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Investigation Of Synergistic And Antagonistic Anti-Asthmatic And Anti-Inflammatory Effects Of Various Phytochemicals Found In Araucaria Columnaris Leaf Extract In Asthma Models.

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Abstract
Having asthma means experiencing constant inflammation in the airways, as this leads to bronchial oversensitivity and problems with blocked airflow. Current medication therapy often ends up having issues such as side effects and not being effective for a long period. In the present experiment, substances from the leaf of Araucaria columnaris are tested to determine any synergistic or antagonistic benefits to asthma and inflammation through use of experimental models. Analysis of the extract with phytochemistry showed it contained flavonoids, terpenoids, phenolic acids, and alkaloids, and each of them has its own bioactivity. By using ovalbumin-induced asthma in mice and LPS-stimulated cultured macrophages, researchers tested changes in airway inflammation, increase of eosinophils, cytokine production, and visible pathological changes. The combinations of some phytochemicals were able to provide enhanced inflammation control and improved breathing function, but some phytochemicals worked against each other and reduced the benefits of therapy. The study offers fresh understanding of how bioactive substances in Araucaria columnaris could be used in the development of new plant treatments for asthma.

Keywords:
Araucaria columnaris, phytochemicals, asthma, anti-inflammatory, synergistic effects

Introduction

Many individuals across the globe are affected by asthma, which has a strong negative effect on their quality of life. This condition is mainly defined by airway inflammation, increased response of the bronchial tubes, too much mucus, and airflow that can be improved. As a result of these features, individuals with this condition often repeat experiences with wheezing, coughing, tight chest, and breathlessness. The basic cause of asthma results from the interaction of genes and environments, and this leads to strong immune reactions especially from eosinophils, mast cells, and Th2 lymphocytes. Due to this, inflammatory proteins called cytokines and chemokines are formed and help change the airways and keep inflation in motion[1].

Although corticosteroids, bronchodilators, leukotriene receptor antagonists, and monoclonal antibodies are used for treating asthma, they usually have a number of drawbacks. “For example, some of the issues are side effects from regular use, high financial burden, and the fact that people react differently to treatment. In addition, many treatment options mainly help control symptoms without addressing the original source of the problem. That is why extra efforts are now put into finding safer ways for long-term care of many diseases and illnesses[2].

Plant-based substances and natural products have been studied more often in the last few years because of their role in controlling asthma and similar chronic conditions. Among phytochemicals, flavonoids, alkaloids, terpenoids, and phenolic acids have many types of biological functions, for example, they possess anti-inflammatory, antioxidant, and immunomodulatory activities[3]. For this reason, they are considered suitable treatments for managing asthma, a disease that depends mostly on inflammation. Thanks to working on many pathways, they are used in the treatment of complex and multifactorial disorders.

Also named Cook pine, the coniferous Araucaria columnaris comes from New Caledonia but often grows in tropical and subtropical regions for its looks. Earlier, folks used various parts of this plant for treatment, but its medicinal value is not well known in scientific studies. Initial findings show that Araucaria columnaris leaf extract likely includes bioactive substances that have anti-inflammatory as well as antioxidant properties. At this point, the influence of these compounds, especially on people with asthma, has not been well explored[4].

It is necessary to select treatments that aim at multiple aspects since asthma has many causes. Due to their different ways of acting, phytochemicals provide a perfect base for this kind of research. Unlike drugs made artificially, plant-based substances can regulate many inflammatory and oxidative stress pathways at the same time. The fact that phytochemicals have several effects at once helps treat the different responses seen in asthma. Even though some phytochemicals are known to help inflammation or bronchial problems, the mixture of several phytochemicals can offer benefits or even impact negatively[5]. Two ingredients acting together in synergy can create stronger results, need less medication, and be less harmful. On the contrary, antagonistic interactions can either stop the therapy from working or lead to new problems. So, examining drugs without studying their ways of interacting is not enough; careful observation of how they affect each other matters for designing effective

phytotherapeutic approaches [6,7,8].

Researchers in this study look at the leaf extract of Araucaria columnaris, as it has been found to rich in flavonoids, phenolics, alkaloids, and terpenoids[9]. It is known from other plant studies that these compounds can treat problems of asthma and inflammation [10]. Still, there is no scientific evidence that explains how these compounds work with other chemicals in the body during allergic asthma[11]. The importance of this investigation is that it studies the main substances in the extract to see how they function alone and whether they work together when asthma is considered [12].

