prepared by dissolving pure histamine in distilled water. The histamine solution was then delivered using a nebulizer inside an aerosol chamber, designed to evenly disperse the histamine particles into the air. When exposed to this aerosol, the guinea pigs developed bronchoconstriction, mimicking an asthmatic episode.

Measurement

The primary measure of bronchoconstriction was the Preconvulsion Time (PCT), defined as the duration from the start of histamine exposure to the onset of preconvulsive dyspnea (severe difficulty in breathing that precedes convulsions). The PCT was recorded for each guinea pig at 1, 4, and 24 hours post-administration of the test compounds. An increased PCT indicates a delay in the onset of bronchoconstriction, suggesting a protective effect against asthma.

The guinea pigs were separated into four groups:

- **Group 1 (Control):** administer the vehicle control, carboxymethyl cellulose (CMC) solution.
- **Group 2 (Test 100 mg/kg):** Administered Alpinia calcarata ethanol extract orally at 100 mg/kg body weight.
- **Group 3 (Test 200 mg/kg):** Administered Alpinia calcarata ethanol extract orally at 200 mg/kg body weight.
- **Group 4 (Standard Treatment):** Administered chlorpheniramine maleate intraperitoneally, a known antihistamine drug, to serve as the positive control.

Calculation of Percentage Protection

The efficacy of the plant extract was determined by calculating the percentage protection against histamine-induced bronchoconstriction. This was done using the following formula:-

where \(E_{ta}\) is the preconvulsion time after administration of the drug and\(E_{tb}\) is the preconvulsion time before administration of the drug.

Collection of Alpinia calcarata Rhizomes

The aerial parts of Alpinia calcarata be successfully collected and authenticated, ensuring that the plant material used in the study was genuine.

Extraction of Plant Material

The rhizome of Alpinia calcarata were cleaned, dried in the shadow, and make into a powder. Soxhlet extraction with ethanol was employed to extract the active constituents from the coarse powder. The yield of the ethanolic extract was 17% w/w, indicating a reasonable extraction efficiency.

Ethanolic extract of Alpinia calcarata Rhizomes: preliminary phytochemical screening

Sl.No:	Constituents	Presence/absence
1	Phenol	+
2	Alkaloids	-
3	Flavonoids	+
4	Tannins	+
5	Carbohydrate	+
6	Saponinglycosides	-
7	Cardiacglycosides	+
8	Anthraquinoneglycoside	-
9	Cyanogenicglycosides	-
10	Proteins	+
11	Fatsandoils	-
12	Steroids	+
13	Aminoacids	+

(+:presence , -absence)

Acute Toxicity Studies

An acute toxicity study be conducted to assess the safety of the ethanol extract of Alpinia calcarata rhizomes using Swiss albino mice, following OECD guidelines 423. The extract was administer at different doses to determine its safety profile.

- **Dose (2000 mg/kg):** No deaths or health issues were observed, indicating that the extract is well-tolerated at this high dose.
- **Doses (low :100 mg/kg and high :200 mg/kg):** Base on the safety seen at 2000 mg/kg, these lower doses were chosen for further investigation into the extract's therapeutic effects.

The absence of adverse effects at the highest dose suggests that the ethanolic extract has a good safety margin, making it a promising candidate for further pharmacological studies.

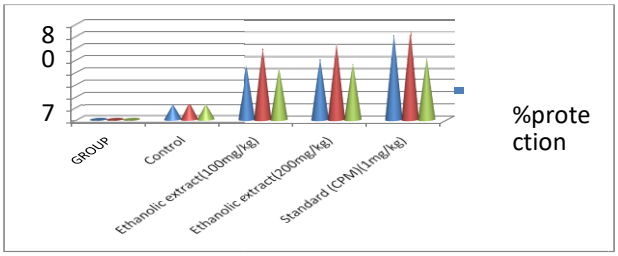
In Vivo Assessment of Anti-Asthmatic Efficacy: Histamine Aerosol-Induced Bronchoconstriction in Guinea Pigs

Group	Latent period of convulsion			
	Before	1hour	4hour	24hour
Control	15.3±0.18	17.36±0.18	17.63±0.186	17.4±0.12
Ethanolic extract(100mg/kg)	15.71±1.01	28.65±0.25	38.38±0.05*	27.2±0.23
Ethanolic extract(200mg/kg)	14.71±0.57	29.5±3.08	39.36±1.04*	27.4±0.35
Standard(CPM)(1mg /kg)	17.46±0.69	59.25±0.03*	67.26±1.01**	35.5±0.45

	% protection		
GROUP	1HR	4HR	24HR
Control	11.86	13.21	12.06
Ethanolic extract(100mg/kg)	45.16	59.06	42.24
Ethanolic extract(200mg/kg)	50.13	62.62	46.31
Standard (CPM)(1mg/kg)	70.53	74.04	50.81

Table No. 5: Percentage Protection Against Histamine-Induced Bronchoconstriction in Guinea Pigs Treated with Alpinia calcarata Rhizome Extracts

%Protection of the plant Alpinia calcarata rhizomes against histamine induced broncho constriction in guineapig



- **Histamine Exposure:** A 0.2% histamine solution was nebulized to trigger asthma symptoms in guinea pigs. The onset of preconvulsive dyspnea (PCD), an early sign of severe asthma, was recorded.
- **Preconvulsion Time (PCT):** This is the point in time from the start of histamine exposure to the start of PCD, which indicates asthma severity.

