The Anti-Asthmatic Effect of the Combined Yerba Buena (*Mentha arvensis Linn.*) and Oregano (*Coleus amboinicus Lour.*) Leaves in BALB/c Mice Model of Allergic Asthma

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ABSTRACT

Introduction. Asthma is an IgE-mediated inflammatory response characterized by hyperresponsiveness, airway inflammation, and reversible airflow obstruction. Currently, asthma affects 12 - 22% of the population in the Philippines. Anecdotal reports showed that yerba buena (*Mentha arvensis Linn.*) and oregano (*Coleus amboinicus Lour.*) are utilized for treating asthma in the folk culture.

Objective. The objective of this study was to determine the effect of combined Yerba Buena (*Mentha arvensis Linn.*) and Oregano (*Coleus amboinicus Lour.*) leaves extract in asthma-induced mice.

Methods. This study investigated the anti-asthmatic activity of the aqueous and methanolic extracts of the combined herbs in asthma-induced mice using immunoglobulin E (IgE) as a parameter.

Results. Aqueous- and methanol-treated mice has 50% and 60% reduction in the IgE level, respectively (p = 0.018). The extracts exhibited a significant (p = 0.001) anti-inflammatory activity in mice that further proved its effect on IgE. Moreover, lung histopathology also established the potential effect of the extract through the widening of the alveoli on treated mice.

Conclusion. Combined Yerba Buena and Oregano aqueous and methanol extracts may have a potential health benefit against asthma.

Key Words: yerba Buena, oregano, anti-asthma, anti-inflammation, IgE

Introduction

Asthma is a chronic disease characterized by airway inflammation, hyperresponsiveness, and reversible airflow obstruction.[1] These pathologic changes are due to different allergic triggers like pollen, dust mites, and fumes that can be found in the environment. In the Philippines, asthma affects 12% of adolescents and 17-22% of adults.[2]

During an asthmatic reaction, increased level of the different cytokines such as IL – 4, IL – 5, and OVA-specific IgE among the allergic asthmatic patients were observed.[3] IL-4 is the principal Th2 cytokine for the production of IgE and developing Th2 from a naïve CD4+ T cells.[4] It contributes to the airway obstruction in asthma by inducing the expression of mucin gene and mucus hypersecretion.[5] IL-5 also plays important role as a mediator in the activation of eosinophils in the bronchial mucosa. It influences adhesion, membrane receptor expression, chemotaxis, and mediator synthesis.[6] Lastly, IgE is a key mediator in the inflammatory response, particularly for type I hypersensitivity.[7] Allergen-specific IgE binds to high affinity Fc receptor of the mast cells. This causes the mast cells to degranulate and releases chemical mediators responsible for inflammation.[8]

Currently, asthma is treated with bronchodilators and anti-inflammatory drugs. However, with prolonged usage, patients experience tachycardia, sweating, tremors, dizziness, and agitations. Anecdotal reports on Yerba Buena (*Mentha arvensis Linn*) and Oregano (*Coleus amboinicus Lour*) showed effectivity against asthma.[9,10]

Given the potential of these plants with no current studies regarding their anti-asthmatic activity, it is hypothesized that combined Yerba Buena (*Mentha arvensis Linn.*) and Oregano (*Coleus amboinicus Lour*) leaf decoction is effective in reducing IgE levels among asthma-induced mice.

Objectives

The general objective of this study was to determine the effect of combined Yerba Buena (*Mentha arvensis Linn.*) and Oregano (*Coleus amboinicus Lour.*) leaves extract among asthma-induced mice. Specifically, this paper wanted to:
1. Utilize OVA-specific IgE as a marker responsible for asthmatic reaction.
2. Determine the anti-inflammatory activity of the combined methanolic and aqueous extracts.
3. Determine its phytochemical components such as alkaloids, cyanin, coumarins, and flavonoids that have anti-inflammatory activity.

Methods

Plant

Yerba Buena (*Mentha arvensis Linn.*) leaves were collected from a plantation in Baguio City while Oregano (*Coleus amboinicus Lour.*) leaves were collected from a farm in Morong, Bataan. Total weights of 4 kilograms (kg) of leaves for each plant were collected. Both samples were authenticated at the Bureau of Plant Industry (BPI) in Manila.