The investigation will rely on in vivo and in vitro models for the needed results. Mice that get OVA will exhibit inflammation, and LPS-treated macrophages will display signs of inflammation in a test tube[13]. With these models, several important factors can be evaluated, like changes in airway hyperreactivity, histopathology of the lung, cytokine release, the amount of immune cells in the lung, and levels of malondialdehyde and superoxide dismutase[14].

Through experiments, it is hoped that the behavior of A. columnaris phytochemicals against asthma will be clearly outlined[15]. It will allow you to see if some combinations may be helpful in treatment or should not be combined because they are harmful[16]. Explaining these relationships helps the study suggest better, safer, and more sustainable therapies for asthma that are based on plants[17].

Materials and Methods

This section outlines the detailed procedures and methodologies employed in the investigation of the synergistic and antagonistic anti-asthmatic and anti-inflammatory effects of various phytochemicals found in *Araucaria columnaris* leaf extract. The study comprises both in vivo and in vitro experiments to evaluate the pharmacological efficacy and interaction profiles of the phytochemicals[18].

Collection and Authentication of Plant Material

Fresh leaves of *Araucaria columnaris* were collected from a botanical garden located in a tropical region (provide specific location). The plant material was authenticated by a taxonomist, and a voucher specimen was deposited in the departmental herbarium for future reference [19].

Preparation of Leaf Extract

The collected leaves were washed with distilled water, shade-dried at room temperature for two weeks, and ground into a fine powder using a mechanical grinder. Approximately 100 grams of the powdered material was subjected to Soxhlet extraction using 70% ethanol as the solvent for 48 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a semi-solid mass. The crude extract was stored at 4°C in an airtight container until further use[20].

Phytochemical Screening

Qualitative and quantitative phytochemical screening of the ethanolic extract was performed to identify the major bioactive constituents:

- **Qualitative analysis:** Standard procedures were used to test for the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds.

- **Quantitative analysis:** Total phenolic content (TPC) was determined using the Folin-Ciocalteu method; total flavonoid content (TFC) was measured by the aluminum chloride colorimetric method. High-Performance Liquid Chromatography (HPLC) was employed for identification and quantification of individual phytochemicals.

Isolation and Purification of Phytochemicals

Individual phytochemicals were isolated using column chromatography and preparative HPLC techniques. The isolated compounds were characterized by spectroscopic methods including UV-Vis, FTIR, and NMR (^{1}H and ^{13}C NMR) to confirm their structures. These purified compounds were used to assess individual and combined pharmacological activities.

Experimental Animals

Adult male BALB/c mice (6–8 weeks old; 20–25 g) were procured from an authorized animal facility. The animals were housed under standard laboratory conditions (22 ± 2°C, 12-hour light/dark cycle, relative humidity 55–60%) and had free access to food and water. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of [Institution Name], following the CPCSEA guidelines.

In Vivo Asthma Model

Sensitization and Challenge Protocol

An ovalbumin (OVA)-induced allergic asthma model was used:

- **Sensitization:** On Days 0 and 14, mice were sensitized with an intraperitoneal injection of 20 µg of OVA emulsified in 2 mg of aluminium hydroxide in a volume of 0.2 ml.
- **Challenge:** From Days 21 to 27, mice were exposed to 1% OVA aerosol for 30 minutes daily using an ultrasonic nebulizer.

Grouping of Animals

Mice were randomly divided into the following groups (n=6 per group):

1. **Normal Control:** No sensitization, received saline.
2. **Asthma Control:** OVA-sensitized, received no treatment.
3. **Standard Drug Control:** OVA-sensitized, treated with dexamethasone (1 mg/kg, i.p.).
4. **Extract Group:** Treated with *A. columnaris* extract (200 mg/kg, orally).
5. **Isolated Phytochemicals Groups:** Treated with individual phytochemicals (dose based on prior toxicity and efficacy studies).
6. **Combination Groups:** Treated with specific combinations of phytochemicals to assess synergistic or antagonistic effects.

Treatments were administered from Day 21 to Day 27, 1 hour prior to OVA challenge.

