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ORIGINAL ARTICLE

Phase III study of Phlai capsules in the treatment of allergic rhinitis: A randomized, double-blind, placebo-controlled trial

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Abstract

Background: Preclinical studies demonstrated anti-inflammatory effects of *Zingiber montanum* (J.König) Link ex Dietr. (Phlai). However, its clinical effect on allergic rhinitis (AR) is not evident.

Objective: We sought to assess the efficacy and safety of Phlai for treating AR.

Methods: A phase 3, randomized, double-blind, placebo-controlled study was conducted. Patients with AR were randomized into three groups and received Phlai 100 mg or Phlai 200 mg or placebo once a day for four weeks. The primary outcome was a change in the reflective total five symptom score (rT5SS). The secondary outcomes were the change in the instantaneous total five symptom score (iT5SS), the reflective individual symptom scores (rhinorrhea, nasal congestion, sneezing, itchy nose, itchy eyes), Rhinoconjunctivitis Quality of Life-36 Questionnaire (RCQ-36) score, peak nasal inspiratory flow (PNIF), and adverse events.

Results: Two hundred and sixty-two patients were enrolled. Compared with placebo, Phlai 100 mg improved rT5SS [adjusted mean difference (aMD) -0.62; 95%CI -1.22, -0.03; p = 0.039], rhinorrhea (aMD -0.19; 95%CI -0.37, 0.002; p = 0.048), itchy nose (aMD -0.24; 95%CI -0.43, -0.05; p = 0.011), and itchy eyes (aMD -0.19; 95%CI -0.36, -0.02; p = 0.033) at week 4. Nasal obstruction, sneezing, iT5SS, overall RCQ-36 score, PNIF did not reach statistical significance. Phlai 200 mg did not bring additional benefits compared to 100 mg. Adverse events were similar among groups.

Conclusion: Phlai was safe. At four weeks, there were small improvements in rT5SS, together with the individual symptoms of rhinorrhea, itchy nose, and itchy eyes.

Key words: *Zingiber montanum*, *Zingiber cassumunar*, Phlai, herbal medicine, rhinitis, allergic, allergy, quality of life

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Introduction

Allergic rhinitis (AR) causes a significant impact on quality of life, school, and work performance leading to high medical costs. The prevalence of AR is escalating and is reported to be approximately 50% in Thailand.[1,2] A proportion of patients are unsatisfied with standard treatments, including oral antihistamines, intranasal corticosteroids, and leukotriene receptor antagonists[3] and would prefer to use complementary alternative therapies to alleviate the nasal symptoms.[4] A growing body of evidence suggests the benefits of herbal medicine for treating AR worldwide.[4] The beneficial effects of herbal medicine can be achieved by several mechanisms of action. Herbal medicine modulates the immunological activities of mast cells and brings anti-allergic effects which impede the release of histamine, leukotriene, cytokine, and chemokine from inflammatory cells. Additionally, anti-inflammatory effects inhibit the production of inflammatory cells such as mast cells, basophils, eosinophils, and monocytes.[4-6] Furthermore, nasal symptoms including sneezing, itching, rhinorrhea, nasal obstruction become clinically well-controlled.[4]

Plants in the Zingiberaceae family, including *Zingiber officinale*,[7] *Zingiber zerumbet*,[8] and *Zingiber cassumunar*[5,6] have anti-allergic[5,8,9] and anti-inflammatory properties.[10] *Zingiber montanum* (J.König) Link ex A.Dietr. (synonym: *Zingiber cassumunar* Roxb.), locally known as "Phlai" in Thai,[11] has a potent bioactive component called compound D [E-4-(3′,4′-dimethoxyphenyl) but-3-en-1-ol] in its rhizomes.[12] Phlai cream, with its anti-inflammatory effect, has been used in muscle strain.[13] Pulverized rhizome of Phlai can improve the symptom severity in adult patients with asthma.[14] In the previous studies, Phlai capsules (100 and 200 mg) decreased bronchial hyperresponsiveness in asthmatic patients with AR.[15] Moreover, these two doses showed an antihistaminic effect on skin testing in patients with AR.[5] However, neither efficacy nor safety of Phlai in treating patients with AR has been investigated in large clinical trials.

The aims of this study were to assess the clinical effectiveness and safety of Phlai extracts for treating patients with AR. We hypothesized that Phlai would effectively control the nasal and ocular symptoms with no serious adverse effects.

Methods

Study design and participants

We conducted a phase 3, randomized, double-blind, placebo-controlled trial in the outpatient departments of seven University hospitals in Thailand: (1) Endoscopic Nasal and Sinus Surgery Excellent Center, King Chulalongkorn Memorial Hospital, (2) Siriraj Hospital, (3) Center of Excellence in Otolaryngology-Head & Neck Surgery, Rajavithi Hospital, (4) Phramongkutklao Hospital, (5) Center of Excellence for Allergy, Asthma and Pulmonary Diseases, Thammasat University Hospital, (6) Srinagarind Hospital, and (7) Songklanakarin Hospital. The study was approved by the Central Research Ethics Committee (CREC036/2019) and the Institutional Review Board of all study sites. The study process was explained in detail, including medications and all investigations. All patients had ample time to ask questions which included potential risks and benefits from the study and provided written informed consent; the trial was conducted following the Declaration of Helsinki. This study was registered at ClinicalTrials.gov (Study ID: NCT04182919).

Patient population

Adults presented to the outpatient departments with AR symptoms were screened for eligibility. The inclusion criteria were: (1) Age 18-50 years; (2) Allergic rhinitis following diagnostic criteria of ARIA guideline;[16] (3) Not currently using intranasal corticosteroid for two weeks, systemic corticosteroid for four weeks, oral antihistamine for one week, nasal decongestant for one week, and leukotriene receptor antagonists for one week; (4) Daily reflective total five symptoms score (rT5SS) of 2-10 per day for three consecutive days (total score = 15) and not greater than 10 on any day during the past week, representing the patients with active AR symptoms. Reflective total five symptoms score (rT5SS) was a sum of individual AR symptoms of rhinorrhea, nasal obstruction, itchy nose, sneezing, and itchy eyes. All individual AR symptoms were scored on a 4-point scale (0 = no symptom present, 1 = mild symptom that does not interfere with any activities, 2 = moderate symptom that slightly interferes with daily activities or sleep, 3 = severe symptom that significantly bothers daily activities or sleep). Diagnosis of AR was confirmed by skin prick test. Seven common allergens in Thailand were tested for sensitization, comprising *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cockroach, dog hair, cat hair, careless weed, and paragrass (AllerVACtest®10, Greater Pharma, Bangkok, Thailand).[17]

Exclusion criteria were: (1) Underlying severe medical diseases, e.g., COPD, heart disease, chronic renal failure, chronic hepatic failure; (2) AR and asthma requiring immunotherapy; (3) Receiving antidepressants, sedatives, anxiolytics, opioid or antipsychotics; (4) Uncontrolled asthma requiring inhaled steroids and/or LABA; (5) Previous surgery for nasal polyp or nasal septum deviation; (6) Acute or chronic rhinosinusitis; (7) Pregnancy and lactation; (8) History of allergy to any kind of herb; (9) Refusal to participate.

Randomization and allocation

Randomization, allocation, and blinding were performed by an independent third party who did not involve in clinical practice. A biostatistician generated a central randomization with unequal block sizes with sizes proportional to elements of Pascal's triangle, and a 1:1:1 allocation ratio within each block.[18] A research coordinator performed the patient assignment, concealed and stored the allocation codes. The packages labeled with unique codes were sent to all trial centers.

Preparation Phlai extracts capsules

Each capsule contained 100 mg of Phlai extract equivalent to 4 mg of compound D. Placebo had similar contents with active capsule without compound D. Active capsules and placebo appeared identical. Phlai extract capsules were produced by The Government Pharmaceutical Organization, Bangkok, Thailand. High-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) techniques were used to detect the presence of compound D in Phlai capsules (**Supplementary Table S1**, **Figures S1-S2**). The quality, consistency, and safety of Phlai capsules were established in accordance with FDA standards.

Intervention

There was a 2-week run-in period of withholding medication. Each study participant received two bags containing capsules with identical appearances. They were instructed to undertake one capsule in each bag after dinner per day. Study investigators, care providers, and patients were blinded to treatment allocation. Study participants were randomized into three groups. Group 1 received a 4-week oral administration of 2 capsules of Phlai extracts with a total of 200 mg of Phlai. Group 2 received a 4-week oral administration of one capsule of Phlai extracts and one capsule of placebo with a total of 100 mg of Phlai. Group 3 received a 4-week oral administration of 2 capsules of placebo. The treatment duration was four weeks. There were two follow-up visits at 2 and 4 weeks. The study flow diagram is shown in **Figure 1**.

In the cases of worsening of AR symptoms with T5SS > 10, study participants were permitted to use isotonic nasal saline irrigation as rescue medication provided by researchers.[19] All rescue medicine use was daily self-recorded. Study participants were withdrawn when having one of the following criteria: (1) patient request; (2) serious illness during the study; (3) severe nasal symptoms score with T5SS > 10 without improvement by rescue medicine; (4) pregnancy (**Supplementary Table S2**).

We used an electronic software, REDCap (Vanderbilt University, Tennessee, USA), to manage clinical data. Each study site ensured the accuracy and completeness of the data entered in clinical report forms and the data derived from source documents. All data were inputted in the same coding format. Clinical report forms were kept on the databases with backup files. All data were locked after finishing the data validation. The checklist of reporting adhered to CONsolidated Standards of Reporting Trials (CONSORT) was completed (http://www.consort-statement.org).

