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Page: [O88-109]

Original research article

Symptomatic treatment of mild COVID-19 with *Vitex negundo* (NIRPROMP formulation): A randomized, controlled clinical trial

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ABSTRACT

There are few drugs available for the early treatment of mild COVID-19 infection. This study aimed to assess the efficacy and safety of Lagundi (*Vitex negundo*) tablets and syrup in patients with mild COVID-19 without comorbidities. This was a 2-stage multicenter parallel, randomized clinical trial. Stage 1 was a dose-finding study comparing high with standard-dose Lagundi. Stage 2 was the efficacy study using the dose chosen from stage 1 vs. placebo. Outcome measures included symptom scores, clinical recovery time, disease progression, global evaluation scales (GES), and time to negative RT-PCR. Lagundi and placebo treatments were given for 10 days. For stage 1, there was no significant difference in the time to recovery and global evaluation scales for the two dosage groups (n= 75). Standard dose was chosen for the 2nd stage. In stage 2, baseline characteristics between Lagundi 600 mg three times a day (n=101) and the placebo (n=99) were not significantly different except for BMI. Total symptom scores (-8.0,95% :CI: -13.37,-2.62) and anosmia scores (-4.17,95%CI:-6.35,-1.98) at endline were significantly lower in the Lagundi group compared to the placebo when controlling for visit date and baseline scores signifying greater relief for Lagundi. Scores for other symptoms were lower in the Lagundi group but were not significant. Physician- and patient-rated GES scores were higher for the Lagundi group than placebo (suggesting more relief) but were not statistically significant. RT PCR showed no significant differences between Lagundi and placebo on mean cycle threshold values for days 4, 8, and 12. The mean recovery time (overall and for symptoms) was not significantly different between the two groups. Adverse events were not significantly different between the intervention and placebo.

Keywords: Vitex negundo, COVID-19, SARS-CoV-2, herbal medicine, traditional medicine

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1. Introduction

In the modern era, no other infectious agent has caused such a global impact as the SARS-CoV-2 virus, which has caused at least 540 million infections and more than 6.33 million deaths worldwide since it was discovered in December 2019.[1] As of 17 May 2022, there have been 158 million confirmed COVID-19 cases, and 1.4 million deaths from this disease were recorded in Asia[1]. This is considered an underestimation of the actual number of cases, as many patients are not tested. Few drugs have been recommended for mild infection and reserved for those with a high risk of hospitalization.[2] These include remdesivir, nirmatrelvir/ ritonavir, molnupiravir, and several monoclonal antibodies[2]. Early treatment is deemed to be beneficial for the following reasons: improvement of patient outcomes, averting hospitalizations, preventing transmission by limiting the duration of infectiousness, as well as avoiding the long-term sequelae of COVID-19.[3] Unfortunately, these treatments are not commonly available or are very expensive. Several other drugs, such as hydroxychloroquine[2,4], lopinavir/ ritonavir[2], and Ivermectin[2,5], were initially deemed promising and subjected to randomized clinical trials and systematic reviews but have failed to show benefit for this set of patients.[2]

This ongoing pandemic of COVID-19 has triggered researchers worldwide to discover more treatments, especially for mild, early cases of the disease. Because of the time-consuming new drug development process, drug repositioning may be the fastest way to find solutions to this epidemic. Lagundi (*Vitex negundo*) is one of the Philippines' traditional herbs for cough.[6,7] The National Integrated Research Program on Medicinal Plants (NIRPROMP) formulation of Lagundi (tablet and syrup) has been scientifically validated and registered as herbal medicine since 1994 for treating cough of nonbacterial origin and asthma.[6,7] It is currently listed in the Philippine National Formulary for this same indication.[8]

The early symptoms of COVID-19 include fever, cough, sore throat, myalgia, and fatigue.[9] Several in vitro and in vivo studies have elucidated Lagundi's analgesic, anti-inflammatory[10], and antipyretic properties.[12,13] In silico studies have shown that several compounds found in Lagundi have a high binding affinity to drug targets of SARS-COV-2, making Lagundi a potential antiviral agent[14,15], which is especially true during the early course of the disease[3]. Thus, the repurposing of Lagundi for the treatment of mild COVID-19 seems promising and viable. We assessed the efficacy and safety of the NIRPROMP formulation of Lagundi (*Vitex negundo*) tablets and syrup in patients with mild COVID-19 without comorbidities.

2. Materials and Methods

2.1 Study design and participants

This was a 2-stage adaptive, multicenter parallel, randomized clinical trial. The first phase was a dose-finding single-blinded study conducted from 8 September 2020 to 15 November 2020 in 2 quarantine/ isolation centers in Metro Manila. The second phase was the efficacy study, a multicenter, randomized, double-blind, placebo-controlled clinical trial using the dose chosen from phase 1 and conducted from 1 December 2020 to 1 May 2021 at 7 quarantine/isolation centers.

Study participants were enrolled from the isolation/quarantine centers if they were 19-55 years old, confirmed COVID-19 infections by nasopharyngeal real-time reverse transcription-polymerase chain reaction (RT-PCR), had signs and symptoms of mild COVID-19 based on the Philippine COVID-19 Living Recommendations Severity Classification[15], had a Modified Early Warning Score (MEWS)[16] of less than 5 and willing to adhere to the study procedures and examination schedule. Patients were excluded if they were asymptomatic, known to be allergic to Lagundi syrup or tablet, participated in other clinical trials in the previous month, with any comorbidity or were on maintenance medication, and were pregnant or breastfeeding.

The study was approved by the University of the Philippines Manila Research Ethics Board and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained prior to any screening procedure performed. The protocol was also approved for conduct by the Philippine Food and Drug Administration (2020-CT0553) and registered in the Philippine Health Research Registry (PHRR210126-002992).

2.2 Study drug and placebos

Lagundi 600 mg tablet and Lagundi 600mg/5mL syrup are licensed formulations from the University of the Philippines Manila and are registered as Herbal Medicines by the Philippine FDA. The study drugs and placebos used in this clinical trial were manufactured under Good Manufacturing Practices by NewMarketlink Inc., a licensee of UP Manila. These formulations have undergone Phase 1, 2, and 3 clinical trials and were found to be effective and safe for the treatment of nonbacterial cough and asthma. The recommended dose for adults is 600mg tablet or 600mg/ 5mL syrup three times a day and is the standard dose in Stage 1. Double this dose was given as the High dose. Dose finding was performed as it was unknown which would be more beneficial for the treatment of COVID-19.

2.3 Randomization

For Stage 1, patients were assigned to either Standard dose or High dose Lagundi in a 1:1 ratio. Patients were randomized by quarantine center in permuted blocks of 2,4 and 6 in a randomization sequence prepared by the statistician using Stata 14. 2 (Stata Corp, College Station, TX) statistical software. This randomization sequence was given to the study pharmacist to prepare the different treatment packs for each center.