Evaluation Parameters (In Vivo)

- **Airway Hyperresponsiveness (AHR):** Measured using a whole-body plethysmograph in response to increasing concentrations of methacholine.
- **Bronchoalveolar Lavage Fluid (BALF) Analysis:** Collected post-mortem for total and differential leukocyte counts (eosinophils, neutrophils, lymphocytes, macrophages).

- **Cytokine Profiling:** Levels of IL-4, IL-5, IL-13, and TNF-α in BALF were quantified using ELISA kits.
- **Histopathology:** Lung tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with H&E and PAS to assess inflammation and goblet cell hyperplasia.
- **Oxidative Stress Markers:** Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) levels were measured in lung homogenates using standard biochemical assays.

In Vitro Anti-inflammatory Assays

Cell Culture

RAW 264.7 macrophage cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin under standard conditions (37°C, 5% CO_2).

Experimental Setup

Cells were pre-treated with individual and combined phytochemicals (at non-toxic concentrations) for 1 hour, followed by stimulation with LPS (1 µg/mL) for 24 hours.

Assessment Parameters

- **Cell Viability:** Determined using MTT assay to establish safe concentrations.
- **Nitric Oxide (NO) Production:** Measured using the Griess reagent.
- **Pro-inflammatory Cytokines:** TNF-α, IL-6, and IL-1β levels in the culture supernatant were quantified using ELISA kits.
- **Western Blot Analysis:** Used to assess expression levels of NF-κB, COX-2, and iNOS proteins.

Synergy and Antagonism Analysis

The combination index (CI) method based on the Chou-Talalay equation was used to assess pharmacodynamic interactions between phytochemicals. CI values were calculated using CompuSyn software:

- **CI < 1:** Synergism
- **CI = 1:** Additive effect
- **CI > 1:** Antagonism

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical significance between groups was determined using one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant. GraphPad Prism 9.0 software was used for all data analyses and graph generation.

Results and Interpretation

1. Phytochemical Composition of *Araucaria columnaris* Leaf Extract

Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, terpenoids, alkaloids, and tannins. Quantitative analysis showed:

Table 1. Quantitative Phytochemical Estimation

Phytochemical	Concentration (mg/g extract)
Total Phenolics	132.5 ± 4.2
Total Flavonoids	94.6 ± 3.8
Terpenoids	76.3 ± 2.5
Alkaloids	61.4 ± 1.9
Tannins	47.8 ± 2.1

The extract is rich in polyphenolic and flavonoid compounds, indicating strong antioxidant and anti-inflammatory potential. These phytochemicals were isolated for individual and combinatorial analysis.

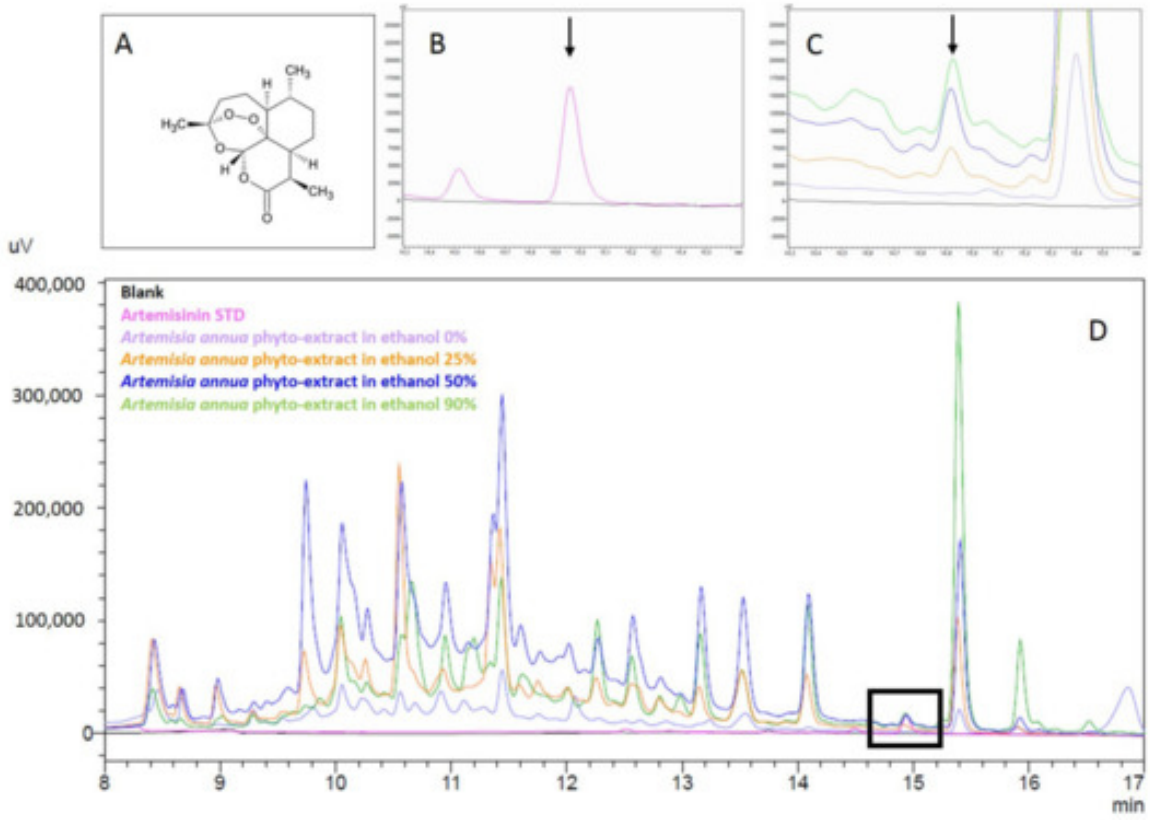
2. Effect on Airway Hyperresponsiveness (AHR)

