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HYSSOP and POLIUM could help to prevent COVID-19 in high-risk population: The results of a parallel randomized placebo-controlled field trial

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This study was conducted to evaluate the effect of HYSSOP (composed of *Hyssopus officinalis* L., *Echium amoenum Fisch & C. A. Mey* and *Glycyrrhiza glabra* L.) and POLIUM (contained *Teucrium polium* L., *Cuscuta epithymum* Murr and *Cichorium intybus* L.) combined distilled herbal medicines compared to placebo in the prevention of COVID-19. This is a double-blind parallel placebo-controlled field trial conducted on 751 asymptomatic individuals whose one of the family members recently had a positive RT-PCR test for COVID-19. They were divided into three groups including POLIUM, HYSSOP and placebo using random blocks with a 1:1:1 allocation ratio. Participants received daily 5 cc (under 12 years) or 10 cc (over 12 years) of allocated oral medications for 20 days. The primary outcome was the frequency of positive RT-PCR test among participants who became symptomatic. The mean age of participants was 36.6. Nineteen participants get infected by COVID-19 during the intervention; fifteen of them belonged to the placebo and four to the POLIUM group. Fisher's exact test indicated significant differences between HYSSOP and placebo ($p<0.001$) as well as POLIUM and placebo ($p=0.009$) groups in terms of COVID-19 confirmed by PCR tests. Cox regression model adjusted for confounders illustrated that the hazard of getting infection by COVID-19 in POLIUM and HYSSOP groups decreased by 66% (OR (95% CI): 0.34 (0.12 to 0.94); $p=0.038$) and 93% (OR (95% CI): 0.07 (0.01to 0.56); $p=0.012$) respectively, compared to placebo .Oral administration of HYSSOP and POLIUM with the other supportive health care could decrease the risk of getting COVID-19 .

Keywords: COVID-19, HYSSOP, Medicinal herbs, POLIUM, Prevention

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Coronaviruses (CoVs) are a group of enveloped viruses with a single-stranded, non-segmented and positive-sense RNA genome[1]. All CoVs are pleomorphic RNA viruses that typically contain crown-shaped peplomers with a size of 80-160 nM and a positive pole of 27-32 kb[2]. The recombination rate of CoV is very high due to transcription errors, which develop continuously and jump in RNA polymerase-dependent RNA polymerase (RdRP)[3]. Coronaviruses, because of their high mutations, are zoonotic pathogens that exist in humans and different animals. CoV infection's clinical features are varied from asymptomatic course to the hospitalization requirement and even intensive care unit involvement by causing infections in the respiratory, gastrointestinal, hepatic and nervous system[4–6].

COVID-19 is caused by a member of the beta-coronaviruses family namely severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[7,8]. SARS-CoV-2 begins its infection through the interaction

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with angiotensin-converting enzyme 2 (ACE2) receptors and transmembrane serine protease 2 (TMPRSS2) on the target cell membrane[9]. The mortality of COVID-19 originates mainly from acute respiratory distress syndrome and severe cytokine release syndrome[10]. Globally, there have been 423,437,674 confirmed cases of COVID-19, including 5,878,328 deaths. Accordingly, 6,942,452 confirmed cases consisting of 135,040 deaths have been reported from Iran till Feb 20, 2022[11].

Besides vaccine development, and scientific efforts in order to protect the high-risk population against COVID-19 by developing prophylactic agents, very limited studies give satisfactory outcomes. Although anti-inflammation and anti-viral agents may have the potential to be prophylactic candidates, their related adverse event limits their usage in the high-risk population. Natural products and herbal medicines are involved in one-third of Food and Drug Administration (FDA)-approved drugs with the potential of disease prevention. Regarding the acceptable toxicity of herbal medicine, they are still a promising resource for drug discovery, in the current crisis of COVID-19 as a prophylactic candidate against COVID-19[12].