Outcome measures

Study participants self-assessed AR symptoms twice a day in the morning (assessing the instantaneous T5SS) and at bedtime (assessing the reflective T5SS). The primary outcome was the reflective total five symptoms score (rT5SS) which evaluated a sum of five AR symptoms of the past 24 hours. The total score was 15. The secondary outcomes were the instantaneous T5SS (iT5SS),

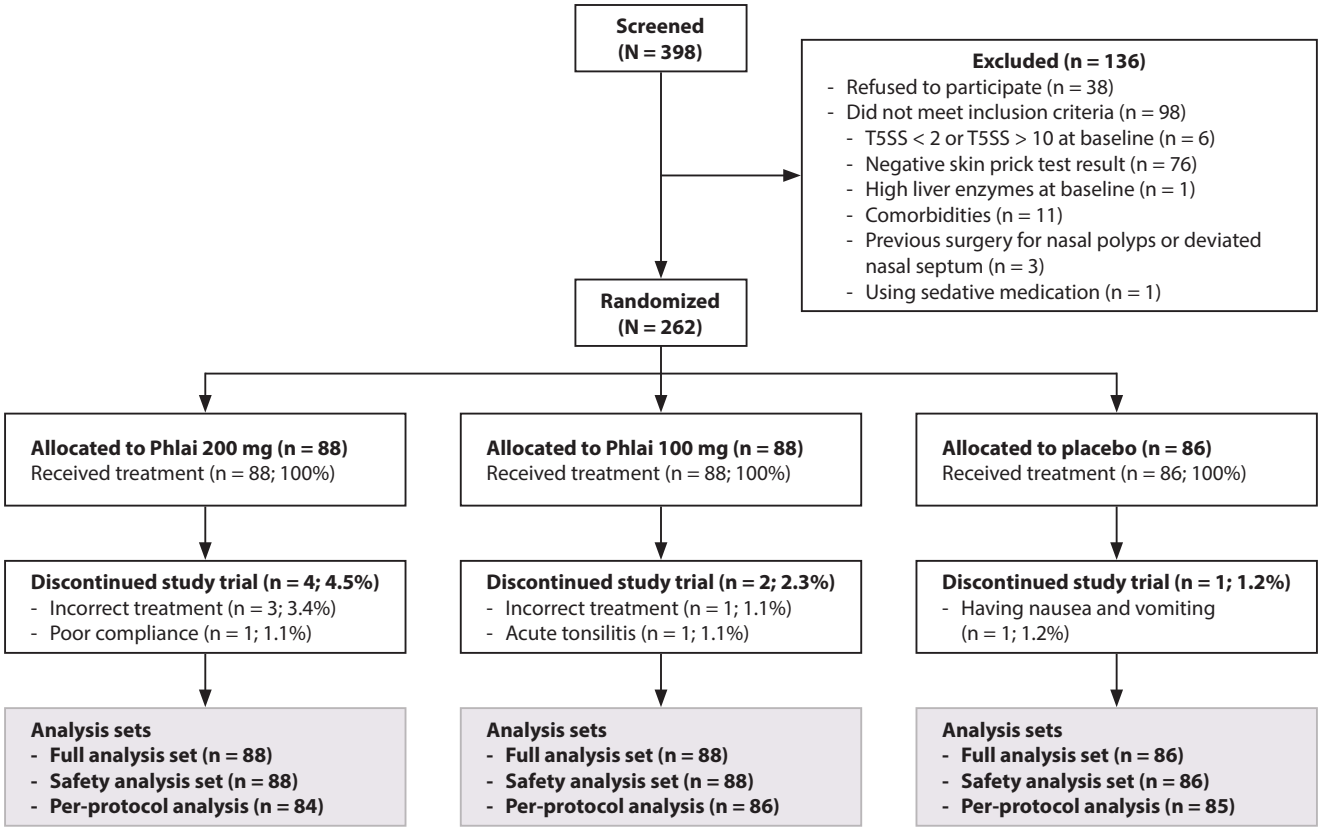

Figure 1. Study design.

reflective individual symptom scores, overall Rhinoconjunctivitis Quality of Life-36 Questionnaire (RCQ-36) score, peak nasal inspiratory flow (PNIF), safety, and compliance. The instantaneous T5SS was an on-spot evaluation. The individual AR symptoms were scored on a 4-point scale (0-3). RCQ-36 is a validated Thai version of RQLQ, comprising 36 items in six domains (symptoms, physical functioning, role limitations, sleep, social functioning, emotions) and two independent items (general health and absenteeism).[20] The score of each item ranged from 1 to 5 (lower is better). The RCQ-36 assessment was performed over the screening period and four weeks after treatment. PNIF meter (Clement Clarke International Ltd, Harlow, UK) was measured three times, and the highest value among three attempts was recorded. Outcome assessors were blinded to the treatment arm when analyzing patient-reported outcomes.

Adverse events were recorded daily. Complete blood counts (CBC), liver functions [aspartate transaminase (AST), alanine transaminase (ALT)] and renal functions [blood urea nitrogen (BUN), and creatinine levels] were measured over periods of screening and four weeks. Study participants recorded the recuse medicine use, concomitant drugs, and remaining capsules for calculating compliance during each week.

Statistical analysis

Sample size assumptions were based on the data from the previous study.[21] T5SS decreased by 1.14 for the active arm versus placebo after 4 weeks of study; the combined standard deviation was 2.5.[21] Enrolling 74 participants (a total of 222 for three arms) would give 80% power to detect this difference in either of the active groups versus the placebo, at a 2-sided significance level of 5%.

All statistical analyses were conducted using Stata 17.0 (StataCorp, College Station, TX). Descriptive data are displayed as mean and standard deviation (SD) for continuous variables and n (%) for categorical variables. Repeated measures data (T5SS, T5SS component symptom scores, PNIF, RCQ-36) were analyzed using a mixed-effects model. The model included treatment group interacted with study week, baseline scores, sex, body mass index (BMI; < 30 vs. ≥ 30), AR symptom severity and duration (ARIA guideline),[16] recruitment season, and a random intercept for subjects. Baseline scores were defined as those on the day before undertaking the intervention. Changes from baseline outcome measures were calculated in the intention-to-treat (ITT) population that included all randomized participants, with missing data were handled by the mixed model under the missing at random assumption. A per-protocol analysis was also conducted, excluding data from participants with protocol violations. Time-to-response analysis was performed using Kaplan-Meier estimates. A patient with a reduction of T5SS from baseline > 1 point was defined as a responder.[21] Minimal clinically important differences (MCID) for T5SS and individual symptoms scores have not been determined. Therefore, we followed the recommendation of Meltzer et al. suggesting any difference between treatment groups ≥ 0.2 times the baseline SD is clinically significant (**Supplementary Table S3**).[22] Changes in overall RCQ-36 score ≥ 0.21 were considered clinically meaningful.[23] The estimated MCID for PNIF in AR was 5 L/min.[22]

Results

Three hundred and ninety-eight patients were screened from February 2020 to November 2021. A total of 262 patients were randomized, 88 to Phlai capsule 200 mg, 88 to Phlai capsule 100 mg, and 86 to placebo (**Figure 2**).

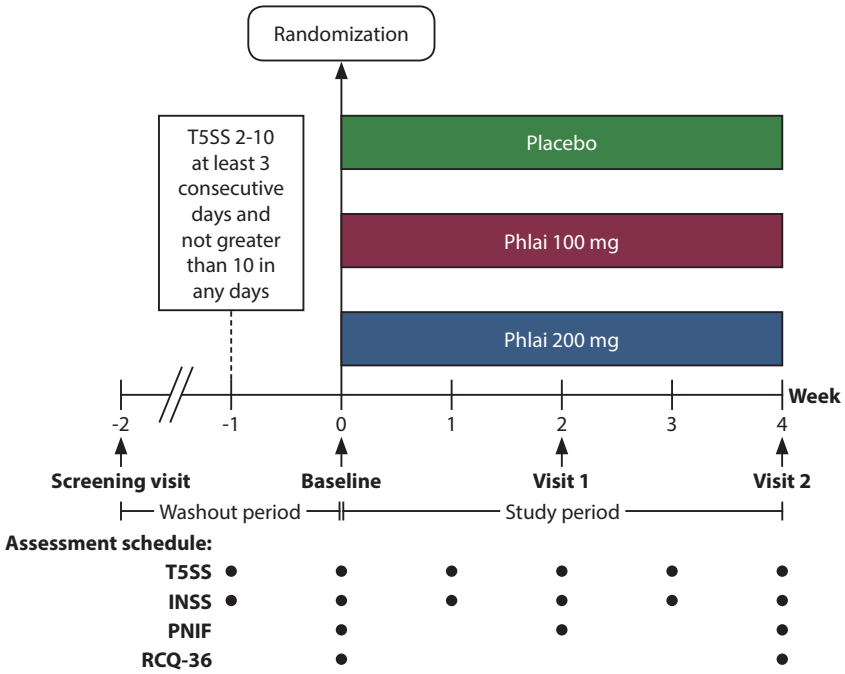

Figure 2. Patient disposition and trial profile.

Table 1. Demographics, baseline characteristics in the three groups.

	Phlai 200 mg (n = 88)	Phlai 100 mg (n = 88)	Placebo (n = 86)	Overall population (n = 262)
Female, n (%)	63 (71.6)	69 (78.4)	55 (64.0)	187 (71.4)
Age (year), mean (SD)	30.2 (7.7)	32.4 (9.0)	31.8 (7.5)	31.3 (8.1)
BMI, mean (SD)	23.5 (4.5)	23.8 (4.0)	23.7 (4.3)	23.6 (4.3)
Characteristics of AR condition, n (%)				
Intermittent	31 (35.2)	32 (36.4)	31 (36.1)	94 (35.9)
Persistent	57 (64.8)	56 (63.6)	55 (63.9)	168 (64.1)
Mild	49 (55.7)	50 (56.8)	50 (58.1)	149 (56.9)
Moderate to severe	39 (44.3)	38 (43.2)	36 (41.9)	113 (43.1)
Season recruitment, n (%)				
Winter	28 (31.8)	22 (25.0)	27 (31.4)	77 (29.4)
Rainy season	46 (52.3)	52 (59.1)	52 (60.5)	150 (57.2)
Summer	14 (15.9)	14 (15.9)	7 (8.1)	35 (13.4)
Allergens, n (%)				
Der p.	82 (93.2)	80 (90.9)	74 (86.1)	236 (90.1)
Der f.	80 (90.9)	75 (85.2)	70 (81.4)	225 (85.6)
Dog	16 (18.2)	16 (18.2)	9 (10.5)	41 (15.7)
Cat	10 (11.4)	6 (6.8)	11 (12.8)	27 (10.3)
Cockroach	31(35.2)	52 (59.0)	34 (39.5)	117 (44.7)
Para Grass	11 (12.5)	5 (5.7)	9 (10.5)	25 (9.5)
Careless Weed	11 (12.5)	6 (6.8)	5 (5.8)	22 (8.4)
Baseline scores, mean (SD)				
T5SS	5.01 (2.35)	5.01 (2.39)	4.74 (2.09)	4.92 (2.28)
Rhinorrhea	1.19 (0.87)	1.09 (0.87)	1.05 (0.84)	1.11 (0.86)
Nasal obstruction	1.16 (0.83)	1.10 (0.86)	1.15 (0.76)	1.14 (0.81)
Itchy nose	0.86 (0.76)	1.07 (0.83)	0.88 (0.74)	0.94 (0.78)
Sneezing	0.95 (0.76)	1.01 (0.73)	0.93 (0.73)	0.97 (0.74)
Itchy eyes	0.84 (0.81)	0.74 (0.81)	0.73 (0.86)	0.77 (0.83)
Overall RCQ-36	2.06 (0.58)	1.93 (0.47)	1.95 (0.56)	1.98 (0.54)
PNIF (L/min)	111.59 (37.78)	113.98 (40.12)	117.10 (43.18)	114.20 (40.30)
Anterior rhinoscopy, n (%)				
Nasal swelling	63 (71.6)	57 (64.8)	61 (70.9)	181 (69.1)
Pale	27 (30.7)	37 (42.1)	31 (36.1)	95 (36.3)
Nasal discharge	16 (18.2)	16 (18.2)	26 (30.2)	58 (22.1)

Footnote: AR, allergic rhinitis; BMI, body mass index; T5SS, total five symptoms score; RCQ-36, The Rhinoconjuntivitis Quality of Life Questionnaire; PNIF, peak nasal inspiratory flow; SD, standard deviation; Der.p, *Dermatophagoides pteronyssinus*; Der f.; *Dermatophagoides farinae*.