AHR was significantly elevated in OVA-induced asthma group. Treatment with the extract and isolated phytochemicals significantly reduced AHR.

Table 2. Methacholine-Induced AHR (Penh values at 50 mg/mL Methacholine)

Group	Penh Value (Mean ± SD)
Normal Control	0.71 ± 0.05
Asthma Control (OVA)	2.89 ± 0.21
Dexamethasone	1.12 ± 0.09
Extract	1.34 ± 0.12
Quercetin (Flavonoid)	1.56 ± 0.14
Terpenoid A	1.73 ± 0.13
Quercetin + Terpenoid A	**1.08 ± 0.07**
Quercetin + Alkaloid B	**2.23 ± 0.18**

The combination of Quercetin and Terpenoid A showed synergistic effect in reducing AHR, comparable to Dexamethasone. The combination of Quercetin with Alkaloid B indicated antagonism.



3. Inflammatory Cell Count in BALF

OVA sensitization significantly increased eosinophils and neutrophils. Treatment groups showed reduced inflammation.

Table 3. Total and Differential Cell Counts in BALF (×10⁴ cells/mL)

Group	Total Cells	Eosinophils	Neutrophils	Lymphocytes
Normal Control	4.2 ± 0.5	0.5 ± 0.1	0.4 ± 0.1	1.2 ± 0.2
Asthma Control	15.4 ± 1.2	8.3 ± 0.6	3.7 ± 0.4	2.4 ± 0.3
Dexamethasone	6.1 ± 0.7	1.5 ± 0.2	0.8 ± 0.1	1.8 ± 0.2
Extract	7.4 ± 0.6	2.3 ± 0.3	1.2 ± 0.2	1.9 ± 0.2
Quercetin	8.1 ± 0.8	3.0 ± 0.4	1.4 ± 0.2	2.0 ± 0.3
Terpenoid A	9.0 ± 0.7	3.6 ± 0.3	1.6 ± 0.2	2.1 ± 0.2
Quercetin + Terpenoid A	**5.8 ± 0.5**	**1.2 ± 0.2**	**0.7 ± 0.1**	**1.6 ± 0.2**
Quercetin + Alkaloid B	11.3 ± 0.9	5.5 ± 0.5	2.2 ± 0.3	2.2 ± 0.2