Animals

Healthy 6-8-week old BALB/c male and female mice weighing 20-40 grams were obtained from the animal laboratory of St. Luke's Medical Center. A total of 5 mice for each treatment (sensitized treated with negative control, sensitized treated with positive control, sensitized treated with aqueous extract, sensitized treated with methanolic extract) were used. The mice were housed in 15 X 5 in^2 cages at the Animal House of the Department of Biochemistry in a 12-hour light and dark cycle. The protocol was approved by the Institutional Animal Care and Animal Use Committee (IACUC) of the National Institute of Health (NIH), University of the Philippines, Manila. No mice escaped and died during the research duration. At the end of this study, mice were sacrificed by cardiac puncture.

Research Design

Pretest-post test study design was used in this study. IgE was used as a marker to measure the efficacy of the extracts. Mice paw edema was used to assess the anti-inflammatory activity of the extracts while lung histopathology was used to verify whether asthma was induced. Phytochemical components were studied using phytochemical analysis and thin layer chromatography (TLC).

Preparation of Plant Extracts

For the methanol extract, Yerba Buena and Oregano leaves were collected, selected, weighed, washed and dried. The collected dried leaves of both plants were mixed with a 1:1 ratio. These were soaked in a pure methanol (1.5 ml) overnight and supernatants were collected after. Extraction was done thrice and a rotary evaporator (rotavap) was used to collect a concentrated supernatant. A small amount of concentrated sample was reconstituted and used for phytochemical screening and TLC.

For the aqueous extract, fresh leaves of Oregano and Yerba Buena with 1:1 ratio were boiled in water for 15 minutes. It was cooled and decanted to get the supernatant. Similar to methanolic extract, the procedure was repeated three times and used for lyophilization. Supernatants of the aqueous extract were frozen and freeze dried under Christ Freeze Drier in the Natural Products Laboratory (NPL). A small portion of the lyophilized aqueous extract was reconstituted for phytochemical screening.

Concentrated supernatant (750 mg/kg) of both aqueous and methanolic extracts were used to determine the anti-asthmatic effect among the asthma-induced mice.

Anti-asthmatic activity

The extracts were used for testing the anti-asthmatic activity of the combined Yerba Buena (*Mentha arvensis Linn.*) and Oregano (*Coleus amboincus Lour.*) (Figure 1).
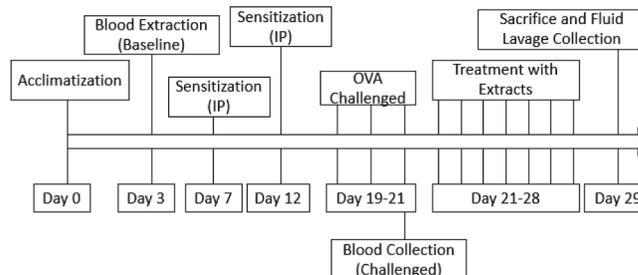

Figure 1. Induction and sensitization of mice with ovalbumin by intraperitoneal injection on day 7 and day 12. Mice were challenged using intranasal ovalbumin solution on day 19 to day 21. Asthma-induced mice were treated with the aqueous and methanol extracts of the combined yerba buena and oregano on day 21 to day 28. Mice were sacrificed on day 29 for the collection of lavage fluid and blood for anti-asthmatic assay using IgE ELISA.

Sensitization of Mice

The mice were sensitized intraperitoneally on day 7 and day 12 with 25μg ovalbumin (OVA) emulsified in 2mg aluminum hydroxide and dissolved in 0.5ml of 0.9% saline solution as described in the paper of Pretorius & Ekpo.[11] The immunization was halted for a week to allow the immune system to mount the reaction against the OVA antigen.[1]

After one week, the mice were challenged daily for three days by intranasal exposure to 1% (w/v) OVA diluted in 100ml of sterile normal saline[4] on day 19 to day 21. The challenge was given twice each day with a 30-minute interval between exposures. This procedure was done to induce inflammatory reactions in the lungs that could lead to asthma.[12,13] On the last day of the challenge, saphenous blood extraction was done for analysis of the serum one hour after the latter intranasal exposure.

Assessment of the Presence of Asthma

Mice activities were observed for signs of difficulty of breathing, such as snuffle sound, loss of appetite and often lying down. Auscultation was done to check for wheezing. Observations were done immediately after, after 10 minutes, and an hour after giving treatment.[12,14]

Treatment Administration

Administration of the treatments was given 30 minutes after the last intranasal challenge (on Day 21). The

treatments were given per orem once a day for seven days.[11] The treatment groups received a dosage of 10 ml/kg normal saline solution (NSS) as a negative control, 0.27 mg/kg dexamethasone as a positive control, 750mg/kg body weight (BW) for the combined Yerba Buena (*Mentha arvensis Linn.*) and Oregano (*Coleus amboinicus Lour.*) aqueous and methanol extracts. Dosages for the negative and positive control were based on previous studies[14,15] and the dosage given for the aqueous and methanol extract was based on a pretest done by the researcher prior the actual experiment. The dosages used in the pretest were 50 mg/kg, 100 mg/kg, and 500 mg/kg. Based on the results from the pretest, the 500 mg/kg arm exhibited a remarkable reduction in the induced baseline asthma, thus, a 50% increase was used as the treatment dosage in the actual experiment to maximize its therapeutic effect.