For Stage 2, patients were assigned either the chosen dose from Stage 1 or a placebo. Patients were randomized similarly to Stage 1. Allocation was made by the unblinded pharmacist and was concealed from the investigators and patients.

2.4 Interventions

For Stage 1, study patients in the Standard dose (S.D.) received Lagundi 600 mg three times a day for ten days or High dose (H.D.) Lagundi 1.2 g three times a day for ten days, either as a tablet or syrup, depending on the participant's preference. The Lagundi tablet and syrup used were the NIRPROMP formulation provided by New Marketlink.

For Stage 2, study patients received either a Lagundi dose based on Stage 1 or a placebo of the same preparation preferred by the participant. The placebos were also provided by New Marketlink Inc, which were tablets and syrup made of the same ingredients and excipients as the Lagundi products, except for the Lagundi raw material, which had a similar label and packaging.

All patients were given the same standard of care for both stages according to Unified COVID-19 guidelines.[17] This included symptomatic treatment and supportive care as needed. Supportive treatment included appropriate nutrition and hydration, paracetamol for fever, sodium chloride nasal drops for nasal obstruction, vitamins, and the allowed medications.

2.5 Study sites

For Stage 1, the study sites were the Isolation/ Quarantine Center at the Quezon Institute (Q.I.) in Quezon City and the Philippine National Police National Capital Region Police Office (PNP NCRPO) Special Care Facility in Taguig City. For Stage 2, there were seven isolation/ quarantine facilities with enrollment: the Rizal High School in Pasig, the Valenzuela Quarantine Facility in Valenzuela City, the Araullo High School in Manila, the Silungan Molave in University of the Philippines, Diliman, the Q. I. , and PNP NCRPO.

2.6 Procedures

For stages 1 and 2, participants were identified from the admission records of the isolation centers. Newly admitted patients were invited to participate in the study. All

patients were SARS COV2 RT-PCR positive prior to admission to the quarantine centers. After explaining the study procedure and obtaining their written informed consent, participants were screened for eligibility using a health questionnaire, medical interview, and physical examination. Baseline laboratory measurements were also performed, including complete blood count, random blood sugar, and liver and renal function tests. On the same day, eligible patients were randomized and given treatment according to their assigned treatment arm, depending on the phase. Participants were followed up on days 4, 8, 12, 16, and 20. A medical interview was performed on follow-up days, including elucidating adverse effects and performing a physical examination. If a participant was sent home, the participant was followed up by telephone, and a medical interview was conducted. Laboratory exams were repeated within 1-4 days of the last dose. Diary cards were given to the participants to monitor compliance and document adverse events.

RT-PCR sample collection

For stage 2, it was aimed to have at least 50 participants from each arm undergo serial nasopharyngeal RT-PCR on Days 4, 8, 12, 16, and 20 or until two negative swabs were obtained.

Study personnel collected nasopharyngeal and oropharyngeal swabs using Dacron-tipped swabs at different study sites. Swabs were stabilized in various Universal Transport Media (UTM) (Sansure, Biotech, Hunan, China; Sanli, Liuyang, China) , temporarily stored at 4^0C, transferred to freezers (-80 ±20^{o}C), and tested within 24 – 72 hours.

SARS-CoV-2 real-time RT-PCR testing

The RT-PCR samples were submitted to the COVID-19 Laboratory of the National Institutes of Health, University of the Philippines. The swabs were heat-inactivated at 65^0C for 10 minutes. Viral RNA was extracted using the PANAMAX viral DNA/ RNA extraction kits (Panagene Inc, South Korea) and the PANAMAX 48 automated nucleic acid extraction machine (Panagene Inc, South Korea) or extracted manually using the Sansure RNA one-step nucleic acid release reagent (Sansure, Biotech, Hunan, China) or GenAmplify RNA extraction kit (Manila HealthTek, Manila, Philippines), following manufacturers' instructions. Briefly, specimens were thawed, and an aliquot of 200 µl (Sansure) or 140 µl (GenAmplify) of each specimen was used for RNA extraction following the manufacturer's instructions for each kit. We used the Sansure (Sansure Biotech, Hunan, China) and GenAmplify (Manila HealthTek, Manila, Philippines) SARS-CoV-2 rRT-PCR kits following testing conditions and Ct cut-off values indicated by each manufacturer. A negative result was defined as the absence of both PCR amplification products for the ORF1ab or N gene targets after 40 cycles or both the RdRp and E gene targets after 38 cycles for the Sansure and GenAmplify SARS-CoV-2 kits, respectively. Extracted nucleic acid was amplified using the following PCR machines: Bio-Rad CFX96 (Bio-Rad Laboratories Inc., CA, USA) or ABI 7500 Fast (Applied Biosystems, CA, USA).

2.7 Outcomes

Outcome measures for Stage 1 were time to clinical recovery, disease progression, and adverse events. The outcome measures for Stage 2 included symptom scores, time to clinical recovery, progression of the disease, RT-PCR values over time, and adverse events.

Time to clinical recovery was defined as the time from initiation of study treatment until normalization of fever, respiratory rate, oxygen saturation, and alleviation of cough. Other outcomes included comparing the number of participants who had disease progression between both arms. Disease progression was defined as a Modified Early Warning Score (MEWS) of 5 or higher.[17] Safety outcomes included all adverse events which occurred during treatment and were classified according to the National Cancer Institute Common Terminology Criteria of

Adverse Events version 5.0. All serious adverse events were to be reported to the UPMREB and PhilFDA.

For Stage 2, additional outcomes included symptom scores and obtaining cycle threshold (Ct) values from repeat RT-PCR testing and time to a negative RT-PCR result. Symptom scores were elicited from the patient at every visit on a 10-point Likert scale, with "0" meaning no symptoms and "10" meaning worst symptoms. Specific symptoms which were scored by the participants were cough, colds, sore throat, anosmia or smell, ageusia or taste, body malaise, fatigue, and fever. The total symptom score was an aggregate of all the symptom scores of the patient, with "0" as no symptoms and with "80" as the highest score if it was worst for all symptoms asked.

2.8 Statistical analyses

For Stage 1 dose-finding studies, respiratory tract disease effects usually require 30 patients per group, and for viral diseases, 40 cases per group. [17] Thus, a sample size of 40 per group for the dose-finding study was used.[17]

For Stage 2, we estimated a sample size of 186 (93 in each arm) with the primary outcome measure of time to clinical recovery to measure a desired/assumed 20% difference in the proportion of patients whose symptoms resolve at seven days (e.g., control = 50%, intervention = 70%) = 186 (93 in each arm) using power at 80% , Alpha = 0.05, and using Stata version 14.2. We added 7-10 % to account for dropouts producing a sample size of 100 per arm.