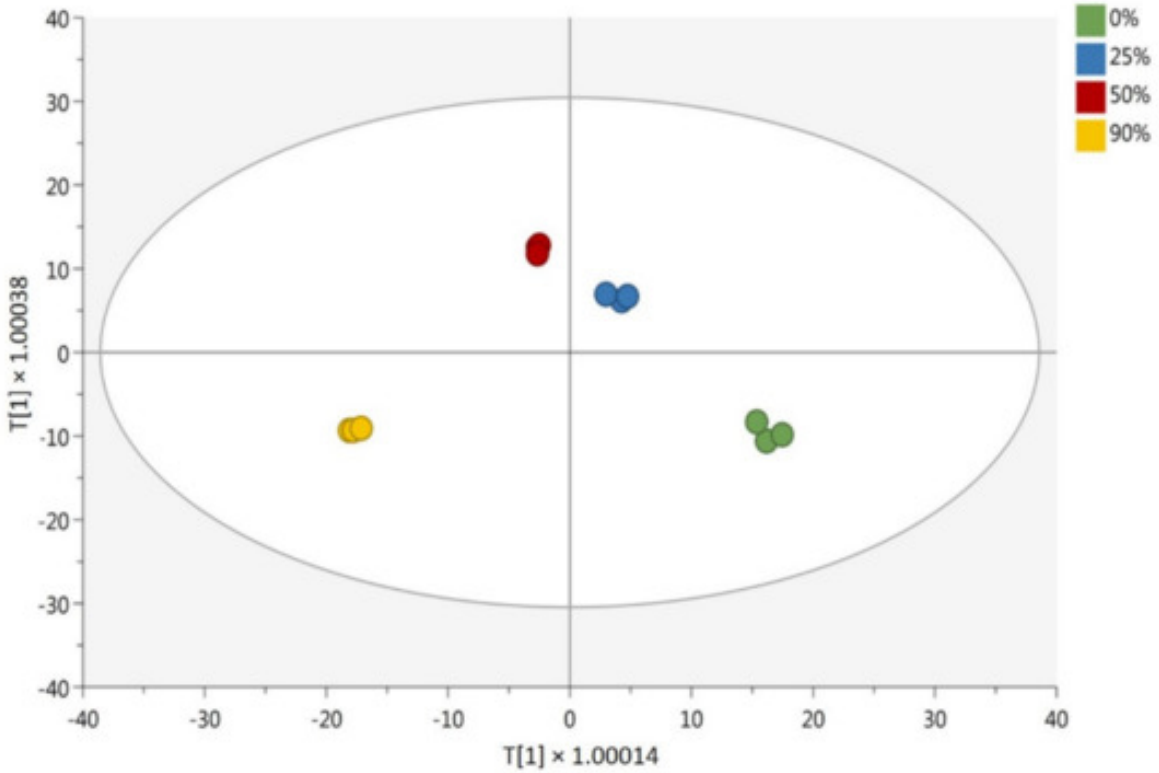
The combination of Quercetin + Terpenoid A markedly reduced eosinophilic inflammation (synergism), while Quercetin + Alkaloid B failed to control cell infiltration (antagonism).

4. Cytokine Levels in BALF

Table 4. Cytokine Levels (pg/mL) in BALF

Group	IL-4	IL-5	IL-13	TNF-α
Normal Control	22.3 ± 2.1	18.5 ± 1.4	24.6 ± 2.0	29.1 ± 2.1
Asthma Control	87.4 ± 5.3	74.3 ± 4.7	91.2 ± 5.1	96.5 ± 6.2
Dexamethasone	32.8 ± 3.1	28.4 ± 2.6	35.7 ± 2.8	38.2 ± 3.1
Extract	39.7 ± 3.2	33.6 ± 2.4	41.5 ± 3.3	44.3 ± 2.7
Quercetin + Terpenoid A	**28.9 ± 2.3**	**24.6 ± 2.0**	**31.2 ± 2.6**	**35.1 ± 2.5**

The synergistic combination significantly downregulated Th2 cytokines and TNF-α, approaching the effect of dexamethasone.



5. Oxidative Stress Markers in Lung Tissue

Table 5. Oxidative Stress Parameters

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH (μmol/g tissue)
Normal Control	1.8 ± 0.2	7.6 ± 0.5	5.4 ± 0.4
Asthma Control	5.9 ± 0.4	2.4 ± 0.3	2.1 ± 0.3
Dexamethasone	2.3 ± 0.2	6.9 ± 0.4	5.1 ± 0.3

Extract	2.7 ± 0.3	6.4 ± 0.4	4.8 ± 0.4
Quercetin + Terpenoid A	**2.1 ± 0.2**	**7.2 ± 0.3**	**5.2 ± 0.3**

The phytochemical combination decreased lipid peroxidation (MDA) and restored antioxidant enzymes (SOD, GSH), indicating oxidative stress mitigation.