Measurement of OVA-specific serum levels of IgE

Blood samples were collected before sensitization, after the last OVA challenge, and two hours after giving the last treatment. The samples were centrifuged to collect the serum[14,16,17] and the OVA-specific serum level of IgE was measured using ELISA.

Lung Histopathology

The lavaged lungs of the mice[18] were stored in 10% formaldehyde before slide preparation. The prepared slides were composed of lungs cut cross-sectionally and were stained with hematoxylin and eosin stain to check for the presence of epithelial thickening, subepithelial folding, intraluminal debris, and inflammatory infiltrates. Slides were read blindly by the Department of Pathology in UP Manila. The above parameters were used to establish induction of asthma and the efficacy of the extracts.

Anti-inflammatory Activity

The anti-inflammatory activity was assessed every 30 minutes by measuring the right and left paw using a caliper. Baseline paw diameter was measured before inducing carrageenan-paw edema. A 0.05 ml Carrageenan was injected on the right paw while NSS was injected on the left paw of the mice. A 750 mg/kg (p. o.) of methanol and aqueous extract were given to two groups while 100 mg/kg Indomethacin was given as a positive control and a 0.9 % NSS was given as a negative control. This was based from the standard protocol done by Feitosa, Rocha, Ribeiro, & Lima.[19]

Phytochemical Analysis

Thin Layer Chromatography

Concentrated supernatant (300 mg/ml) of methanolic extract was used in TLC. Different combinations of chloroform and methanol solvent, and hexane and ethyl acetate were prepared and used. When the mobile phase was 1 cm away from the top edge, the TLC plate was dried, and visualized under visible light, a hand-held UV-lamp for short and long wavelengths, and via spraying a solution with 10% sulfuric acid. The solvent that produced the best trail in the TLC plate was used.

After TLC was done, an aliquot of the extracts was used for phytochemical analysis. This was used to qualitatively describe the different components present in the extract. This study tried to determine the presence of reducing sugars, steroids, glycosides, tannins, coumarins, anthraquinones, flavonoids, saponins, and terpenoids from the given extract since these secondary metabolites are believed to carry the anti-asthmatic property of the plants.

Statistical Analysis

Values in the graph were all presented in mean $\pm$ standard error of the mean (SEM) in 95.0% confidence interval (CI). The graphs shown were made from the Microsoft Excel 2007. Statistical analyses, using one-way analysis of variance (ANOVA), were all done in IBM SPSS Statistics version 21.

Results

Anti-asthmatic Activity

To analyze the IgE levels, serums during without asthma, during the induction of asthma, and during asthma with treatment of the extracts were collected and were used for the investigation (Figure 2).

Asthma was successfully induced as shown by the increase in serum IgE levels after the intranasal exposure of OVA (p = 0.018). The combined aqueous extract was able to reduce the serum IgE level by 50% (p = 0.001) while the methanol extract was able to reduce the serum IgE level by 60% (p = 0.01).
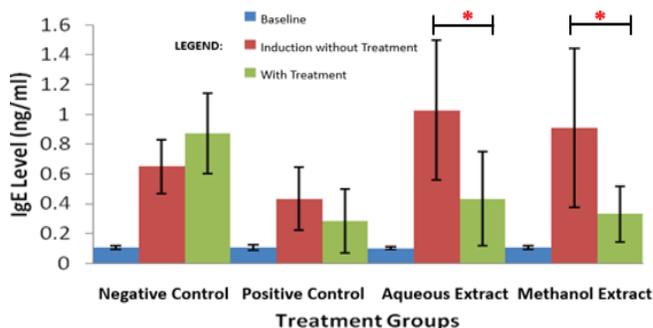