Only confirmed COVID-19 participants were included in the efficacy analysis of the study. Normally distributed continuous data were presented as means with their standard deviation, and the means were compared using t-tests. Non-normally distributed continuous data were presented as means and compared using the Wilcoxon rank-sum test. Linear regression was performed on the patient-rated global evaluation scale, physician-rated global evaluation scale, total symptom score, and for each symptom score.

Comparison of treatment and control arms using Ct values

We compared the Ct values of the gene targets between the treatment group and controls. We used measures of central tendency (mean, median), measures of dispersion (range, standard deviation, upper and lower quartiles), scatterplots, and trend analysis to calculate the slope and the trendline reliability (R^2) using the data analysis tool of Microsoft Excel 365 (Microsoft, WA, USA). We also used the two-sample Student's t-test assuming unequal variances to compare the means of the Ct values of the specific gene targets between the treatment and control arms using Prism version 9.2.0 (Graphpad Software, CA, USA). Two-tailed P values $<.05$ were considered statistically significant.

Time to negative using Ct values between treatment and control arms

To compare the time to negative between the treatment and control arms using the Ct values of the different gene targets, we used the Ime4::lmer function in R version 4.0.5 (R Development Core Team, 2021) to generate a linear mixed effect model fit by maximum likelihood assuming the dependent variable as the Ct value and the explanatory variables as the treatment group (as fixed intercept), day of illness (as random slope) , target gene (as random intercept) and the subject (as random intercept). Patients whose Ct values did not reach the Ct value for a negative RT-PCR interpretation across all the serial PCR tests were excluded from the final analysis.

Adverse events were listed individually per dose level and summarized as frequencies. Pre-study and post-study findings of physical examination, vital sign variables, laboratory variables, SpO2, and MEWS were recorded separately.

Both intention-to-treat analyses and per-protocol analyses were performed. Subgroup analysis may be performed to

compare any difference in the efficacy or safety of the different formulations

3. Results

3.1 Stage 1 dose finding trial

Between 1 September 2020 to 15 November 2020, we screened 295 RT-PCR-positive patients for eligibility. Figure 1 summarizes the patients' screening, recruitment, follow-up, and analyses. Two hundred twenty patients were either excluded due to not meeting the inclusion criteria or declining participation. A total of 75 patients were randomized, and all were included in the analyses of Stage 1.

The baseline demographic characteristics (see Table 1) were not significantly different between the high-dose Lagundi and the standard-dose Lagundi groups. It was noted that the number of days since symptom onset was 11.5± 6.8 days for the high-dose Lagundi and 9.6± 5.7 for the standard dose. The laboratory characteristics were not significantly different except for the random blood sugar, alanine transaminase, and aspartate aminotransferase. The average value was higher in the high-dose group (Supplement S1. The mean clinical recovery time was not significantly different from both groups. It was 6.58±4.54 for high dose lagundi and 6.54±4.01 for the Standard dose (P-value= 0.962). No patient progressed to moderate or severe from both doses.

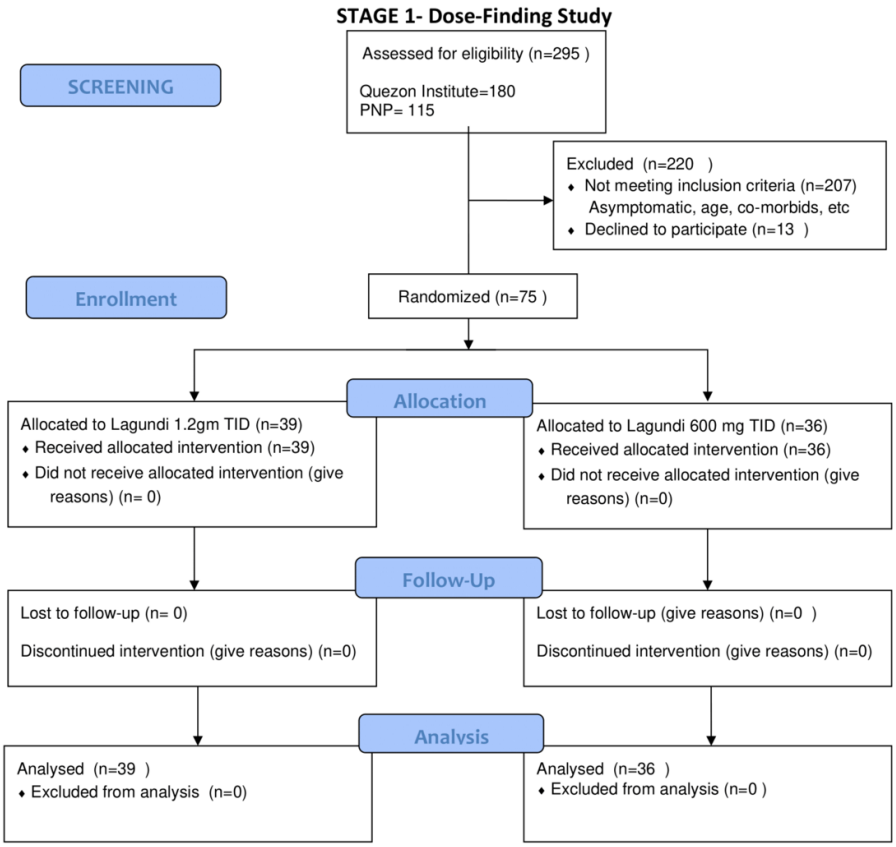

Fig. 1. Consort diagram for Stage 1 dose-finding study.

Table 1 Demographic characteristics of mild COVID-19 patients enrolled in stage 1.

Characteristic	High dose Lagundi	Standard dose Lagundi	P-value
Age (Mean, SD), n=75	32.2, 9.83	30.8, 6.7	0.443
Sex – Male	25 (58.1%)	18 (41.9%)	0.217
Sex - Female, (n=75)	14 (43.8%)	18 (56.3%)	
MEWS (Mean, SD) N=75	1.45, 0.65	1.63, 081	0.291
No. of days since symptom onset (Mean, SD), n=73	11.2, 6.7	9.6, 5.9	0.298
BMI (Mean, SD), n=75	23.7, 3.9	24.3, 3.4	0.466

Table 2 Physician-rated and Patient-rated Global Evaluation Scales on day 4, by treatment group.

	Fair n (%)	Good n (%)	Very Good n (%)	P-value
Patient-rated				
High dose Lagundi	7 (19.4)	23 (63.9)	6 (16.7)	0.142
Low Dose Lagundi	15 (37.1)	18 (51.4)	4 (14.1)	
Physician-rated				
High dose Lagundi	9 (25)	22 (61)	5 (13.9)	0.259
Low Dose Lagundi	16 (40)	17 (48.6)	4 (11.4)	

Repeat laboratory exams were within normal limits for both arms. Few cases of diarrhea and pruritus were reported as adverse events, and no difference was seen between both groups (Supplement S2). No serious adverse events were reported in any group. Due to the lack of difference in results, we decided that the Standard dose shall be used for the Stage 2 trial.