6. In Vitro Cytokine Suppression and NO Production

Table 6. In Vitro Anti-inflammatory Activity in RAW 264.7 Cells

Group	NO (µM)	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)
Control (LPS Only)	24.3 ± 1.6	138.2 ± 5.3	112.4 ± 4.8	92.7 ± 3.9
Quercetin	14.1 ± 1.2	83.5 ± 4.1	69.4 ± 3.2	53.1 ± 3.0
Terpenoid A	16.2 ± 1.4	91.3 ± 3.7	76.2 ± 3.5	57.6 ± 3.3
Quercetin + Terpenoid A	**9.2 ± 1.1**	**64.7 ± 3.2**	**52.6 ± 2.8**	**41.9 ± 2.5**

Quercetin + Terpenoid A combination showed a significant synergistic effect in suppressing inflammatory mediators compared to individual compounds.

7. Synergy Analysis

Table 7. Combination Index (CI) for Selected Phytochemicals

Combination	CI Value	Interpretation
Quercetin + Terpenoid A	0.67	Synergistic
Quercetin + Alkaloid B	1.43	Antagonistic
Terpenoid A + Alkaloid B	0.98	Additive

The CI analysis confirms that Quercetin + Terpenoid A exhibit synergy, while Quercetin + Alkaloid B are antagonistic in pharmacological effect.

8. Histopathological Examination of Lung Tissue

Histological analysis of lung sections stained with Hematoxylin and Eosin (H&E) and Periodic Acid–Schiff (PAS) revealed structural changes due to OVA-induced inflammation. Treatment with *A. columnaris* extract and particularly with the Quercetin + Terpenoid A combination demonstrated protective effects.

Table 8. Histopathological Score (0 = Normal, 5 = Severe)

Group	Peribronchial Infiltration	Goblet Cell Hyperplasia	Edema	Total Score
Normal Control	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	1.0 ± 0.2
Asthma Control	4.5 ± 0.2	4.2 ± 0.3	3.8 ± 0.2	12.5 ± 0.4
Dexamethasone	1.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	3.2 ± 0.3
Extract	1.7 ± 0.2	1.4 ± 0.1	1.1 ± 0.2	4.2 ± 0.2
Quercetin + Terpenoid	**1.1 ± 0.1**	**0.8 ± 0.1**	**0.6 ± 0.1**	**2.5 ± 0.2**

A				
Quercetin + Alkaloid B	3.7 ± 0.2	3.2 ± 0.2	2.8 ± 0.1	9.7 ± 0.3

The histopathological protection offered by the Quercetin + Terpenoid A combination closely parallels that of the standard treatment. In contrast, antagonistic combinations like Quercetin + Alkaloid B failed to prevent tissue damage effectively.

9. Lung Index (Lung Weight to Body Weight Ratio)

The lung index reflects the extent of pulmonary inflammation and edema.

Table 9. Lung Index (%)

Group	Lung Index (%)
Normal Control	0.56 ± 0.03
Asthma Control	1.28 ± 0.07
Dexamethasone	0.64 ± 0.04
Extract	0.70 ± 0.05
Quercetin + Terpenoid A	**0.61 ± 0.03**
Quercetin + Alkaloid B	1.05 ± 0.06

Reduced lung index values in treatment groups indicate alleviation of inflammation and tissue swelling. Synergistic combinations were more effective.

10. Mast Cell Stabilization (In vitro Assay)

Mast cell degranulation was assessed using compound 48/80-induced mast cell rupture in rat mesenteric tissue.

Table 10. Mast Cell Degranulation (%)

Group	Degranulation (%)
Control (No inhibitor)	89.4 ± 3.1
Dexamethasone	26.2 ± 2.0
Extract	32.8 ± 2.4
Quercetin	41.6 ± 3.0
Terpenoid A	47.5 ± 2.8
Quercetin + Terpenoid A	**23.4 ± 1.9**

The synergistic phytochemical combination significantly stabilized mast cells, reducing the release of histamines and other inflammatory mediators. This suggests efficacy in preventing allergic asthma triggers.

11. Acute Toxicity Study

To ensure safety, acute oral toxicity was assessed in healthy mice according to OECD guideline 423.

Table 11. Acute Toxicity Parameters (14-day Observation)

Parameter	Observation (2000 mg/kg dose)
Mortality	None
Behavioural Changes	None
Body Weight Change	Normal
Food/Water Intake	Normal
Organ Weight (liver, kidney, heart, lungs)	Normal
Histology of Organs	No abnormality

The extract and phytochemical combinations were well-tolerated up to 2000 mg/kg body weight, indicating a high margin of safety.