The baseline demographics and clinical characteristics were comparable across randomized groups (**Table 1**). Females comprised 70.4% of study participants. Persistent AR accounted for 64.1% of study participants. Forty-four percent of participants had moderate to severe AR symptoms. The majority (97.3%) of study participants completed the study. Patients who were lost to follow-up were 4.5%, 2.3%, and 1.2% in Phlai capsule 200 mg, Phlai capsule 100 mg, and placebo groups, respectively.

Reflective total five symptom score (rT5SS)

Adjusted changes in rT5SS over the study period are shown in **Table 2** and **Figure 3**. Compared to placebo, there was a statistically significant improvement in rT5SS after four weeks [adjusted mean difference (aMD) -0.62; 95%CI -1.22, -0.03; p = 0.039] in the group receiving Phlai capsule 100 mg. In the group receiving Phlai capsule 200 mg, rT5SS also improved compared to placebo with a change of similar magnitude (aMD -0.49; 95%CI -1.09, 0.10; p = 0.17).

Table 2. Intention-to-treat analysis of adjusted mean change from baseline to week 4 of patient-reported symptoms and objective assessments parameters.

Outcome	Group	Adjusted mean change from baseline at week 4 (95% CI)	Difference between groups			
			vs Placebo (95% CI)	*p* value	Phlai 200 vs 100 mg (95% CI)	*p* value
rT5SS (Scale 0-15)	Phlai 200 mg	-2.39 (-2.81, -1.97)	-0.49 (-1.09, 0.10)	0.17	0.13 (-0.46, 0.73)	0.66
	Phlai 100 mg	-2.53 (-2.95, -2.11)	-0.62 (-1.22, -0.03)	0.039		
	Placebo	-1.90 (-2.32, -1.48)				
iT5SS (Scale 0-15)	Phlai 200 mg	-2.12 (-2.53, -1.71)	-0.32 (-0.89, 0.26)	0.28	0.25 (-0.32, 0.82)	0.39
	Phlai 100 mg	-2.37 (-2.77, -1.97)	-0.57 (-1.14, 0.00)	0.05		
	Placebo	-1.80 (-2.21, -1.39)				
Rhinorrhea (Scale 0-3)	Phlai 200 mg	-0.53 (-0.66, -0.40)	-0.27 (-0.46, -0.09)	0.004	-0.09 (-0.27, 0.10)	0.36
	Phlai 100 mg	-0.44 (-0.57, -0.31)	-0.19 (-0.37, 0.00)	0.048		
	Placebo	-0.26 (-0.39, -0.12)				
Nasal obstruction (Scale 0-3)	Phlai 200 mg	-0.42 (-0.56, -0.28)	0.07 (-0.13, 0.26)	0.50	-0.04 (-0.23, 0.16)	0.69
	Phlai 100 mg	-0.38 (-0.52, -0.25)	0.11 (-0.09, 0.30)	0.28		
	Placebo	-0.49 (-0.63, -0.35)				
Itchy nose (Scale 0-3)	Phlai 200 mg	-0.46 (-0.59, -0.33)	-0.07 (-0.26, 0.11)	0.44	0.17 (-0.02, 0.35)	0.08
	Phlai 100 mg	-0.63 (-0.76, -0.50)	-0.24 (-0.43, -0.05)	0.011		
	Placebo	-0.39 (-0.52, -0.25)				
Sneezing (Scale 0-3)	Phlai 200 mg	-0.52 (-0.64, -0.39)	-0.09 (-0.26, 0.09)	0.33	0.03 (-0.15, 0.20)	0.75
	Phlai 100 mg	-0.54 (-0.67, -0.42)	-0.12 (-0.29, 0.06)	0.20		
	Placebo	-0.43 (-0.55, -0.30)				
Itchy eyes (Scale 0-3)	Phlai 200 mg	-0.47 (-0.59, -0.35)	-0.12 (-0.30, 0.05)	0.16	0.06 (-0.11, 0.24)	0.47
	Phlai 100 mg	-0.53 (-0.65, -0.41)	-0.19 (-0.36, -0.02)	0.033		
	Placebo	-0.34 (-0.47, -0.22)				
Overall RCQ-36 (Scale 1-5)	Phlai 200 mg	-0.38 (-0.47, -0.30)	-0.03 (-0.15, 0.09)	0.60	0.01 (-0.11, 0.13)	0.85
	Phlai 100 mg	-0.40 (-0.48, -0.31)	-0.04 (-0.16, 0.08)	0.48		
	Placebo	-0.35 (-0.44, -0.27)				
PNIF (L/min)	Phlai 200 mg	4.10 (-1.22, 9.43)	-0.40 (-7.95, 7.14)	0.92	0.59 (-6.91, 8.09)	0.88
	Phlai 100 mg	3.51 (-1.77, 8.80)	-0.99 (-8.51, 6.53)	0.70		
	Placebo	4.50 (-0.84, 9.85)				

Footnote: rT5SS, reflective total five symptoms score; iT5SS, instantaneous total five symptom score; RCQ-36, The Rhinoconjuntivitis Quality of Life Questionnaire; PNIF, peak nasal inspiratory flow; SD, standard deviation; CI, confidence interval.

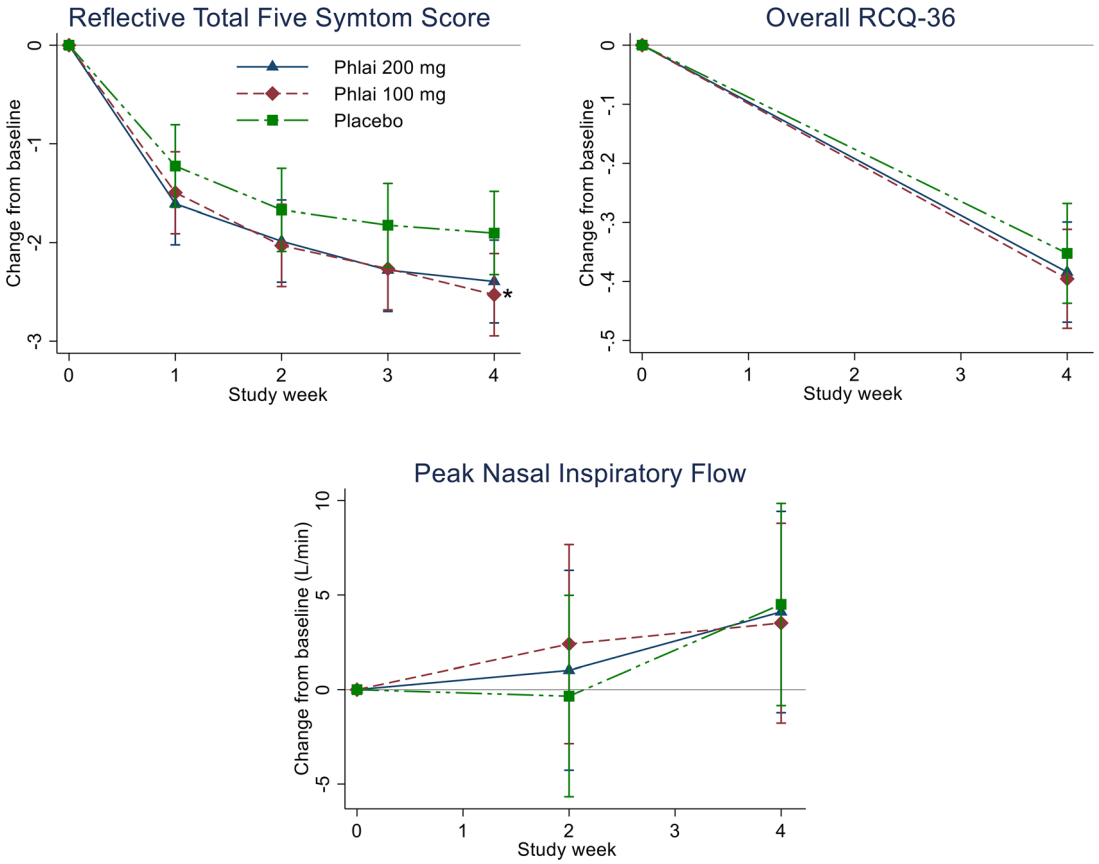

Figure 3. Adjusted changes from baseline to week 4 in reflective total five symptoms score, overall RCQ-36 score, and nasal peak inspiratory flow during the study period. Data are least-squares means ± 95%CI for the full analysis set. Total five symptoms score range, 0-15. Overall RCQ-36 range, 1-5. *$p < 0.05$ Phlai 100 mg versus Placebo.