In this study, guinea pigs developed PCD after exposure to histamine. The PCT values served as a baseline for evaluating the extract's anti-asthmatic effects.

Efficacy of Ethanol Extract:

- **200 mg/kg Dose:** The extract significantly delayed the onset of convulsions after four hours of histamine exposure, providing 62.62% protection, indicating strong anti-asthmatic effects.
- **100 mg/kg Dose:** This lower dose offered 45.16% protection after one hour and 59.06% after four hours, maintaining the same protection level after 24 hours.

Comparison with Standard Medication:

- **Chlorpheniramine Maleate:** This standard medication showed high protective effects with 75% protection after one hour and 74% after four hours.
- **Control Group**: The control group treated with carboxymethylcellulose had minimal protection, only 11.86% after one hour and 13.21% after four hours.
- The 200 mg/kg dose of the plant extract provided 50.13% protection after one hour, 62.62% after four hours, and 46.31% after 24 hours.
- While the extract was less effective than chlorpheniramine maleate, it still demonstrated significant anti-asthmatic activity, especially at the higher dose.

These findings suggest that the ethanol extract of Alpinia calcarata has notable anti-asthmatic properties, with 200 mg/kg dosage providing the best results.

Conclusion

The ethanolic extract of Alpinia calcarata rhizomes underwent preliminary phytochemical screening, revealing the presence of phenols, flavonoids, tannins, carbohydrates, cardiac glycosides, proteins, steroids, and amino acids. Acute toxicity studies conducted on Swiss albino mice showed that the extract was well-tolerated at a high dose of 2000 mg/kg, with no adverse effects observed, suggesting its safety for further pharmacological evaluation. In vivo assessment of the anti-asthmatic efficacy of the extract was performed using histamine aerosol-induced bronchoconstriction in guinea pigs. The results demonstrated that both doses (100 mg/kg and 200 mg/kg) significantly delayed the onset of convulsions (preconvulsive dyspnea) and offered considerable protection against histamine-induced bronchoconstriction. The 200 mg/kg dose showed 62.62% protection after four hours, while the 100 mg/kg dose provided 59.06% protection. Although the extract was less effective than the standard medication chlorpheniramine maleate, which provided over 70% protection, the extract still exhibited significant anti-asthmatic activity, particularly at the higher dose. Overall, the ethanolic extract of Alpinia calcarata rhizomes demonstrates promising anti-asthmatic properties and a favorable safety profile, warranting further investigation into its therapeutic potential.

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Conflict of Interest Statement

No conflict of interest.

Ethics Approval and Consent to Participate

Not applicable.

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References

1. Attanasi M, Consilvio NP, Rapino D, Nicola MD, Scaparrotta A, Cingolani A, Petrosino MI, Filippo PD, Di Pillo S, Chiarelli F. Bronchial hyperresponsiveness to mannitol, airway inflammation and Asthma Control Test in atopic asthmatic children. Arch. Med. Sci. 2016;12:137–44.
2. Arora P, Ansari SH, Anjum V, Mathur R, Ahmad S. Investigation of anti-asthmatic potential of Kanakasava in ovalbumin-induced bronchial asthma and airway inflammation in rats. J. Ethnopharmacol. 2016;197:242–9.
3. Ledford DK, Wenzel SE, Lockey RF. Aspirin or other nonsteroidal inflammatory agent exacerbated asthma. J. Allergy Clin. Immunol. Pract. 2014;2:653–7.
4. Varner AE. Beneficial effect of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors in patients with asthma during viral infection. J. Infect. Dis. 2002;186:723.
5. Huang CQ, Li W, Wu B, Chen WM, Chen LH, Mo GW, Zhang QF, Gong L, Li J, Zhang HC, Zhu HM, Zeng QZ. Pheretima aspergillum decoction suppresses inflammation and relieves asthma in a mouse model of bronchial asthma by NF-kappaB inhibition. J. Ethnopharmacol. 2016;189:22–30.