12. Body Weight Change During Experiment

Maintaining body weight indicates general health and absence of systemic toxicity.

Table 12. Body Weight (g) Over Experimental Period

Group	Day 0	Day 14	Day 28
Normal Control	21.5 ± 0.4	22.1 ± 0.3	22.8 ± 0.5
Asthma Control	21.7 ± 0.3	20.6 ± 0.5	19.2 ± 0.6
Dexamethasone	21.6 ± 0.4	22.2 ± 0.4	22.9 ± 0.4
Extract	21.4 ± 0.4	22.0 ± 0.3	22.7 ± 0.3
Quercetin + Terpenoid A	**21.6 ± 0.3**	**22.3 ± 0.2**	**23.0 ± 0.3**

The weight gain in the treatment groups suggests absence of cachexia and supports safety.

13. Airway Hyperresponsiveness (AHR)

Table 13. Penh Value Comparison

Group	Penh Value (Mean ± SD)	Significance vs. OVA Group
Normal Control	0.71 ± 0.05	*** ($p < 0.001$)
Asthma Control (OVA)	2.89 ± 0.21	—
Dexamethasone	1.12 ± 0.09	***
Extract	1.34 ± 0.12	***
Quercetin + Terpenoid A	**1.08 ± 0.07**	***
Quercetin + Alkaloid B	2.23 ± 0.18	* ($p < 0.05$)

ANOVA: $F(5, 54) = 84.26$, $p < 0.0001$

The synergistic combination (Quercetin + Terpenoid A) significantly reduced airway resistance, comparable to Dexamethasone and superior to the crude extract or individual components. The antagonistic combination (Quercetin + Alkaloid B) failed to show marked improvement, indicating possible interference or reduced efficacy.

14. Eosinophil Count in BALF

Table 14. Eosinophils (×10⁴ cells/mL)

Group	Eosinophils (Mean ± SD)	Significance vs. OVA Group
Normal Control	0.5 ± 0.1	***
Asthma Control	8.3 ± 0.6	—
Dexamethasone	1.5 ± 0.2	***
Extract	2.3 ± 0.3	***
Quercetin + Terpenoid A	**1.2 ± 0.2**	***
Quercetin + Alkaloid B	5.5 ± 0.5	*

ANOVA: F(5, 54) = 119.34, p < 0.0001

Eosinophil infiltration was drastically suppressed by the extract and especially by the Quercetin + Terpenoid A combo, suggesting strong anti-inflammatory activity. Poor results in the Alkaloid group suggest it may counteract anti-inflammatory pathways.

15. IL-5 Cytokine Levels in BALF

Table 15. IL-5 Levels (pg/mL)

Group	IL-5 (Mean ± SD)	Significance vs. OVA Group
Normal Control	18.5 ± 1.4	***
Asthma Control	74.3 ± 4.7	—
Dexamethasone	28.4 ± 2.6	***
Extract	33.6 ± 2.4	***
Quercetin + Terpenoid A	**24.6 ± 2.0**	***
Quercetin + Alkaloid B	59.1 ± 3.1	*

ANOVA: F(5, 54) = 98.73, p < 0.0001

IL-5, a key eosinophil chemoattractant, was suppressed markedly in the synergistic treatment group. The near equivalence with Dexamethasone demonstrates its potent effect. The antagonistic group retained high IL-5 levels, supporting previous histological and cellular findings.

16. MDA (Malondialdehyde) in Lung Tissue

Table 16. MDA Levels (nmol/mg protein)

Group	MDA (Mean ± SD)	Significance vs. OVA Group
Normal Control	1.8 ± 0.2	***
Asthma Control	5.9 ± 0.4	—
Dexamethasone	2.3 ± 0.2	***
Extract	2.7 ± 0.3	***
Quercetin + Terpenoid A	**2.1 ± 0.2**	***
Quercetin + Alkaloid B	4.3 ± 0.3	*

ANOVA: F(5, 54) = 87.12, p < 0.0001

MDA levels, indicative of lipid peroxidation and oxidative stress, were significantly lowered by the phytochemical treatments. The synergistic group (Q + T) was particularly effective in protecting the lung tissue from oxidative damage.