3.2 Stage 2 Lagundi vs. placebo clinical trial

Baseline characteristics

Between 1 December 2020 and 15 April 2021, 1069 patients with mild COVID-19 disease confirmed by RT-PCR were screened at seven quarantine centers. Fig. 2. summarizes the screening, enrollment, and follow-up of the participants. Eight hundred sixty-six patients were excluded due to non-eligibility, declined to participate, or enrolled in another study. A total of 203 participants were enrolled, 101 participants were analyzed from the Lagundi group, and 99 were analyzed in the placebo group.

The baseline characteristics of the participants randomized to the Lagundi were not significantly different from those of the placebo group except for BMI (Table 3) . The participants from the Lagundi group had a mean age of 31.4$\pm$7.7 years old, while the placebo was 30.9$\pm$7, which was not statistically significant. The baseline MEWS score was very low for both groups. The average number of days from symptom onset was six

for both groups. The baseline laboratory values were also not significantly different between the Lagundi and placebo groups except for hemoglobin, although they were still within normal limits (Supplement S3). The most common symptom for both groups was cough, and the least frequent was fever (Table 4). The proportion of participants with different symptoms was not significantly different between arms. A similar number of patients also chose tablets or syrup among the intervention groups.

The baseline symptom scores were also not significantly different between the 2 groups (Table 4).

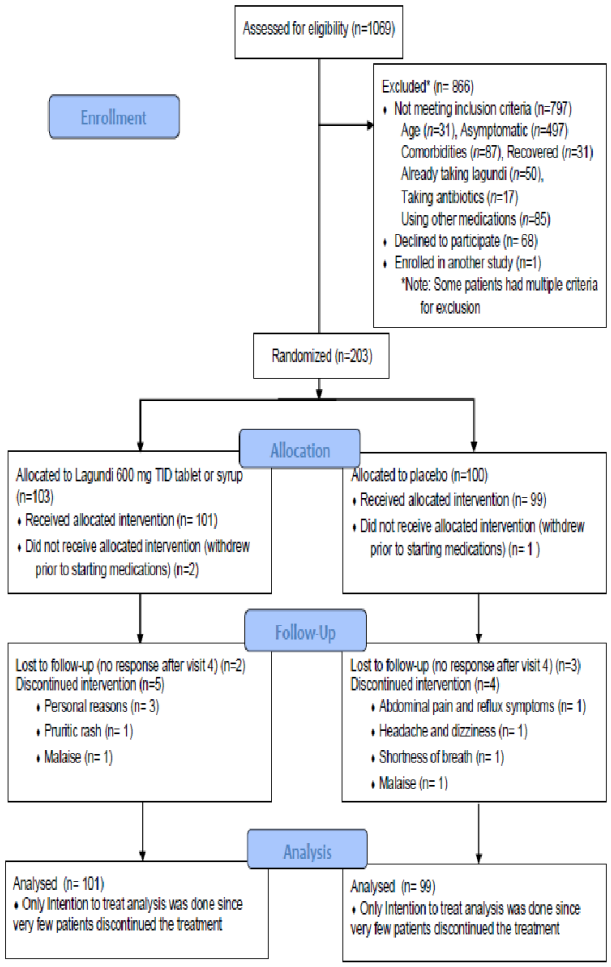

Fig. 2. Consort diagram of the Stage 2 randomized, control trial of Lagundi vs. placebo.

Outcomes

Symptom scores and global evaluation scales

Using linear regression, the Total symptom scores (-8.0, 95% : CI -13.37, -2.62) and anosmia scores (-4.17, 95% CI: -6.38, -1.98) were significantly lower in the Lagundi group compared to the placebo group, when controlling for visit date and baseline scores. A lower score signifies having less severe symptoms, and thus the results favor the Lagundi group (Table 5). Individual symptom scores for other symptoms (cough, colds, ageusia, body malaise, fatigue) were generally lower in the Lagundi group, but the difference did not reach statistical significance. Fig. 3. and 4 show the Waffle plots for anosmia and the Total symptom score of Lagundi and Placebo. Patient global evaluation scale (GES) scores were slightly higher for the Lagundi group than the placebo group (-1.0, 95% CI -0.17, 2.17), suggesting more improvement, but were not statistically significant. The same trend was seen for the Physician GES.

Recovery time

There was no significant difference between the two arms for the time to total clinical recovery and time to clinical recovery of cough, sore throat, anosmia, and ageusia (Table 6). There were too few people with a fever to use this as an outcome.

Progression of disease

For both the Lagundi and placebo participants, no patient progressed to moderate or severe based on the modified early warning score.

Comparison of RT-PCR Ct values

The mean number of days for the treatment arm patients (n= 31) to reach a negative SARS-CoV-2 RT-PCR test result was 12.03 days (range of 4 – 20 days; 95% CI: 10.65, 13.41), while the mean number of days for the control arm (n=38) to reach a negative SARS-CoV-2 RT-PCR test result was 11.29 days (range of 2 – 21 days; 95% CI: 9.84, 12.74). We also

performed the Student's t-test (assuming unequal variances) , which showed a two-tailed P-value of 0.45.

The analysis of the serial nasal swabs of RT-PCR cycle threshold results of the participants in each arm showed no significant differences in Lagundi vs. Placebo on the mean of collection days (Day 4, 8, and 12). Based on the linear mixed-effects model, Ct values increase by 1.06 points (95% CI: 0.91, 1.20) per additional illness day. There was no significant difference in the timing of collection between Lagundi and the placebo.

Comparing the mean of Lagundi vs. placebo Ct values according to collection day (Day 4, 8, and 12) and gene target did not show any significant difference across Day 4, 8, and 12 and between all gene targets (Lagundi vs. placebo) . There was a trend toward a steeper rise (higher slope) among Lagundi vs. placebo across all gene targets using the average Ct value per day of illness. (Figures 5 to 8.) Visual inspection showed a similar increase between treatment and control Ct values across all target genes as the day of illness increased. A limitation is that many days of illness have only 1 data point.

Among treated individuals, the gene targets' mean Ct values increased significantly from Day 4 to Day 8 and Day 8 to Day 12. Adjusting for within-subject variation, treatment had no statistically significant effect on the Ct-value rate increase. Adjusting for within-subject variation, the type of target gene being tested was not statistically associated with the rate of increase of Ct-value.

Safety

Overall, Lagundi was well tolerated, with few adverse events and no serious events. Among the elicited adverse events were diarrhea, pruritus, and nausea. As seen in Table 7, there was no significant difference between the Lagundi and placebo arm. For nausea, only one patient from the placebo group complained of nausea.

Table 3 Demographic characteristics of patients enrolled in stage 2.