Although the difference was not statistically significant, this change was greater than the MCID determined by the recommended method. There was no statistically significant additional benefit of high dose (200 mg) over the low dose (Phlai capsule 100 mg) over all follow-up (**Supplementary Figure S3**).

rTNSS responder analysis

More patients achieving a reduction of rT5SS from baseline > 1 point were observed in groups receiving Phlai capsule 100 mg (85.2%) and Phlai capsule 200 mg (81.4%) compared to placebo group (72.1%) at 4 weeks. The difference in responder rate between Phlai capsule 100 mg and placebo reached the statistical significance (p = 0.044) (**Supplementary Table S4 and Figure S4**).

Instantaneous total five symptom score (iT5SS)

Compared to placebo, a trend was observed toward a greater reduction of iT5SS after week 4 in Phlai capsule 100 mg group (aMD -0.57; 95%CI -1.14, 0; p = 0.05) (**Table 2**).

Reflective individual symptom scores

Compared to placebo, Phlai capsule 100 mg showed the statistically significant improvements in rhinorrhea (aMD -0.19; 95%CI -0.37, 0.002; p = 0.048), itchy nose (aMD -0.24; 95%CI -0.43, -0.05; p = 0.011), and itchy eyes (aMD -0.19; 95%CI -0.36, -0.02; p = 0.033) at 4 weeks. Improvement in itchy nose (aMD -0.2; 95%CI -0.39, -0.02; p = 0.03) and itchy eyes (aMD -0.18; 95%CI -0.35, -0.01; p = 0.04) in the Phlai capsule 100 mg group were significantly better than placebo from week 1 (**Table 2**, **Figure 4**). The Phlai capsule 100 mg changes in nasal obstruction and sneezing were not statistically significant over follow-up. In the Phlai capsule 200 mg group, rhinorrhea was the only symptom which showed a significant improvement versus placebo at 3 weeks (aMD -0.24; 95%CI -0.43, -0.05; p = 0.01) and 4 weeks (aMD -0.27; 95%CI -0.46, -0.09; p = 0.004) (**Table 2**, **Figure 4**).

RCQ-36

Improvements in overall RCQ-36 score were greater in the active treatment groups versus placebo, but the differences were not statistically significant. (**Table 2**, **Figure 3**).

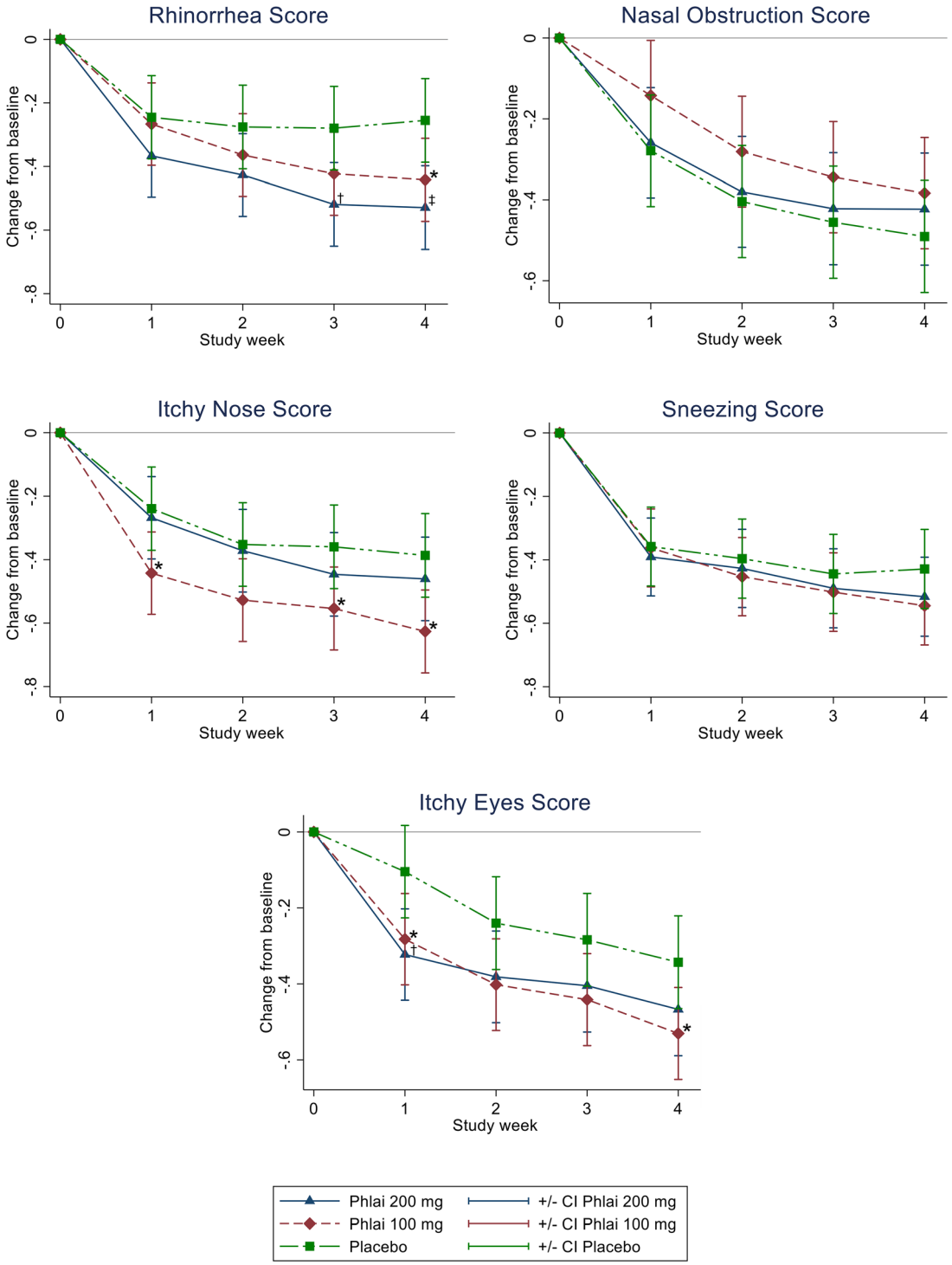

Figure 4. Adjusted changes from baseline to week 4 in individual reflective symptom scores (rhinorrhea, nasal obstruction, itchy nose, sneezing, itchy eyes). Data are least-squares means ± 95%CI for the full analysis set. Individual symptoms score range, 0-3. *$p < 0.05$ Phlai 100 mg versus Placebo; †$p < 0.05$ Phlai 200 mg versus Placebo, ‡$p < 0.01$ Phlai 200 mg versus Placebo.

PNIF

Participants in all groups had improvements in peak nasal inspiratory flow, but the improvements in the active treatment groups were not statistically better than placebo. (**Table 2**, **Figure 3**).

Safety and adverse events

During the study period, 48.9%, 55.7%, and 55.8% of patients in Phlai capsule 200 mg, Phlai capsule 100 mg, and placebo groups experienced ≥ 1 adverse event. The most common adverse events were sedation, dizziness, dry mouth/nose, and headache. The frequencies of common adverse events were not different among three groups. There was no significant change in the laboratory tests in any group (**Supplementary Tables S5-S6**). Rescue medicine usage was similar for all groups, with 5.6%, 6.8%, and 7% of participants in Phlai capsule 200 mg, Phlai capsule 100 mg, and placebo groups, respectively. Medication adherence to treatment was high over the study period, with of percentage of self-reported doses in Phlai capsule 200 mg, Phlai capsule 100 mg, and placebo groups of 93.2%, 95.5%, and 94.2%, respectively (**Supplementary Tables S7-S8**). Three patients (0.01%) took concomitant drugs: Phlai capsule 200 mg group, one patient took a decongestant, and another an antidepressant; Phlai capsule 100 mg group, one participant used an intranasal corticosteroid.

Per-protocol analysis

The per-protocol analysis results were similar to the ITT analysis, with most outcome measures in the active treatment groups showing greater symptom improvement and quality of life versus placebo groups (**Supplementary Table S9**; **Figures S5-S6**).

Subgroup analysis

Although there was some evidence of differential effect on rT5SS at week 4 based on different types of allergens present, BMI, severity of AR, duration of AR, and recruitment season, none of the interaction tests reached nominal significance (**Supplementary Figures S7-S8**).

Discussion

In this randomized controlled trial of Phlai extract in patients with AR, the 100 mg and 200 mg doses provided an additional reduction in our main outcome of rT5SS, relative to the placebo group. The reduction in rT5SS to week 4 was statistically significant, and although the high dose did not show a significant difference to placebo, the mean change in both active groups relative at week 4 was above the threshold for a clinically significant improvement.[22]

The anti-inflammatory and anti-allergic effects of Phlai extract are mediated by compound D.[5,6,8-10,12] In a molecular docking and dynamic simulation study, compound D bound to the 5-lipoxygenase (5-LO) enzyme at the same binding site as arachidonic acid and Zileuton.[24] Therefore, it is likely that anti-asthmatic effects of compound D are mediated by competitive inhibition with arachidonic acid at the 5-LO binding site.[24] Preclinical studies revealed anti-inflammatory, anti-histaminic activity,[9] smooth muscle relaxant.[6] Furthermore, Phlai suppressed inflammation and hypersensitiveness of airway epithelium in response to house dust mites.[25]

Apart from compound D, there are several other active compounds in Phlai.[26,27] The Phlai extract formulation used in our study differed from those from other literature. We used two doses of 100 and 200 mg equivalent to 0.1-1.0 mg/kg Compound D. These doses of Phlai standardized extract represented the current use as an anti-allergic agent in Thai traditional medicine.[12] The two doses of Phlai capsules of 100 and 200 mg had been investigated by a previous study for the suppressive effects on wheal response. They showed no significant difference compared with 10 mg of loratadine.[5] We, therefore, used the doses of Phlai of 100 and 200 mg because there has been evidence showing the equivalence in antihistaminic effects between Phlai and loratadine, a standard treatment for allergic rhinitis.[16] It should be noted that when the other study used a different extract formulation of Phlai for the assessment of the suppressive effects on wheal response, different findings were reported. Dried Phlai powder showed a less wheal size reduction than oral chlorpheniramine in skin test response.[28]

Approximately two-thirds of study participants receiving Phlai experienced reduction in AR symptoms from the first visit onwards, versus 50% of the controls (**Figure S2**). The findings from our study showed that the magnitude of additional benefit of high dose over the low dose did not achieve the significance over all follow-ups regarding responder rate and effect on reduction of rT5SS. Our findings were in line with the previous study showing no difference between the two doses in antihistaminic activity.[5] There is a need for compartment analysis in clinical studies to determine the pharmacokinetic parameters of Phlai extract. It was hypothesized that Phlai capsules used in our trial were partly biotransformed into other chemicals before providing maximum therapeutic effects at the sites of action.