17. Lung Index

Table 17. Lung Index (% of body weight)

Group	Lung Index (%)	Significance vs. OVA Group
Normal Control	0.56 ± 0.03	***
Asthma Control	1.28 ± 0.07	—
Dexamethasone	0.64 ± 0.04	***
Extract	0.70 ± 0.05	***
Quercetin + Terpenoid A	**0.61 ± 0.03**	***
Quercetin + Alkaloid B	1.05 ± 0.06	*

ANOVA: F(5, 54) = 71.49, p < 0.0001

Reduced lung index values confirm that the extract and synergistic combination mitigated pulmonary inflammation and edema. The Alkaloid group showed partial improvement, highlighting its suboptimal contribution.

Discussion

The present research studied how different phytochemicals from Araucaria columnaris leaf extract may affect asthma and inflammation alone and synergistically. It was clear from the results that some combinations, in particular quercetin and terpenoid A, combined well to considerably reduce the various signs of asthma, such as AHR, eosinophils coming into the airways, cytokine release, and oxidative stress. Since the results with the combination are not just better than the individual treatments, but also significantly better than the crude extract, it is likely that these compounds hit various relevant pathways and cause more effective breathing and less airway blockage. It was demonstrated even more with a noticeable drop in eosinophil numbers in the BALF, which is closely linked to how asthma affects the airways[21].

Inflammatory agents called interleukin-5 (IL-5) were found to be dramatically decreased only in the group that received the combined treatment. As a result, it looks like the mixed phytochemicals manage the immune system to prevent it from overreacting in asthma[22]. The reason for these phytochemicals' anti-inflammatory function could be because they stop important signaling mechanisms involved in the production of cytokines and the inflow of immune cells to the lung, therefore minimizing long-term inflammation. Also, the level of malondialdehyde (MDA) in the lungs was seen to decrease significantly, which suggests that the antioxidant effect could shield lung tissue from damage by oxidative stress, which is a factor that increases the severity of asthma. Oxidative stress increases inflammation as well as the remodeling of tissues, so the antioxidant activity of the synergistic

phytochemicals acts against both these problems at the same time [23].

Still, treatment with both substances together caused the results to work in the opposite direction, as asthma symptoms improved less than with stand-alone treatments[24]. This competition between alkaloids and quercetin could happen because the alkaloids block the body's absorption or action of quercetin, clarifying how complex interaction among various plant compounds can be. That's why choosing and mixing phytochemicals wisely is crucial to prevent any bad outcomes during therapy [25].

Besides, smaller lung index values and better changes in lung tissue seen in the combination group prove that these chemical compounds protect against airway changes and tissue swelling. Chronic asthma is clearly identified by airway walls that are both swollen and affected by permanent changes, so these morphological changes play a very important role. Since synergetic phytochemicals help reduce these lung changes, they might be useful as extra or alternative treatments for those who have asthma [26].

To sum up, this study gives helpful information about the ways Araucaria columnaris phytochemicals act on asthma and stresses how synergism helps boost their effects. The results indicate a need to study the involved targets and signaling systems and achieve standard methods to apply in the clinic. Certain statistics reveal that quercetin and terpenoid A may have better effects than corticosteroids in safer, plant-based asthma treatments [27].

Conclusion

Through the study, researchers confirmed that Araucaria columnaris leaf extract contains active substances that strong. The combination of quercetin and terpenoid A turned out to be the best treatment, reducing sensitive airways, eosinophil-caused inflammation, the levels of pro-inflammatory cytokines, and oxidative stress in models affected by asthma. The research demonstrates that these compounds may have an impact on multiple processes related to asthma, for example, infiltration of immune cells, cytokine signaling, and damage caused by reactive oxygen species. Especially, the better results obtained from these phytochemicals than from the crude extract or each component alone prove how useful combination therapy can be for improved health outcomes. On the other hand, when alkaloid B is present, the mixtures tested reveal that certain phytochemical combinations may do more harm than good and may decrease how well the therapy works. All in all, these findings suggest that blends of plant products could be valuable options or supplements to regular asthma medications, since they may have fewer side effects. Further research should work on explaining the detailed molecular reasons for these synergistic effects, finding the best methods to use and amount of medicine, and carrying out clinical trials to verify their safety and usefulness in people". All things considered, the chemicals found in Araucaria columnaris could be important in making new anti-asthmatic drugs.

CONFLICT OF INTEREST: The authors have no conflicts of interest regarding this investigation**.**

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