Characteristic	Standard dose Lagundi (n=101)	Placebo (n=99)	P-value
Age (Mean, SD)	31.5, 7.8	30.9, 7.5	0.606*
Sex – Male	62 (61%)	64 (65%)	0.633
Sex – Female	39 (39%)	35 (35%)	
MEWS (Mean, SD)	0.09, 0.38	0.06, 0.24	0.790*
No. of days since symptom onset (Mean, SD)	6.2, 3.5	6.2, 3.9	0.963
BMI (Mean, SD)	24.6, 3.4	26.1, 3.6	**0.003**
Patient's Global Evaluation Scale (Mean, SD)	7.2, 1.9	7.3, 1.5	0.545
Physician's Global Evaluation Scale (Mean, SD)	7.3, 1.8	7.4, 1.4	0.705

Table 4 Proportion presenting with symptoms, and symptom scores by treatment group at baseline.

Symptom	No. of Patients (%)		P-value**	Symptom Score * (mean, SD)		P-value***
	Lagundi	Placebo		Lagundi	Placebo	
Cough	61 (60%)	58 (59%)	0.794	2.5, 2.5	1.9, 2.2	0.202
Colds	49 (49%)	51 (51%)	0.671	1.9, 2.3	2.1, 2.5	0.556
Sore throat	38 (38%)	33 (33%)	0.526	1.1, 1.8	1.2, 2.0	0.677
Anosmia	44 (44%)	52 (52%)	0.205	3.1, 4.0	3.3, 3.8	0.493
Ageusia	35 (35%)	42 (42%)	0.259	2.2, 3.4	2.6, 3.4	0.340
Body malaise	22 (22%)	20 (20%)	0.784	1.0, 2.1	0.7, 1.7	0.646
Fatigue	22 (21%)	17 (17%)	0.411	1.1, 2.3	0.7, 1.7	0.339
Fever	7 (7%)	6 (6%)	0.803	0.3, 1.1	0.3, 1.1	0.824
Total symptom score****				13.1, 9.5	12.8, 8.6	0.768

*On a scale of 0 - 10, with 10 as the most severe
**Pearson Chi-square
***Wilcoxon rank-sum test
****Highest possible score = 80,

Table 5 Number of patients who chose tablet or syrup depending on the study site and treatment group.

Site	Lagundi		Placebo		Total
	Tablet	Syrup	Tablet	Syrup	
PNP – NCRPO Facility	37	27	37	26	127
Valenzuela Quarantine Facility	12	2	8	4	26
Quezon Institute	8	4	7	4	23
Araullo High School	2	4	0	6	12
PGH Extension Facility	0	2	4	0	6
Rizal High School	1	0	3	0	4
Silungan Molave	1	1	0	0	2
Total	61	40	59	40	200

Table 6 Linear regression results of the effect of treatment on individual symptoms, total symptom score, and patient and physician global evaluation scales (GES), controlling for visit date.

Total symptom score	**Coeff.**	**S.E.**	**95% CI**		**P-value**
			LL	**UL**	
	-8.0	2.74	-13.37	-2.62	**0.004**
Cough	-0.67	0.67	-1.99	0.65	0.321
Colds	-0.33	0.68	-1.66	0.99	0.622
Sore throat	-0.67	0.56	-1.77	0.44	0.235
Anosmia	-4.17	1.11	-6.35	-1.98	**<0.001**
Ageusia	-1.67	0.96	-3.56	0.22	0.084
Body malaise	- 0.17	0.55	-1.25	0.92	0.764
Fatigue	- 0.33	0.54	-1.39	0.73	0.538
Fever	-3.90e-14	0.35	-0.69	0.69	1.000
GES (Patient-rated)	1.0	0.60	-0.17	2.17	0.094
GES (Physician-rated)	1.0	0.57	-0.11	2.11	0.078

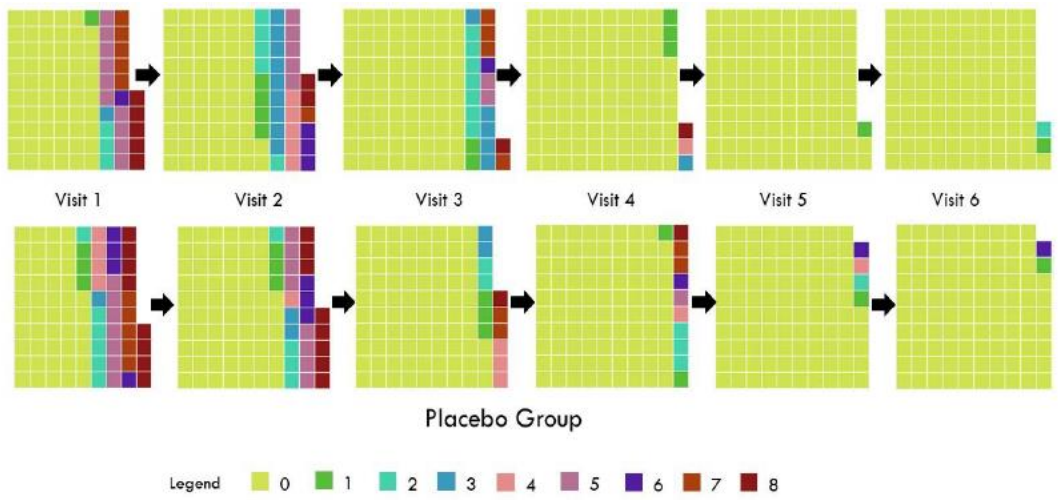

Fig. 3. Waffle plots of symptom scores for anosmia between Lagundi and placebo groups.

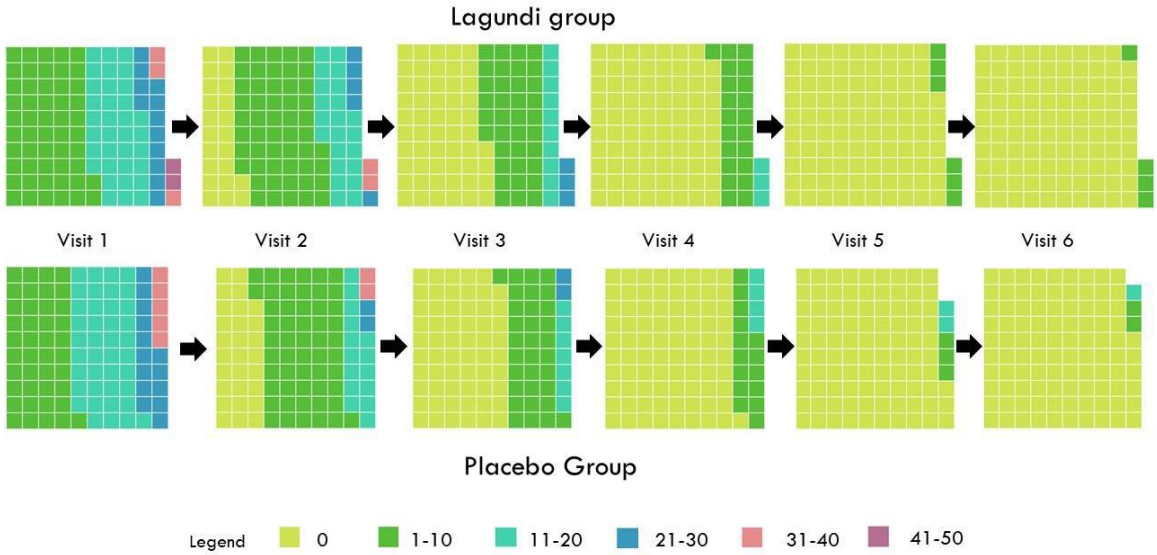

Fig. 4. Waffle plots of symptom scores for total symptom score between Lagundi and placebo groups.