The AR individual symptoms which improved in the active treatment groups over placebo were rhinorrhea, itching nose, and itching eyes reached statistical significance and MCID at one week (itching nose and itching eyes) and three weeks (rhinorrhea) and maintained thereafter. These symptoms are related to early phase allergic hypersensitivity[16] which is consistent with the findings of a previous study showing inhibitory effect of compound D during the early phase on wheel and flare response to histamine and house dust mite.[5] Another other study by Limvuttegrijerat et al.[29] showed that a human pulmonary mucoepidermoid cell line significantly decreased phorbol12-myristate 13-acetate (PMA)-induced mucin (MUC2 and MUC5AC) production and gene expression when pretreated with Phlai for two hours. These latter studies provide some evidence on the mechanism by which Phlai suppresses hypersecretion of mucus and improved rhinorrhea symptoms.

In contrast, our study did not demonstrate beneficial effects of Phlai on the late phase hypersensitivity response. Nasal obstruction improvements in the treatment groups were not different from the placebo group. Over two-thirds of participants in this study had persistent AR and hypertrophic inferior turbinate (**Table 1**) that could bring refractoriness to treatment. In addition, disease-specific quality of life in the treatment groups was not significantly different from the placebo group. Phlai improved nasal obstruction and disease-specific quality of life with greater reduction than the MCIDs when compared to baseline, but the comparison versus placebo was not statistically significant. Placebo arms in previously published randomized controlled trials of allergic diseases typically have a strong effect when evaluated by subjective rather than objective biochemical parameters.[30] In addition, when the questionnaire data of Israeli nurses' AR symptomatology while wearing face masks during the COVID-19 pandemic were evaluated, a decrease in symptom severity with mask usage was revealed when compared with no mask.[31] Since this study was conducted during the COVID-19 pandemic, and wearing masks was a habit for all study participants, face mask usage possibly minimized exposure of the respiratory system to provocative allergens and reduced allergic rhinitis symptom severity in all participants including the placebo group.

Our study found that Phlai was safe with no moderate or serious adverse effects, which was in line with the safety profile of 12-week consumption of Phlai capsules in healthy adult volunteers.[32] Based on the findings of this study, we suggest Phlai can be as considered as an alternative treatment for patients with AR who suffer from rhinorrhea, itching nose, and itching eyes. Further randomized controlled trials which compare Phlai with standard treatments are required to determine its role for the step-up and the step-down approach in the clinical practice guideline.

This study has limitations in several aspects. First, the lower initial rT5SS score in the placebo group may make it harder to reach statistical difference among group after the treatment. Second, face mask usage by study participants may have confounded the study results by reducing exposure to airborne allergens. Third, biochemical parameters such as cytokine levels were not measured, and these would have provided more robust evidence of a therapeutic effect.

Conclusion

Phlai capsules improved reflective total five symptom score, rhinorrhea, itchy nose, and itchy eyes after four weeks. However, nasal obstruction, sneezing, overall RCQ-36, and PNIF did not reach a statistically significant difference compared to the placebo control group. Further randomized controlled studies are warranted to investigate the long-term effects of Phlai capsules.

Declaration of competing interest

- Kornkiat Snidvongs received Honoraria for speaking at symposia from Organon, Mylan, and Menarini.
- Minh P Hoang, Kachorn Seresirikachorn, Wirach Chitsuthipakorn, Jompol Samuthpongtorn, Kajohnkiat Prasittivatechakool, Pongsakorn Tantilipikorn, Orapan Poachanukoon, Pornthep Kasemsiri, Virat Kirtsreesakul, Jesada Kanjanaumporn, Songklot Aeumjaturapat, and Supinda Chusakul declare that they have no conflict of interest.

Authorship contribution

- Minh P Hoang: study design, data collection, data analysis, drafting the article, final approval
- Kachorn Seresirikachorn: data collection, editing the article
- Wirach Chitsuthipakorn: data collection, editing the article
- Jompol Samuthpongtorn: data collection
- Kajohnkiat Prasittivatechakool: data collection, editing the article
- Pongsakorn Tantilipikorn: data collection, editing the article
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- Virat Kirtsreesakul: data collection, editing the article
- Jesada Kanjanaumporn: data collection, editing the article
- Songklot Aeumjaturapat: data collection, editing the article
- Supinda Chusakul: data collection, editing the article
- Kornkiat Snidvongs: conception, study design, drafting the article, final approval

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Availability of data

The data in this study are available to other researchers upon request.

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Supplemental materials

Table S1. Quality of control data of Phlai capsule.

Test Parameter	Requirement	Result
Description	Hard capsule (plant origin - HPMC) no.0, yellow opaque cap and body, containing light yellowish-brown powder.	Pass
Identification	HPTLC fingerprinting The sample preparation showed carmine-red band due to (E)-4-(3',4'-dimethoxyphenyl) but-3-en-1-ol (compound D) corresponding in position and color to those obtained from standard preparation and it showed major bands at Rf values about 0.12, 0.18, 0.24, 0.32, and 0.38 respectively HPLC fingerprinting HPLC chromatogram of sample preparation showed (E)-4-(3',4'-dimethoxyphenyl) but-3-en-1-ol corresponding to that obtained from standard preparation and its showed cyclohexene derivatives peaks at relative retention times about 1.7, 4.6, 5.1 and 5.3, respectively, relative to 1.0 for (E)-4-(3',4'-dimethoxyphenyl) but-3-en-1-ol	Pass Pass
Uniformity of weight (mass)	±7.5% deviation of the average weight	Pass
Disintegration time	≤ 30 minutes	6 minutes
Loss of drying	≤ 5.0 % w/w	1.0% w/w
Content of (E)-4-(3',4'-dimethoxyphenyl) but-3-en-1-ol	90.0 -110.0 % of the labeled amount of (E)-4-(3',4'-dimethoxyphenyl) but-3-en-1-ol	97.1% L.A.

Table S2. Instruction for medications used during the study period.

Medications not allowed to use during the study period
• Systemic, inhaled, INCSs • Oral, intranasal antihistamines • Oral, topical decongestants • Oral antileukotrienes • Antidepressants, sedative, anxiolytic, opioids, neuroleptic • Intranasal cromolyn
Medications allowed for use during the study period
• Saline nasal irrigation (in case of severe worsening of symptoms linked to A.R. [T5SS >10]) • Acetaminophen • Nonsteroidal anti-inflammatory drugs
The process when encountering patients' acute upper respiratory tract infections during the study period
• Contact investigator team for clinical evaluation and record the event • Trial medication use still following the protocol • Allowed symptomatic treatments, including saline nasal irrigation, acetaminophen, and antibiotics (when have the signs/evidence of infection)

Abbreviation: INCS, intranasal corticosteroid; AR, allergic rhinitis, T5SS, total five symptoms score

Table S3. Minimal clinically important differences of T5SS and individual symptom scores.

Outcomes	MCID*
T5SS	0.46
Rhinorrhea	0.17
Nasal congestion	0.16
Itchy nose	0.16
Sneezing	0.15
Itchy eyes	0.17

*MCID: difference between treatment groups ≥ 0.2 times the baseline S.D. is clinically significant. T5SS, total five symptoms score; MCID, minimum clinically important difference

Table S4. Time advance of Phlai: treatment week at which responder rates were achieved.

Reduction of rT5SS from baseline >1 point			
Responder rate	**Phlai 200 mg**	**Phlai 100 mg**	**Placebo**
25%	1	1	1
50%	1	1	1
75%	3	2	-

Abbreviation: rT5SS, reflective total five symptom score

Table S5. Adverse events from treatment.

	Phlai 200 mg (n = 88)	Phlai 100 mg (n = 88)	Placebo (n = 86)
All adverse event n (%)			
Any-on treatment event	43 (48.9)	50 (56.8)	48 (55.8)
Grade 1	42 (47.8)	49 (55.7)	47 (54.7)
Grade 2*	1 (1.1)	1 (1.1)	1 (1.2)
Leading to study withdrawal	0 (0.0)	1 (1.1)	1 (1.2)
Most common adverse events n (%)**			
Sedation	23 (26.1)	19 (21.6)	28 (32.6)
Dizziness	11 (12.5)	10 (11.4)	8 (9.3)
Dry mouth/nose	20 (22.7)	28 (31.8)	27 (31.4)
Headache	6 (6.8)	10 (11.4)	6 (7.0)

*No grade 3 or 4 events were experienced by participants.
** Reported in 5% or more patients in any treatment group

Table S6. Changes in completed blood test, liver function, renal function tests: from baseline to end of treatment.

		Phlai 200 mg (n = 88)	Phlai 100 mg (n = 88)	Placebo (n = 86)
RBC (10^6/μL)	Mean (95%CI)	0.01 (-0.03, 0.05)	0.02 (-0.03, 0.06)	0.02 (-0.02, 0.07)
	p value	0.57	0.49	0.26
Hemoglobin (g/dL)	Mean (95%CI)	0.01 (-0.11, 0.12)	0.04 (-0.08, 0.15)	0.08 (-0.03, 0.2)
	p value	0.93	0.52	0.16
WBC (10^3/μL)	Mean (95%CI)	-0.2 (-0.57, 0.17)	0.15 (-0.22, 0.52)	0.08 (-0.29, 0.46)
	p value	0.29	0.42	0.66
Lymphocytes (%)	Mean (95%CI)	0.25 (-1.18, 1.69)	-0.27 (-1.69, 1.15)	0.35 (-1.09, 1.8)
	p value	0.73	0.71	0.63
Eosinophils (%)	Mean (95%CI)	-0.23 (-1.84, 1.38)	0.26 (-1.33, 1.85)	-0.24 (-1.86, 1.37)
	p value	0.78	0.75	0.77
Platelets (10^3/μL)	Mean (95%CI)	0.10 (-0.17, 0.37)	-0.11 (-0.38, 0.16)	-0.13 (-0.4, 0.15)
	p value	0.47	0.41	0.37
AST (U/L)	Mean (95%CI)	-0.03 (-0.09, 0.03)	-0.02 (-0.07, 0.04)	-0.02 (-0.08, 0.04)
	p value	0.29	0.62	0.59
ALT (U/L)	Mean (95%CI)	-0.01 (-0.3, 0.29)	0.14 (-0.15, 0.43)	0.04 (-0.26, 0.33)
	p value	0.95	0.36	0.81
Creatinine (mg/dL)	Mean (95%CI)	-4.27 (-9.68, 1.13)	-1.15 (-6.5, 4.19)	-3.60 (-9.04, 1.84)
	p value	0.12	0.67	0.20
BUN (mg/dL)	Mean (95%CI)	0.09 (-1.37, 1.56)	-0.75 (-2.20, 0.71)	0.14 (-1.34, 1.62)
	p value	0.90	0.31	0.85

Abbreviation: RBC, red blood cell; WBC, white blood cell; AST, aspartate transaminase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.