Hyssopus officinalis L. is a plant belonging to the Lamiaceae family and is rich in aromatic volatile constituents. Its essential oil contains high amounts of iso-pinocamphone, *cis*-pinocamphone, terpinene-4-ol, β-pinene, pinocarvone, p-cymene, caracerol, elemol, and myrtenal. In addition to the regulatory function of *Hyssopus officinalis* L. on the immune system, its anti-inflammatory impact on the asthmatic mouse model was affirmed[13]. *Hyssopus officinalis* L. has been used in traditional herbal medicines due to its antiseptic, cough relieving and expectorant properties[14]. *Echium amoenum* Fisch & C. A. Mey has been used to treat cough, dyspnea, sore throat, and pneumonia[15]. *Glycyrrhiza glabra* L. as an anti-inflammatory agent is applied to treat allergic reactions, rheumatism and osteoarthritis[16].

Teucrium polium L. belongs to the Lamiaceae family. Monoterpenes compose 66.94% of the oil and the effectiveness of this medicinal herb has been attributed to them. The percentage of the sesquiterpenes and sesquiterpenoid constituents is relatively low (33.06%)[17]. Antioxidant, antiviral, antibacterial, anti-fungal, anti-inflammatory, anti-analgesic and antipyretic effects of *Teucrium polium* L. have been known action which is mainly due to the phenolic content[18-23]. In modern medicine, *Cuscuta epithymum* Murr extract has shown antimicrobial and immune-stimulant properties[24]. Numerous studies have demonstrated antimicrobial, antioxidant, anti-inflammatory and anti-allergic effects of *Cichorium intybus* L.[25-30].

In terms of side effects, some of the complications of HYSSOP and POLIUM, such as the possibility of developing seizures in children, allergic reactions and miscarriage are related to very high doses of this plant (10-15 drops). No side effects have been reported with low doses such as those used in this study[18,31]. In traditional Iranian medicine, no special complication for these plants has also been reported[32].

In addition to the other public health measures taken to prevent COVID-19, natural products can be used to boost our immunity[33,34]. Despite the healing properties and beneficial pharmacological effects of the mentioned medicinal plants, according to the comprehensive literature search conducted by the authors, no controlled trials have been found that investigate the efficacy of these compounds on the prevention or treatment of COVID-19 separately or in combination. Therefore, this trial was performed to evaluate the effect of POLIUM and HYSSOP combined distilled herbal medicines in the prevention of COVID-19 in high-risk individuals exposed to the virus due to their above-mentioned properties.

Materials and Methods

Study design and participants

This study is a double-blind placebo-controlled field trial with three parallel arms that was conducted in Tabriz, Iran. The target population was the high-risk households which were defined as family members who have recently been in contact with a confirmed case of COVID-19 using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) during the last 14 days and met the eligibility criteria. Inclusion criteria included: age of 7 years and older, having a signed informed consent form to participate in the study, not participating in another clinical trial at the same time. Exclusion criteria were: people with suspicious, probable, or definite symptoms of the disease and in need of hospitalization, or receiving COVID-19 medicine on an outpatient basis, participants with history of seizure, pregnant or breastfeeding women, having liver or renal failure according to the individual or family declarationand history of allergies to plant products.

Primary outcome included the frequency of positive specific COVID-19 tests among participants who became symptomatic during the study and secondary outcomes comprised the frequency of disease symptoms including dry cough, chills, sore throat, dyspnea/ difficulty in breathing, fever (body temperature equal or more than 37.8°C) after the beginning of the intervention, frequency of side effects and satisfaction with medications in the study groups.

The sample size was calculated according to the study of Luo *et al.*[35] in the field of the effect of medicinal plants on the prevention of SARS and MERS coronaviruses with RR: 0.36, α =0.05, β =0.05, and power =95% equal to 91 people that considering a 10% drop for each group, the final sample size was estimated 100 people for each group, totally 300 people for three groups. In this study, 300 households instead of 300 individuals, i.e., 100 eligible households were considered for each group. Each household was a research unit and all eligible members received an identical intervention.

Sampling

After the approval of the Ethics Committee of the Vice Chancellor for Research and Technology of Tabriz University of Medical Sciences (IR.TBZMED.REC.1399.026), registration of the study in the Iranian registry of clinical trials (IRCT20200503047280N1), obtaining a letter of introduction from the university, and coordination with the officials of Tabriz Health Vice-Chancellor, individuals who were newly diagnosed with the disease were identified in each of the health centers of Tabriz and their family phone numbers were obtained. After making a phone call and explaining the objectives and method of this research, they were recruited into the study upon their eligibility and satisfaction to participate in the study. A signed informed consent form was obtained from all members of eligible households.