Table 7 Stage 2 mean clinical recovery time in days by treatment group (ITT).

	Lagundi Mean (S.D.)	**Placebo Mean (S.D.)**	**P-value***
Clinical recovery time in days	8.62 (4.87)	7.51 (4.05)	0.103
Clinical recovery from cough in days,	6.0 (4.0)	5.4 (3.5)	0.456
Clinical recovery from throat discomfort	3.8 (1.9)	4.1 (2.4)	0.636
Clinical recovery from anosmia	5.6 (3.8)	5.2 (3.7)	0.767
Clinical recovery from ageusia	5.3 (3.6)	4.6 (3.1)	0.550

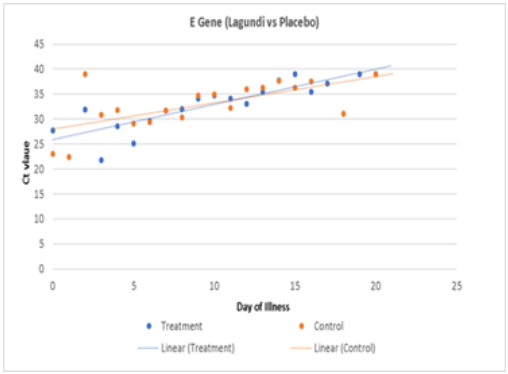

Fig. 5. Scatter plot of E gene Ct values of Lagundi treatment versus placebo according to day of illness.

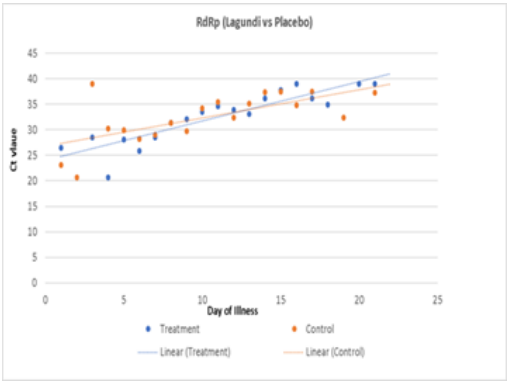

Fig. 6. Scatter plot of RdRp gene Ct values of Lagundi treatment versus placebo according to day of illness.

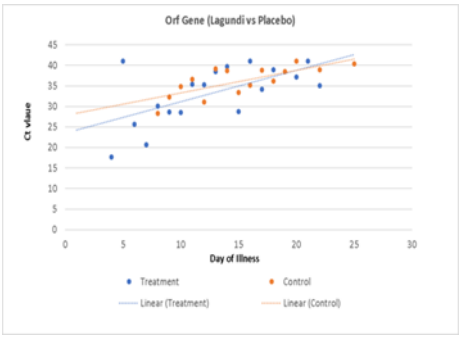

Fig. 7. Scatter plot of Orf gene Ct values of Lagundi treatment versus placebo according to the day of illness.

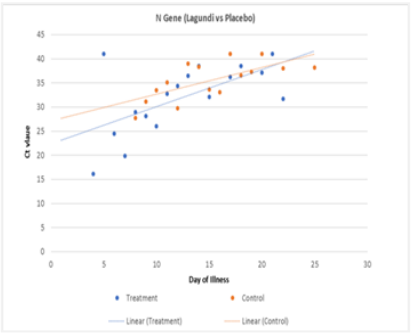

Fig. 8. Scatter plot of N gene Ct values of Lagundi treatment versus placebo according to day of illness.

Table 8 Occurrence of adverse events among patients by group.

Diarrhea time	Severity	Standard dose Lagundin (%)	Placebo n (%)
Diarrhea	Grade 1	2 (2%)	3 (3%)
Pruritus	Grade 1	2 (2%)	3 (3%)
Nausea	Grade 1	0 (0%)	1 (1%)

4. Discussion

In this randomized, double-blind, placebo-controlled trial of symptomatic adults with confirmed COVID-19, a ten-day course of Lagundi showed a statistically significantly lower score for anosmia and total symptom score during treatment compared to placebo. As a result, the Lagundi intervention group experienced less anosmia over time than the placebo group. The total symptom score was also significantly lower in patients treated with Lagundi compared to placebo. On each of the follow-up days, for the Lagundi group, the mean difference in the symptom score for cough, colds, sore throat, and other symptoms had a negative score, which signifies lower scores (greater alleviation) for this group compared to placebo. Still, the difference did not achieve statistical significance. Additionally, there was no statistically significant difference between Lagundi and placebo in terms of how long it took for each symptom to subside.

The capacity of Lagundi to lessen anosmia symptoms during successive visits, beginning at visit 2 or the fourth day after taking the medication, was viewed as its most notable benefit compared to placebo. The incidence of anosmia in this study was 48%. Another research review reported a wide range of incidences of anosmia at 19. 4-98. 7% . [18] Several factors were found to affect this prevalence. Older age, being male, and severe disease were associated with *de*creased prevalence of chemosensory (smell and taste) dysfunction. [19] Asians had a lower chemosensory dysfunction at 17% compared to Caucasians (54.85%). It is theorized that virus entry to the olfactory epithelium may also be affected by virus mutation and patient genetics.[19]

The underlying pathogenesis for anosmia may be a direct effect of the virus on the function of sustentacular cells, which process odorants by endocytosing the odorant-binding protein complex and maintaining the epithelial cell's integrity. [20]

Since these cells abundantly express ACE2 and TMPRSS2 proteins, the Coronavirus accumulates in the sustentacular cells, and olfactory sensation is impaired. It is theorized that an inflammation-mediated loss of odorant receptor expression also contributes to anosmia.[20] A postmortem cohort study also supports this theory. Dissection of the olfactory bulb and olfactory tract showed axon pathology and microvasculopathy in COVID-19 patients with smell alterations.[21] The pathology was not due to viral damage but local inflammation. Thus, Lagundi may improve anosmia symptoms due to its anti-inflammatory effects.