Table S7. Summary of rescue medicine use to randomized therapy: by week and randomized arm (intention-to-treat analysis).

Study week	Rescue Medicine	N (%)		
		Phlai 200 mg (n = 88)	Phlai 100 mg (n = 88)	Placebo (n = 86)
Week 1	Yes	1 (1.1)	2 (2.3)	1 (1.2)
	No	87 (98.9)	86 (97.7)	85 (98.8)
Week 2	Yes	3 (3.4)	4 (4.5)	2 (2.3)
	No	85 (96.6)	84 (95.5)	84 (97.7)
Week 3	Yes	3 (3.4)	2 (2.3)	3 (3.5)
	No	85 (96.6)	86 (97.7)	83 (96.5)
Week 4	Yes	4 (4.5)	3 (3.4)	2 (2.3)
	No	84 (95.5)	85 (96.6)	84 (97.7)

Table S8. Summary of adherence to randomized therapy: by week and randomized arm (intention-to-treat analysis).

Study week	Rescue Medicine	N (%)		
		Phlai 200 mg (n = 88)	Phlai 100 mg (n = 88)	Placebo (n = 86)
Week 1	≥ 80%	86 (97.7)	86 (97.7)	85 (98.8)
	< 80%	2 (2.3)	2 (2.3)	1 (1.2)
Week 2	≥ 80%	84 (95.5)	87 (98.9)	82 (95.4)
	< 80%	4 (4.5)	1 (1.1)	4 (4.6)
Week 3	≥ 80%	83 (94.3)	87 (97.7)	84 (97.7)
	< 80%	5 (5.7)	2 (2.3)	2 (2.3)
Week 4	≥ 80%	83 (94.3)	84 (95.5)	85 (98.8)
	< 80%	5 (5.7)	4 (4.5)	1 (1.2)

Table S9. Per-protocol analysis of adjusted mean change from baseline of patients-reported symptoms and objective assessments parameters at week 4.

Outcome	Group	Adjusted mean change from baseline at week 4 (95% CI)	Difference between groups			
			vs Placebo (95% CI)	*p* value	Phlai 200 vs 100 mg (95% CI)	*p* value
rT5SS (Scale 0-15)	Phlai 200 mg	-2.42 (-2.84, -1.99)	-0.52 (-1.12, 0.08)	0.09	0.11 (-0.49, 0.70)	0.72
	Phlai 100 mg	-2.53 (-2.95, -2.11)	-0.63 (-1.22, -0.03)	0.039		
	Placebo	-1.90 (-2.32, -1.48)				
iT5SS (Scale 0-15)	Phlai 200 mg	-2.14 (-2.55, -1.73)	-0.34 (-0.92, 0.23)	0.28	0.22 (-0.35, 0.80)	0.45
	Phlai 100 mg	-2.37 (-2.77, -1.96)	-0.57 (-1.14, 0.01)	0.05		
	Placebo	-1.80 (-2.21, -1.39)				
Rhinorrhea (Scale 0-3)	Phlai 200 mg	-0.54 (-0.67, -0.41)	-0.29 (-0.47, -0.10)	0.003	-0.09 (-0.28, 0.09)	0.34
	Phlai 100 mg	-0.45 (-0.58, -0.32)	-0.19 (-0.38, -0.01)	0.040		
	Placebo	-0.25 (-0.39, -0.12)				

Table S9. Per-protocol analysis of adjusted mean change from baseline of patients-reported symptoms and objective assessments parameters at week 4.

Outcome	Group	Adjusted mean change from baseline at week 4 (95% CI)	Difference between groups			
			vs Placebo (95% CI)	*p* value	Phlai 200 vs 100 mg (95% CI)	*p* value
Nasal obstruction (Scale 0-3)	Phlai 200 mg	-0.42 (-0.56, -0.28)	0.07 (-0.12, 0.27)	0.47	-0.04 (-0.23, 0.16)	0.72
	Phlai 100 mg	-0.38 (-0.52, -0.25)	0.11 (-0.09, 0.30)	0.28		
	Placebo	-0.49 (-0.63, -0.35)				
Itchy nose (Scale 0-3)	Phlai 200 mg	-0.47 (-0.60, -0.33)	-0.09 (-0.28, 0.10)	0.34	0.16 (-0.03, 0.34)	0.10
	Phlai 100 mg	-0.62 (-0.75, -0.49)	-0.25 (-0.43, -0.06)	0.009		
	Placebo	-0.38 (-0.51, -0.24)				
Sneezing (Scale 0-3)	Phlai 200 mg	-0.52 (-0.65, -0.40)	-0.09 (-0.27, 0.09)	0.32	0.02 (-0.16, 0.19)	0.84
	Phlai 100 mg	-0.54 (-0.66, -0.42)	-0.11 (-0.28, 0.07)	0.23		
	Placebo	-0.43 (-0.56, -0.31)				
Itchy eyes (Scale 0-3)	Phlai 200 mg	-0.47 (-0.59, -0.34)	-0.12 (-0.30, 0.05)	0.16	0.06 (-0.11, 0.23)	0.48
	Phlai 100 mg	-0.53 (-0.65, -0.41)	-0.18 (-0.36, -0.01)	0.033		
	Placebo	-0.34 (-0.47, -0.22)				
Overall RCQ-36 (Scale 1-5)	Phlai 200 mg	-0.38 (-0.47, -0.29)	-0.03 (-0.15, 0.09)	0.63	0.01 (-0.11, 0.13)	0.82
	Phlai 100 mg	-0.39 (-0.48, -0.31)	-0.04 (-0.16, 0.08)	0.47		
	Placebo	-0.35 (-0.44, -0.27)				
PNIF (L/min)	Phlai 200 mg	3.87 (-1.50, 9.24)	-0.62 (-8.20, 6.97)	0.87	0.32 (-7.22, 7.87)	0.93
	Phlai 100 mg	3.55 (-1.76, 8.85)	-0.94 (-8.48, 6.60)	0.81		
	Placebo	4.49 (-0.87, 9.84)				

Abbreviations: rT5SS, reflective total five symptoms score; iT5SS, instantaneous total five symptom score, RCQ-36, The Rhinoconjuntivitis Quality of Life Questionnaire; PNIF, peak nasal inspiratory flow; S.D., standard deviation; CI, confidence interval.

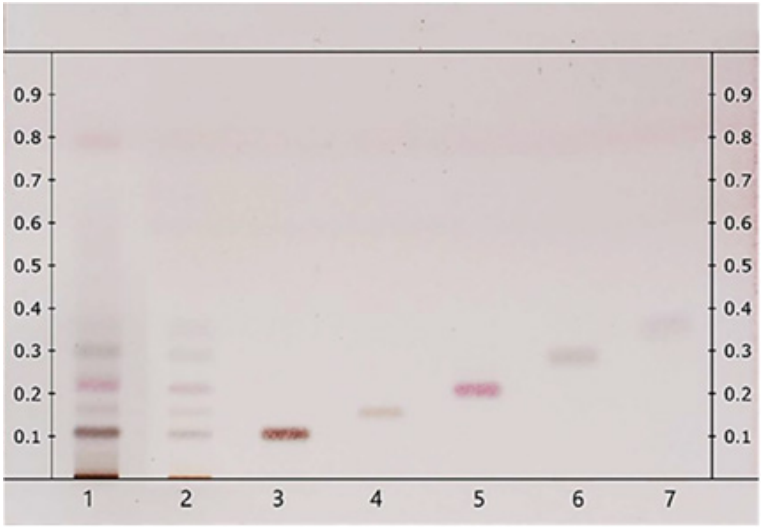

Figure S1. HPTLC fingerprinting of the sample preparation and standard preparation in daylight after spraying with p-Anisaldehyde-Sulfuric acid reagent. 1 = Phlai extract capsules sample solution; 2 = Mixture standard solution; 3 = Compound D standard solution; 4 = Compound C standard solution; 5 = Compound C′ standard solution; 6 = Compound B standard solution; 7 = Compound B′ standard solution.

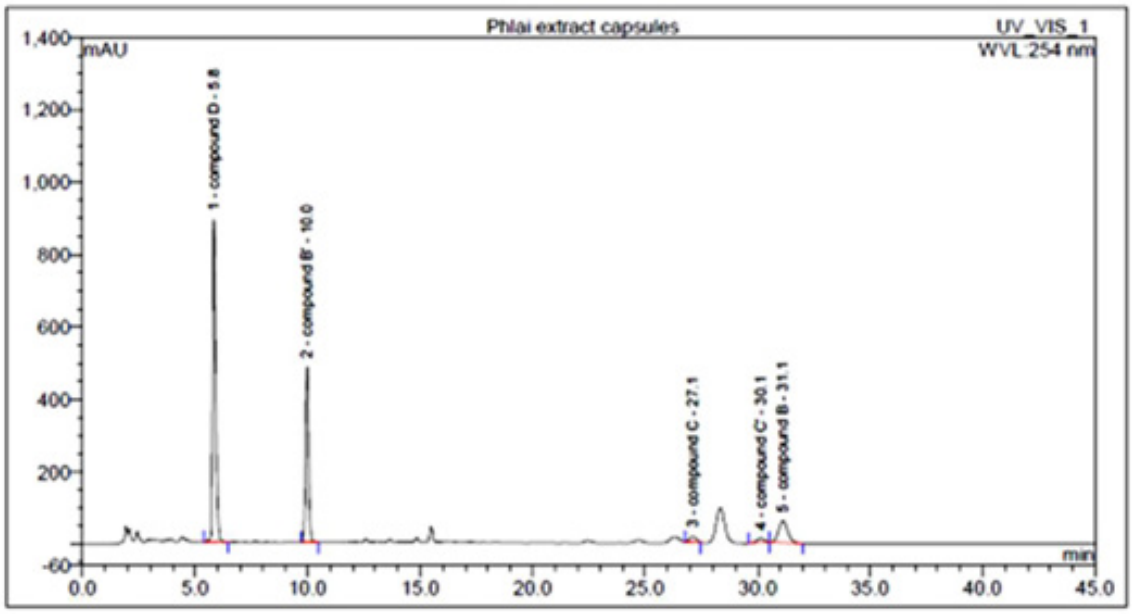

Figure S2. HPLC fingerprinting of the sample preparation.