Figure 2. Mean IgE level for each treatment group. Baseline data were found equal across the treatment group (blue bars). There was an increase in the IgE level after induction of intranasal albumin (red bars). Significant reduction of IgE level was observed after treatment of the aqueous and methanol extract with 750 mg/kg concentration (*p = 0.018) (green bars).
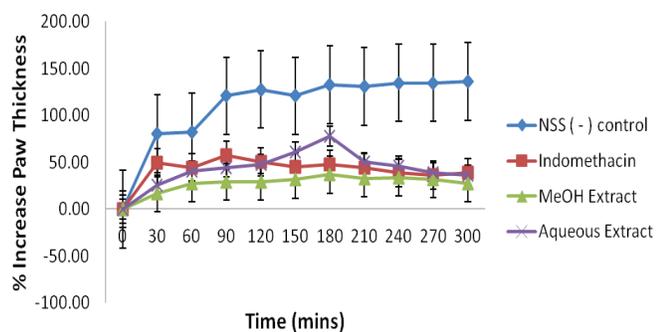
Figure 3. Anti-inflammatory activity. There was a significant reduction of inflammation in the aqueous and methanolic extract treatment groups as compared with the positive extract (p = 0.001). This data supports the potential inhibitory activity of the Yerba Buena and Oregano against asthma.

Anti-inflammatory Activity

Anti-inflammatory activity shows that both the aqueous and methanolic extracts of Yerba Buena and Oregano extracts are comparable with the positive control which is Indomethacin (Figure 3).

As shown in Figure 3, the aqueous and methanolic extracts of Yerba Buena and Oregano were also able to demonstrate anti-inflammatory activity by reducing the paw thickness by 92%, similar to Indomethacin, the positive control (p = 0.001).

Lung Histopathology

Figure 4 shows the comparison among the untreated sensitized mice to treated sensitized mice. Thickening of the epithelium and folding of the subepithelium were manifested in the negative control, signifying increased inflammatory activity. Interestingly, the positive, aqueous, and methanol extracts treated mice were found to have less thickening, less folding, and lesser inflammatory infiltrates that may support IgE level reduction and anti-inflammatory activity.

Phytochemical Screening and Analysis

Dried leaves that were extracted using 100% methanol in soxhlet apparatus were greenish-black in color while boiled fresh leaves produced brownish-yellow lyophilized powder. Table 1 shows the percent yield collected for both aqueous and methanolic extracts.

Table 1. Percent Yield for Aqueous and Methanolic Extracts

	Aqueous Extract	Methanol Extract
Weight of Sample	102 g	50 g
Weight of Extract	1.43 g	6. 9261 g
Percent Yield	1.40%	13.85 %

From several trials of the combination of methanol and chloroform solvents, and hexane and ethyl acetate solvents,

a good solvent system was derived. A 70-30 mixture of hexane-ethyl acetate solvent system has separated the extract into 15 different components; however, the TLC plate cannot differentiate these 15 components.

On one hand, phytochemical screening revealed different possible phytochemical compounds present in the aqueous and methanolic extracts. It shows that there are five compounds that are similar to both aqueous and methanol extracts. These are proteins, alkaloids, cyanins, coumarins, and flavonoids (Table 2). Furthermore, the aqueous extract was also found to have carbohydrates, terpenes, quinines, phenols and tannins.
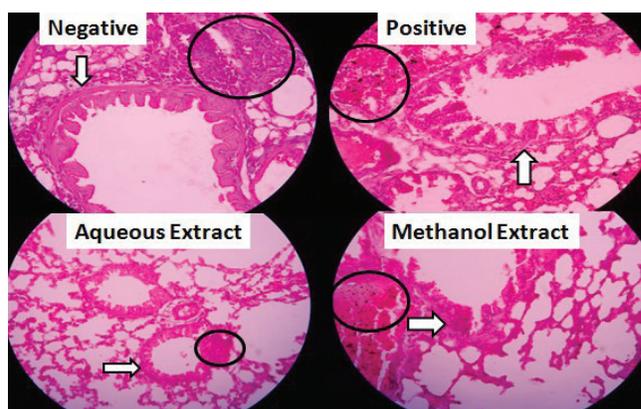

Figure 4. Lung histopathology of mice among negative control, positive control, aqueous extract and methanol extract. Arrows point to the epithelial cell lining of the lungs. Circles show the inflammatory infiltrates (stain used was hematoxylin and eosin with a magnification of x400). Thinning and reduction of inflammatory infiltrates further validated the potential curative effect of the combined Yerba Buena and Oregano against asthma.