Anti-inflammatory activities were found in the iridoid and flavonoid fractions of the Lagundi extract (See Table 8) . Specifically, the fractions that contain the iridoids, which contain agnuside lagundinin, 2' -parahydroxybenzoyl mussaenosidic acid,[25] and the flavonoids casticin and chrysoplenol D[26] were found to have anti-inflammatory potential. The anti-inflammatory action of Lagundi is partly explained by its ability to inhibit chemotaxis, phagocytosis, and complement activation via alternative and classical pathways.[25]

The other symptoms of cough, colds/ nasal obstruction, sore throat, ageusia, body malaise, and fatigue showed a trend for better symptom relief over time for the Lagundi group, but this did not reach statistical significance. But since these scores were totaled per patient, the total symptom scores differed statistically. Thus, the benefits of symptomatic relief are consistent with the NIRPROMP formulation of Lagundi's different pharmacologic effects, which include increased airflow/ bronchodilation, antitussive, analgesic, and antipyretic effects (Table 8).

For clinical recovery time, Lagundi and placebo were not statistically different. This may be because treatment was administered quite late to the patients, at around the 6th day after the onset of symptoms. This was not within our control since our team would recruit and screen patients on the day of their admission into the quarantine facility. The delays for admission included the lag in time for patients to seek medical attention, especially if they only have mild symptoms, the turnaround time for the results of the RT-PCR, and the time for the coordination with the LGU or administrators of the quarantine facility. For most antivirals, the greatest efficacy is seen at least within 2-5 days of the onset of symptoms. For Oseltamivir, the antiviral of choice for influenza, the drug should be administered within 48 hours of illness to see its greatest effect.[40] The first antiviral agent registered in the U. K. for treating mild to moderate COVID-19 is molnupiravir. According to its product monograph, it should be administered within 5 days from the onset of illness to decrease hospitalizations. If, indeed, Lagundi also has antiviral activity, as evidenced by at least 3 in silico studies on several target sites of SARS COV2 (RdRP, Mpro, and 3 virulence factors) , then its pharmacologic activity would be most evident in the 1st five days of illness. Unfortunately, this window was missed in this clinical trial. It would still be prudent to pursue in vitro antiviral studies using Lagundi.

No patients in Stage 1 (High dose and standard dose Lagundi) nor in Stage 2 for the Lagundi and control groups progressed to moderate or severe disease. The main factor which probably contributed to this was that the patients were young, with a mean age of 31 yrs old without comorbidities, meaning they were at lower risk for disease progression.

When the reverse transcriptase-polymerase chain reaction test for SARS-COV2 was initially developed as a diagnostic tool, it was hoped that it would also be a good test of cure if a negative result would be found in a COVID-19 patient during the time we developed this research protocol.

Table 9 Beneficial pharmacologic effects of Lagundi for COVID-19 diseases.

Pharmacologic activity	Mechanism of action	Isolated fractions/compounds	In vivo/clinical trial
Anti-inflammatory	Inhibits cyclooxygenase-2[22]	Negundin-B[24]	Rat paw edema model [10, 22, 25, 26,27]
		methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl) ethyl) benzoate and 3-(1-hydroxy -2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl) ethyl)benzoic acid [25]	
	Inhibits chemotaxis, phagocytosis, and complement activation[23]	lagundinin, 2-p-hydroxy benzoyl-mussaenosidic acid (or negundoside), casticin, chrysoplenol D[23]	
Analgesic	Prostaglandin inhibition[10]	methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoate (1) and 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoic acid[25]	Rat hot plate and tail flick test [10] acetic acid-induced abdominal constriction assay[25] Clinical trial in post-dental extraction pain[28]
Antipyretic	Prostaglandin inhibition	Unknown	Yeast-induced pyrexia in male rabbits [12] Yeast-induced pyrexia in rats [29]
Bronchodilator	Smooth muscle relaxation [30] involvement of phosphodiesterase inhibitory pathway, and inhibitory effect on Ca^{++} entry[32]	2-p-hydroxybenzoyl-mussaenosidic acid 6-p-hydroxybenzoyl-mussaenosidic acid, lagundinin, agnuside [30] Chrysoplenol D, Luteolin, isoorientin [31]	Carbachol-induced bronchoconstriction in rats and guinea pigs [32] Clinical trial in adults with asthma [33]
Antitussive	Bronchodilation?	Unknown	cough provoked by SO_2 gas in mice [34] Clinical trials using NIRPOMP tablet and syrup [35, 36]
Antiviral	Inhibition of RNA-dependent RNA polymerase of SARS COV-2 [14]	Quercetin, Vitexin in silico[14]	
	Inhibition of virulence factors of SARS-COV2 [37]	Vitexin in silico[37]	
	Inhibition of Mpro of SARS-COV2 [13]	40 compounds from Lagundi[13]	

Unfortunately, the latter did not turn out to be appropriate. Currently, RT-PCR is still the gold standard for diagnosis, but it is not a test of cure nor infectiousness. Later studies showed that RT-PCR could detect SARS-CoV-2 RNA in specimens obtained weeks to months after the onset of COVID-19 symptoms (NIH, 2021) . [38] Studies have reported that when the Ct value is above 34, the live virus has not been detected *in vitro.* [39,40] Previous studies have reported the detection of viral fragments by RT-PCR for several weeks despite clinical improvement. RT-PCR can still be positive due to detecting non-viable RNA fragments reflecting host immune response and cannot be used to determine infectiousness. [41,42] This finding has led to revisions of isolation guidelines to symptom-based evaluation rather than test-based recommendations for release from

isolation.[43] Thus, time to a negative RT-PCR is not an appropriate efficacy outcome. Our study showed no significant difference in time to negative RT-PCR between the Lagundi and placebo groups.

Some researchers have used the cycle threshold (Ct) as a surrogate marker for the viral load, which is inversely related to the number of cycles required to exceed a threshold level.[44] In this study, there was a trend toward a steeper rise (higher slope) among Lagundi vs. placebo across all gene targets using the average Ct value per day of illness. A limitation is that many days of illness have only 1 data point. Visual inspection shows a similar increase between treatment and control Ct values across all target genes as the day of illness increases. There is some indication of faster viral clearance in the Lagundi group, although this data should be further substantiated due to the limited number of samples.

Lagundi was found to have an excellent safety profile for Stage 1 patients, even at the double dose. In Stage 2, the safety profile of Lagundi was further confirmed as there was no significant difference in the prevalence, grade of toxicity, and types of reaction between Lagundi and placebo. This further confirms that the reactions may be due to the disease process or maybe even the excipients of the placebo and Lagundi formulation, although such reports were few and of low grade. No serious adverse events were observed or reported.

5. Conclusion

Lagundi decreased the symptoms and discomfort during mild covid disease, especially for anosmia, and overall relief and discomfort due to other symptoms (significantly compared to placebo). However, it did not decrease the time to recovery of the patients. Lagundi was safely used in these patients without any serious adverse events. Mild adverse events of diarrhea, vomiting, pruritus, and nausea were not significantly different between Lagundi and placebo (events probably caused by the disease itself).