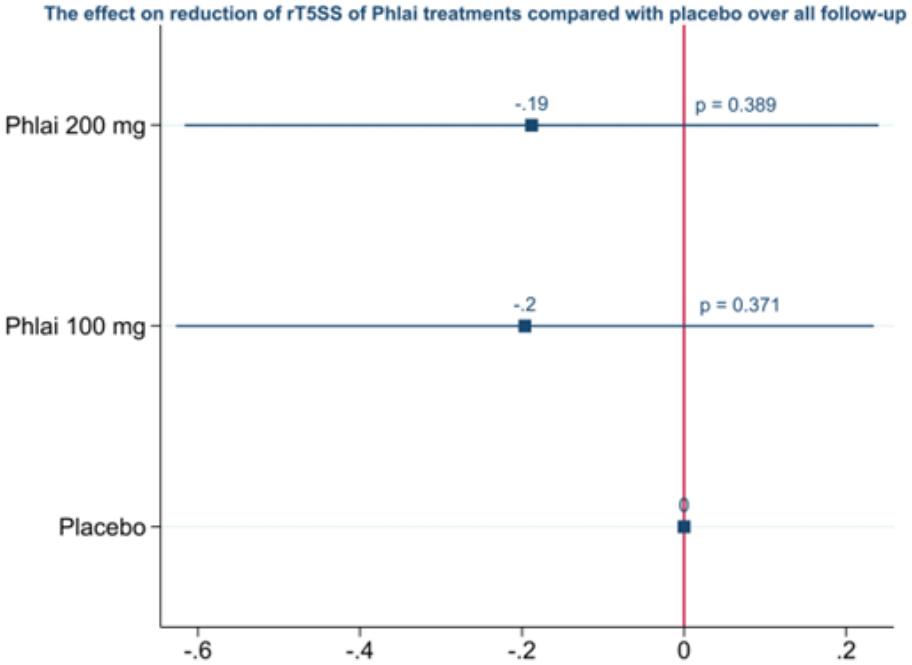

Figure S3. The effect on reduction of rT5SS of active treatments compared with placebo over all follow-up.

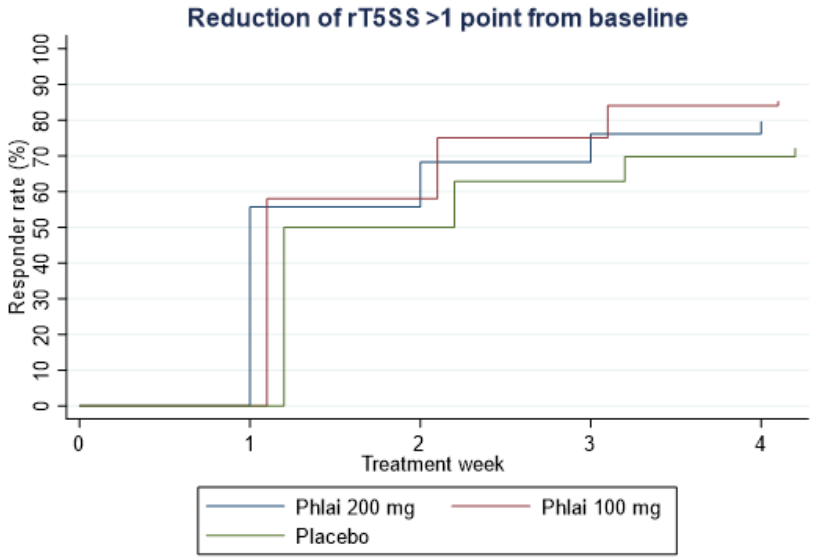

Figure S4. Time-response curves showing the percentage of patients exhibiting reduction of rT5SS from baseline > 1 point by treatment week after treatment with Phlai 200 mg (n = 88), Phlai 100 mg (n = 88), and placebo (n = 86). Data are presented as mean proportion of participants. Phai 200 mg vs Phlai 100 mg: p = 0.34; Phlai 200 mg vs placebo: p = 0.28; Phlai 100 mg vs placebo: p = 0.044.

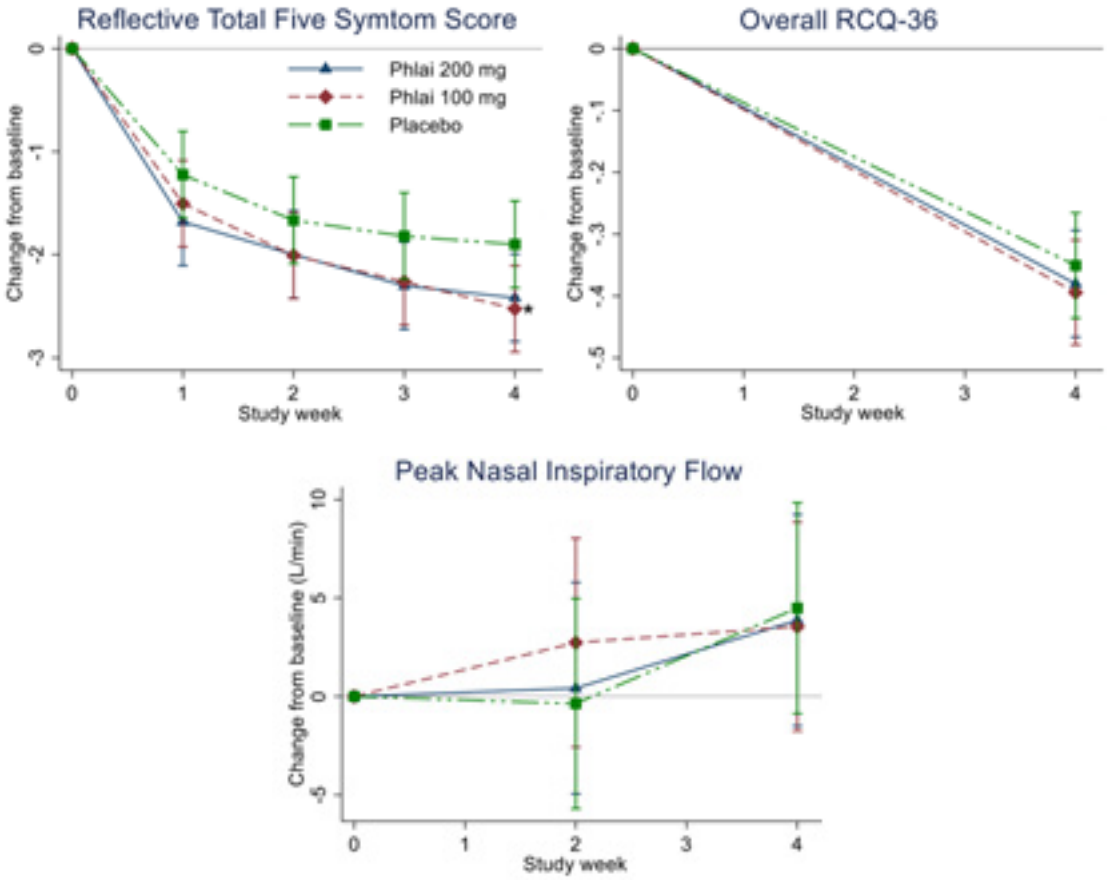

Figure S5. Adjusted changes from baseline to week 4 in reflective total five symptoms score, overall RCQ-36, and nasal peak inspiratory flow. Data are least-squares means ± 95%CI for the per-protocol set. Total five symptoms score range, 0-15. Overall RCQ-36 range, 1-5. *p < 0.05 Phlai 100 mg versus Placebo.