6. Morales DR, Guthrie B, Lipworth BJ, Jackson C, Donnan PT, Santiago VH. NSAID exacerbated respiratory disease: a meta-analysis evaluating prevalence, mean provocative dose of aspirin and increased asthma morbidity. Allergy . 2015;70:828–835.
7. Kidon MI, Kang LW, Chin CW, Hoon LS, Hugo VB. Nonsteroidal anti-inflammatory drug hypersensitivity in preschool children. Allergy Asthma Clin. Immunol. 2007;3:114–22.
8. Chu X, Xu Z, Wu D, Zhao A, Zhou M, Qiu M, Jia W. In-vitro and In-vivo evaluation of the anti-asthmatic activities of fractions from Pheretima. J. Ethnopharmacol. 2007;111:490–5.
9. N.P. Committee, editor. Pharmacopoeia of People›s Republic of China(editionI) China: Medical Science Press; 2015.
10. Bin L. Dictionary of Chinese medicine. Traditional Chinese Medicine Ancient Books Publishing House ; 2008.
11. Zhang M, Li X, Liu Y, Ye F, Qiu G. Effects of extract of dilong (pheretima) on the scalded skin in rats. J. Tradit. Chin. Med. 2006;26:68–71.
12. Tang W, Xie Q, Guan J, Jin S, Zhao Y. Phytochemical profiles and biological activity evaluation of Zanthoxylum bungeanum Maxim seed against asthma in murine models. J. Ethnopharmacol. 2014;152:444–50.
13. Xu SY, Bian RL, Chen X. Experimental Methodology of Pharmacology. The Peoples Medical Publishing House; 2003.
14. Vale N, Gouveia MJ, Botelho M, Sripa B, Suttiprapa S, Rinaldi G, Gomes P, Brindley PJ, Correia da Costa JM. Carcinogenic liver fluke Opisthorchis viverrini oxysterols detected by LC-MS/MS survey of soluble fraction parasite extract. Parasitol. Int. 2013;62:535–42.
15. Zhang S, Lu J, Wang S, Mao D, Miao S, Ji S. Multi-mycotoxins analysis in Pheretima using ultra-high-performance liquid chromatography tandem mass spectrometry based on a modified QuEChERS method. J. Chromatogr B Analyt Technol. Biomed. Life Sci. 2016;1035:31–41.
16. Peng J, Yang D, Huang S, Tan Y, Xiao L, Su W. Pharmacological action and health protection function of Pheretima. Zhong Yao Cai . 2000;23:114–7.
17. Li XH, Zhang DX, Xu JF, Wang WY, Du YM, Zhang Y, Lei YW, Zhang YX. Effect of earthworm decoction on airway inflammation of bronchial asthma in guinea pigs. Zhongguo Zhong Yao Za Zhi . 2007;32:1445–8.
18. Liu CH, Lin YW, Tang NY, Liu HJ, Huang CY, Hsieh CL. Effect of oral administration of Pheretima aspergillum (earthworm) in rats with cerebral infarction induced by middle-cerebral artery occlusion. Afr. J. Tradit. Complement Altern. Med. 2012;10:66–82.
19. Li J, Long XY, He L, Liu F, Shu JQ. Determination of capsaicin in Pheretima aspergillum from different habitats by RP-HPLC. Zhong Yao Cai . 2006;29:448–9.
20. Li XH, Tu XY, Zhang DX, Xu JF, Wang WY, Zhang Y, Du YM. Effects of wuwei dilong decoction on inflammatory cells and cytokines in asthma model guinea pigs. J. Tradit. Chin. Med. 2009;29:220–3.
21. Han RF LH, Wang JW, Cong XJ. Study on clinical effect and immunologic mechanism of infants capillary bronchitis secondary bronchial asthma treated with bacterial lysates Broncho-Vaxom. Eur. Rev. Med. Pharmacol. Sci. 2016;20:2151–5.
22. Al-Daghri NM AM, Draz HM, Abd-Alrahman SH, Yakout SM, Clerici M. Th1/Th2 cytokine pattern in Arab children with severe asthma. Int. J. Clin. Exp. Med. 2014;7:2286–91.
23. Yamada Y, Matsumoto K, Hashimoto N, Saikusa M, Homma T, Yoshihara S, Saito H. Effect of Th1/Th2 cytokine pretreatment on RSV-induced gene expression in airway epithelial cells. Int. Arch. Allergy Immunol. 2011;154:185–94.
24. Kim JY, Kim DY, Lee YS, Lee BK, Lee KH, Ro JY. DA-9601, Artemisia asiatica herbal extract, ameliorates airway inflammation of allergic asthma in mice. Mol. Cells . 2006;22:104–12.
25. Theron AJ, Steel HC, Tintinger GR, Gravett CM, Anderson R, Feldman C. Cysteinyl Leukotriene Receptor-1 Antagonists as Modulators of Innate Immune Cell Function. Clin. Dev. Immunol. 2014;2014:608930.
26. Baraldi E, Carraro S, Alinovi R, Pesci A, Ghiro L, Bodini A, Piacentini G, Zacchello F, Zanconato Cysteinyl leukotrienes and 8-isoprostane in exhaled breath condensate of children with asthma exacerbations. Thorax . 2003;58:505–9.
27. Trueba A, Ryan MW, Vogel PD, Ritz T. Effects of academic exam stress on nasal leukotriene B4 and vascular endothelial growth factor in asthma and health. Biol. Psychol. 2016;118:44–51.
28. Fogarty A, Broadfield E, Lewis S, Lawson N, Britton J. Amino acids and asthma: a case-control study. Eur. Respir. J. 2004;23:565–8.