Table 2. Phytochemical Screening of the Aqueous and Methanolic Extracts

Phytochemicals	Combined Aqueous Extract	Combined Methanol Extract
Carbohydrates & Reducing Sugars	+	-
Proteins & Amino Acids	++	+
Alkaloids	+	+
Glycosides	-	+
Steroids	-	+
Terpenes & Terpenoids	+	-
Quinones	+	-
Cyanin	+	+
Coumarin	+	+
Flavonoid	++	+
Saponin	-	-
Phenol	+	-
Tannin	+	-

Increasing (+) means increasing intensity of the qualitative analysis of the phytochemical component; (-) means phytochemical component not exhibited.

Discussion

Extraction of the Yerba Buena and Oregano for methanol and aqueous yielded only 13.85% and 1.40%, respectively for its lyophilized products. A possible explanation of its low yield is that the extractable components may have different availability due to extraction procedure strength and the extracting solvent used for the endogenous compounds.[20,21]

Phytochemical analysis done using TLC and phytochemical screening, found five compounds, such as proteins, alkaloids, cyanins, coumarins, and flavonoids, that were similar to both aqueous and methanol extracts. Different studies about carrageenan-induced paw edema, lipopolysaccharide-induced edema, and ovalbumin denaturation have shown that these phytochemical compounds possess anti-inflammatory activity. Previous studies have shown that inflammation was reduced by extracts possessing these phytochemical compounds.[22-24]

Although several phytochemical compounds were found to be present, it may be the flavonoids that have the activity against asthma. This possibility was supported by the study of Yang, in which the flavonoid, a purified compound, inhibited the contraction of airway smooth muscle in allergic asthmatic mice was.[25] In addition, this compound isolated from *G. lucidum* was also found to inhibit TNF-a production among asthma patients which was associated with steroid resistant asthma.[26]

Moreover, studies about flavonoids say that they are a prominent inhibitor of cyclooxygenase (COX) or lipooxygenase (LOX) which prevent the synthesis of the prostaglandins (PGs) that suppress the T-cells. Additionally, immune cells communicate with each other through cytokines that are controlled by flavonoids. Various flavonoids inhibit COX2 and the nitric oxide synthase which are relevant in inflammation.[27] Like flavonoids, phenol has also been found to have anti-inflammatory and analgesic properties. Further, phenol was found to inhibit COX activity and silica-induced reactive oxygen species (ROS).[28] Though the mechanisms are not yet fully elucidated, other studies for tannins, quinones, and terpenes also show anti-inflammatory activity.[28,29]

Furthermore, anti-inflammatory assay showed that both the aqueous and methanolic extracts of Yerba Buena and Oregano extracts are comparable to Indomethacin. This suggests that the extracts have a potent anti-inflammatory activity. Although phytochemical compounds were not tested individually, potent anti-inflammatory activity may be because of the flavonoids present based on previous studies.[22,23]

Possible explanation for its mechanism is the reduction in the IgE level. Th2 cytokines is the precursor and has the main key role in inflammation in asthmatic airways. Through these cytokines, B cells switch to IgE resulting to mast cell maturation, eosinophilic inflammation, smooth muscle contraction, and increased mucus production.[30-33] Thus, this extract may be a potential phytomedicine for asthma.

Previous histopathologic studies done on lungs of asthmatic patients have shown that the respiratory epithelium is thicker and has more folds because of airway remodeling which is associated with epithelial cell alteration, submucosal gland hyperplasia, increased in airway smooth muscle mass and increased airway vascularization.[34,35] This results to greater luminal narrowing causing shortness of breath and wheezing.[36] Epithelial thickenings is brought about by the deposition of collagen types I, III and V causing subepithelial fibrosis.[36,37] Furthermore, inflammatory infiltrates were more pronounced in the negative control suggesting that airway inflammation occurred[36] and it happened more on the untreated group suggesting the potential effect of the extracts in airway inflammation. Thus, it is possible that the two extracts may have only exhibited an activity against IgE level.

This was further confirmed by the anti-inflammatory assay using carrageenan-induced paw edema and lung histopathology. Moreover, the extracts' phytochemical constituents such as flavonoids, coumarins and phenol further supports its potential effect against asthma.

Conclusions and Recommendation

The methanol and aqueous extracts of Yerba Buena and Oregano were able to significantly reduce the serum IgE levels in asthma-induced BALB/c mice. The extracts have also shown anti-inflammatory activity as seen in the significant reduction of carrageenan-induced paw edema. Furthermore, lung histopathology revealed reduced epithelial thickenings, foldings and inflammatory infiltrates in the extract-treated mice. These findings support the potential beneficial effect of Yerba Buena and Oregano against asthma. Further investigation on the purified component of the extract is warranted to identify the potent component with anti-asthma properties.

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