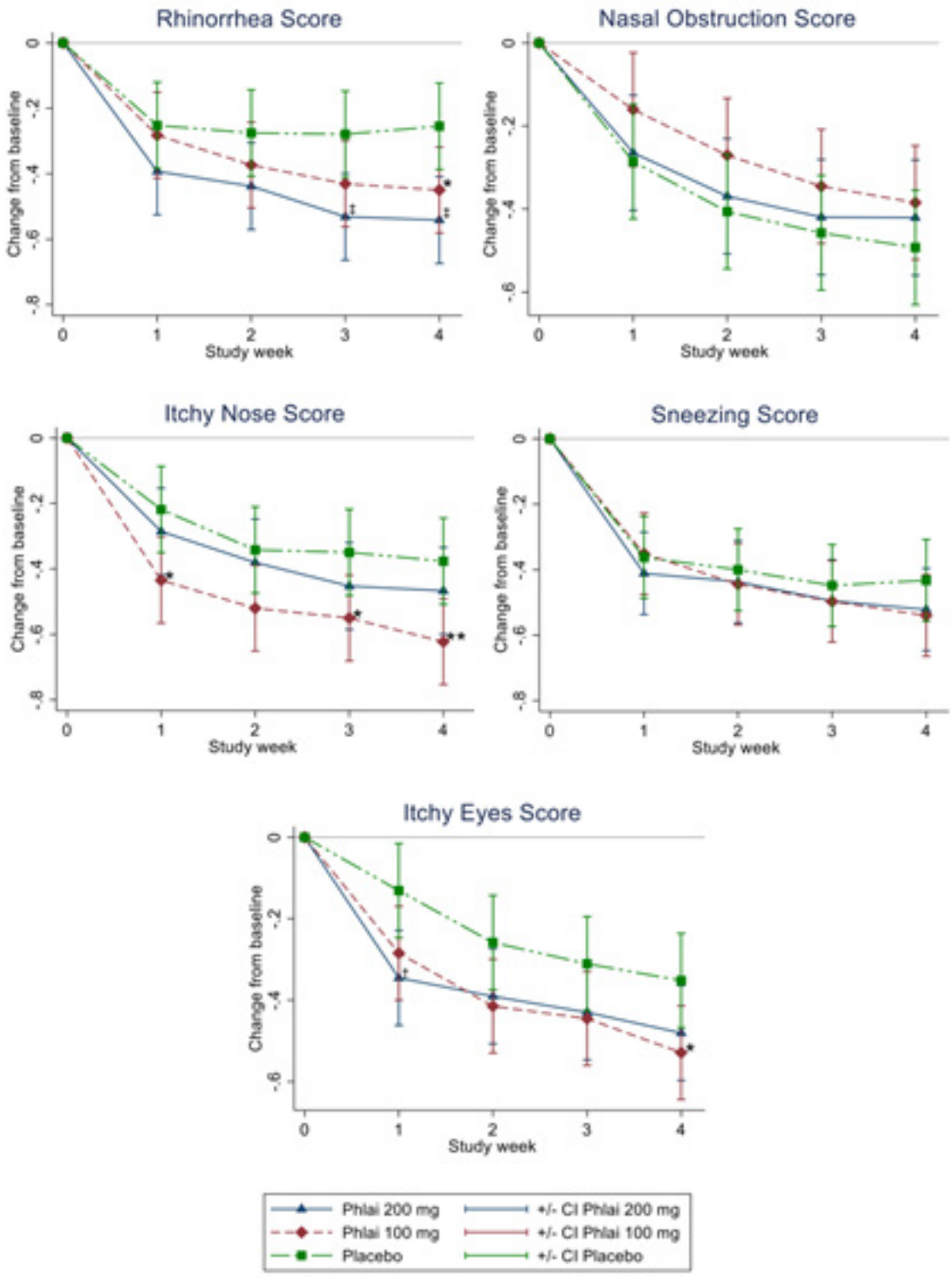

Figure S6. Adjusted changes from baseline to week 4 in reflective individual symptom scores (rhinorrhea, nasal obstruction, itchy nose, sneezing, itchy eyes). Data are least-squares means ± 95%CI for the per-protocol set. Individual symptoms score range, 0-3. *$p < 0.05$ Phlai 100 mg versus Placebo; **$p < 0.01$ Phlai 100 mg versus Placebo; †$p < 0.05$ Phlai 200 mg versus Placebo; ‡$p < 0.01$ Phlai 200 mg versus Placebo.

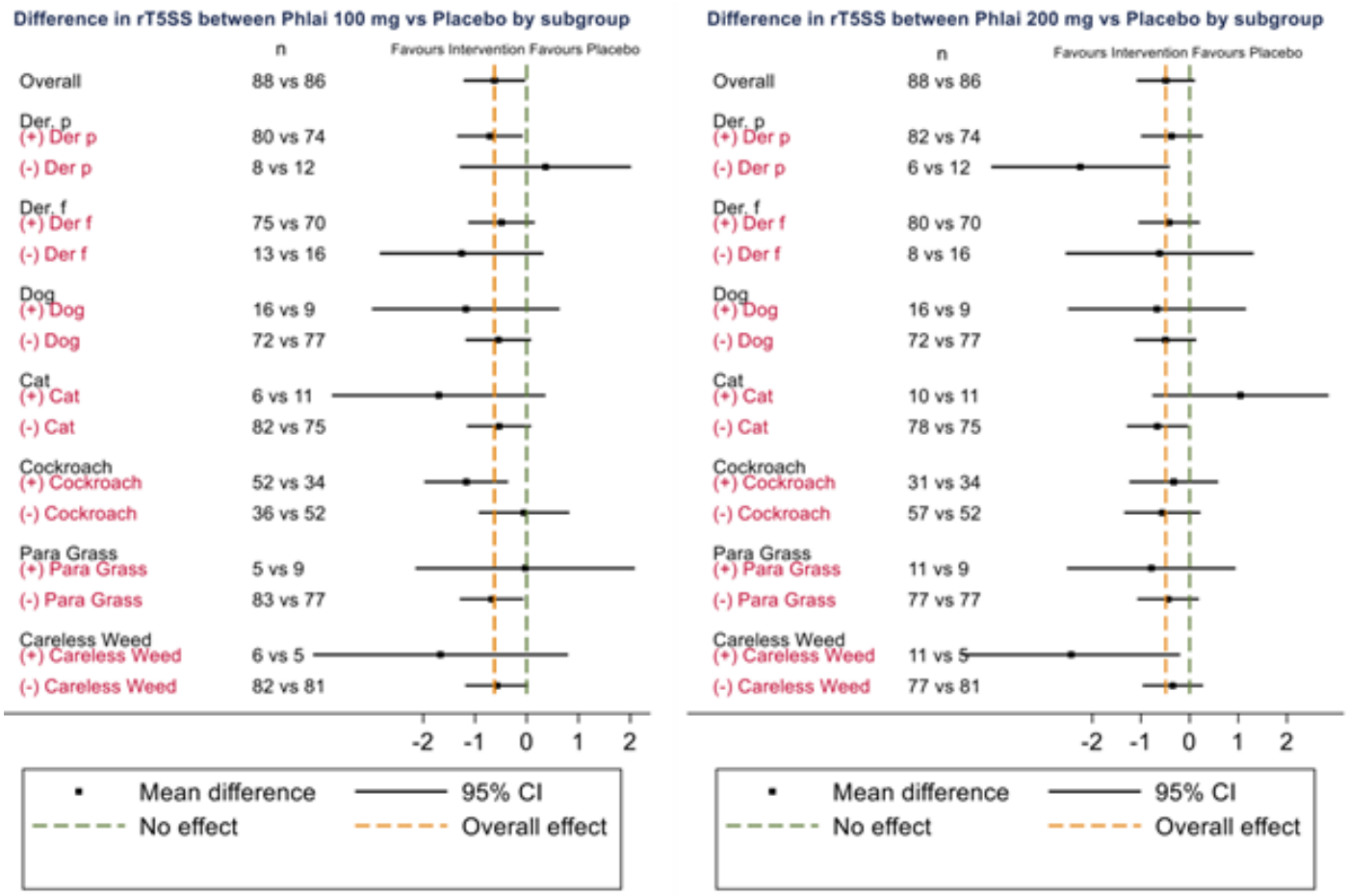

Figure S7. Between-group differences in rT5SS improvement at week 4 by subgroup of different types of allergens present. Data are point estimates and 95%CIs. Number of patients shown as Phlai versus placebo. Der. p: *Dermatophagoides pteronyssinus*; Der. f: *Dermatophagoides farinae*.

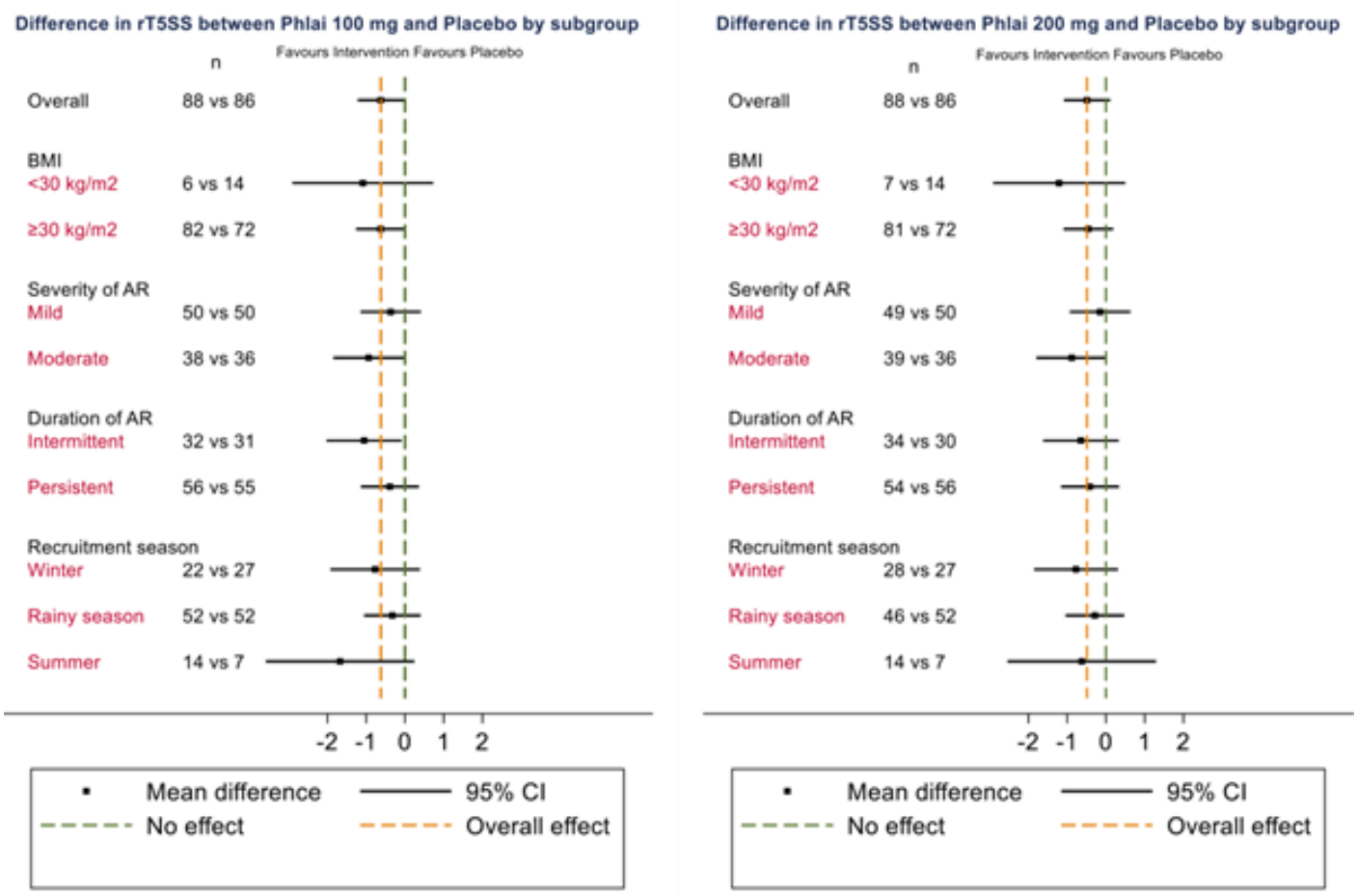

Figure S8. Between-group differences in rT5SS improvement at week 4 by subgroup of BMI, severity of AR, duration of AR, and recruitment season. Data are point estimates and 95% CIs. Number of patients shown as Phlai versus placebo. BMI: body mass index; AR: allergic rhinitis