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2. Philippine National Police National Capital Region Office Special Care Facility
3. Rizal High School and Pasig City Health Department
4. Araullo High School and Manila City Health Department
5. Silungan Molave Quarantine Facility and UP Diliman Health Service
6. Valenzuela Quarantine Facility and Valenzuela City Health Department
7. Philippine General Hospital Extension Facility and Expanded Health Research Office of PGH

Conflicts of interest

The University of the Philippines Manila and the National Integrated Research Program on Medicinal Plants- Institute of Herbal Medicines are the licensors for Lagundi tablet and syrup NIPROMP formulation.

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Supplementary Information

Supplement S1. Baseline Laboratory Values for High-dose and Standard-Dose Lagundi Groups

Laboratory Parameter	Baseline (n=75)			End line (n=70)		
	High dose Lagundi (Mean, SD)	**Standard dose Lagundi (Mean, SD)**	**P-value**	**High dose Lagundi (Mean, SD)**	**Standard dose Lagundi (Mean, SD)**	**P-value**
Hematology						
Hemoglobin (g/dL)	15.31, 1.37	14.63, 1.72	0.062	15.19, 1.38	14.64, 1.66	0.133
Hematocrit	0.45, 0.04	0.43, 0.04	0.083	0.45, 0.10	0.43, 0.04	0.137
RBC count (10^{12} cells/L)	5.15, 0.48	5.10, 0.54	0.656	5.08, 0.50	5.04, 0.52	0.782
MCHC (g/L)	343.7, 10.3	340.4, 16.3	0.299	346.5, 9.4	343.1, 12.3	0.206
WBC count (10^9 cells/L)	7.30, 2.37	6.88, 2.48	0.458	7.92, 1.89	7.58, 2.16	0.495
Neutrophils, %	56.30, 9.19	54.74, 9.06	0.461	57.53, 8.93	57.17, 7.57	0.855
Lymphocytes, %	35.51, 8.24	36.92, 8.71	0.475	33.59, 8.46	34.19, 6.96	0.744
Eosinophils, %	2.68, 0.67	2.71, 0.57	0.808	2.97, 0.63	2.75, 0.50	0.107
Basophils, %	0.11, 0.31	0.11, 0.31	0.969	0.18, 0.39	0.22, 0.42	0.638
Stabs, %	0.33, 0.48	0.36, 0.49	0.852	0.83, 0.39	0.86, 0.36	0.873
Platelet count (10^9 cells/L)	363.19, 98.29	351.61, 94.17	0.604	364.47, 93.49	381.39, 114.46	0.502
Blood Chemistry						
RBS (mg/dL)	97.76, 56.71	76.88, 12.11	**0.030**	85.81, 40.24	79.05, 25.93	0.404
BUN (mg/dL)	9.16, 8.37	9.35, 2.55	0.735	8.68, 1.88	8.93, 2.54	0.642
Creatinine (mg/dL)	0.86, 0.18	0.83, 0.18	0.492	0.86, 0.17	0.85, 0.20	0.817
AST (U/L)	49.58, 40.16	32.36, 14.01	**0.015**	36.10, 17.93	25.85, 9.14	**0.003**
ALT (U/L)	75.28, 77.96	38.67, 23.04	**0.007**	58.18, 50.23	34.19, 20.88	**0.010**

Supplement S2 Occurrence of diarrhea among patients by time period, severity, and group

Diarrhea Time	Severity	High dose Lagundi, N(%)	Standard dose Lagundi, N(%)	P-value
Day 4 (n=73)	Grade 1	0 (0%)	2 (5.4%)	0.225
	Grade 2	1 (2.8%)	0 (0%)	
	None	35 (97.2%)	35 (94.6%)	
Day 8 (n=68)	Grade 1	2 (5.9%)	0 (0%)	0.151
	None	32 (94.1%)	34 (100%)	
Day 12 (n=62)	Grade 1	1 (3.3%)	0 (0%)	0.298
	None	29 (96.7%)	32 (100%)	
Day 16 (n=68)	None	35 (100%)	33 (100%)	
Day 20 (n=71)	None	36 (100%)	35 (100%)	
Total reports		4	2	

Day 4: 2 Grade 1 and 1 Grade 2 on tablet preparation, Day 8: 1 Grade 1 on tablet preparation, Day 12: Grade 1 on tablet preparation

Supplement S3 Mean values of laboratory results at baseline and endline laboratory values for Stage 2, Lagundi vs. Placebo

Laboratory Parameter	Baseline (n=202)			Endline (n=146)			Normal values
	Lagundi (Mean, SD) (N=102)	Placebo (Mean, SD) (N=100)	P-value	Lagundi (Mean, SD)	Placebo (Mean, SD)	P-value	
Hematology							
Hemoglobin (g/dL)	14.52, 1.70	15.08, 1.48	**0.013**	14.39, 1.67	14.79, 1.59	0.135	116-166
Hematocrit	0.44, 0.04	0.45, 0.04	0.052	0.44, 0.05	0.44, 0.04	0.543	35.5-48.6
RBC count (10^{12} cells/L)	5.29, 0.72	5.27, 0.50	0.836	5.23, 0.67	5.19, 0.50	0.683	3.92-5.65
WBC count (10^{9} cells/L)	6.11, 1.71	6.26, 1.53	0.498	7.52, 2.08	7.45, 1.73	0.832	3.4-9.6
Neutrophils, %	50.86, 9.18	51.70, 8.26	0.497	54.90, 7.98	54.01, 7.35	0.153	18-77
Lymphocytes, %	39.80, 9.00	39.26, 8.00	0.651	36.03, 7.22	37.89, 6.57	0.104	10-48
Eosinophils, %	3.20, 1.10	3.04, 0.95	0.283	3.10, 1.00	2.91, 0.95	0.236	0-5
Basophils, %	0.05, 0.33	0.05, 0.22	0.980	0.01, 0.12	0.03, 0.16	0.595	0-2
Stabs, %	0.76, 0.74	0.85, 0.80	0.564	0.71, 0.57	0.88, 0.56	0.248	<1
Platelet count (10^{9} cells/L)	310.71, 105.18	314.71, 99.45	0.781	379.13, 107.02	391.39, 104.82	0.486	135-371
Blood Chemistry							
RBS (mg/dL)	87.42, 53.60	81.94, 36.39	0.397	88.60, 58.99	84.52, 45.21	0.639	65-110
BUN (mg/dL)	10.51, 5.93	9.97, 4.03	0.457	9.86, 3.03	9.70, 2.25	0.726	8-21
Creatinine (mg/dL)	0.89, 0.28	0.94, 0.39	0.286	0.86, 0.23	0.89, 0.24	0.494	0.8-1.3
AST (U/L)	37.64, 25.00	39.91, 25.70	0.526	44.39, 32.12	41.47, 30.91	0.576	5-30
ALT (U/L)	42.57, 35.46	46.38, 32.37	0.426	53.73, 47.62	51.01, 37.45	0.701	5-30