Randomization and intervention

Three hundred households were divided into three groups: two medicinal polyherbs and one placebo group using RAS (Random Allocation Software) by random six- and nine-sized blocks with a 1: 1: 1 allocation ratio. The medicinal polyherbs included two types of combined distilled remedies named POLIUM and HYSSOP.

In this study, the required numbers of herbal medicines bottles were given to the exposed households at the risk of COVID-19 infection based on the number of volunteer eligible household members. The interventional medicines were prepared as combined herbal distillates in white matte bottles with a volume of 500 cc. Placebo and two herbal medications were similar in appearance and odor. All bottles were soaked in an identical herbal solution to blind in terms of smell for 24 h before preparation and labeling and then thoroughly rinsed and dried.

Participants were randomly allocated into the study groups after obtaining a signed consent form (from participants or their parents) and completing demographic and disease-related questionnaires. They received 5 (under 12 years) or 10 cc (over 12 years) oral medications per day after breakfast for 20 days.

The random allocation sequence was generated by the non-involved person in the study. The bottles were numbered from 1 to 300 based on the produced sequence. The first bottle was given to the first eligible household and this process continued until the samples were completed. Follow-up of the recruited people regarding the occurrence of COVID-19 symptoms, taking drugs, need for additional bottles, and investigating the possible occurrence of side effects caused by the drugs was carried out every five days by phone call and filling out the information form. In case of presence of symptom, the person was examined by a physician for further evaluation, and if diagnostic and therapeutic measures were required for disease, the patient was referred to the care centers. The results of follow-ups and specific RT-PCR tests were recorded in the files of individuals. In this study, participants, researchers, analysts and the data monitoring team were blind to the interventions.

All participants were instructed on how to take the medicines and were given a medication daily-use checklist to complete after consumption. It should be noted that all individuals were provided with health advice to prevent COVID-19, according to the protocol of the Iran's Health Ministry.

Preparation of herbal medicines

The two types of combined medicines which were investigated in this research were produced by steam distillation from the leaves, flowers and terminal branches of the herbs or from the whole plant The first combination named POLIUM contained *Teucrium polium* L., *Cuscuta epithymum* Murr and *Cichorium intybus* L. with a ratio of 3: 1: 2 (with the number and date of production and exploitation license 5/80245-733; 10/03/2018 by the Deputy of

Food and Drugs/Iran). The second polyherb, called HYSSOP consisted of *Hyssopus officinalis* L., *Echium amoenum* Fisch & C.A. Mey and *Glycyrrhiza glabra* L.with a ratio of 2: 1: 1 (with the number and date of production and exploitation license 5/80245-733; 10/03/2018 by the Deputy of Food and Drugs/Iran). Two distilled herbal compounds were analyzed for some required indicators. The results have been shown in Table 1. Standardization of herbal medicine was performed based on Phenol and antioxidant capacity.

Assessment of study variables

In this trial, a checklist related to the eligibility criteria for the selection of participants before randomization, an informed consent form, a demographic information questionnaire including age, gender, occupation, education, family income, smoking, supplement use, underlying diseases, immune system deficiencies such as prednisolone therapy more than two weeks, chemotherapy, cancers, organ transplantation and AIDS, weight and height, a questionnaire related to observing the essential tips for disease prevention, a clinical symptoms questionnaire, a daily medication intake checklist, a side effects checklist,and satisfaction with medication scale were used to collect data. Content and face validity was used to determine the validity of the questionnaires; So that they were given to ten academic members, and after collecting their comments, corrections were made on the tools based on the feedback obtained. All RT-PCR tests in this study were evaluated by one type of kit.

To perform a specific COVID-19 test, firstly nucleic acid purification was done using a viral RNA/ DNA extraction kit made by Payesh Gene Company according to the manufacturer's instructions and then the COVID-19 One-Step RT-PCR molecular detection kit made by Danesh Bonyan Pishtaz Teb Company was applied for diagnosis according to the manufacturer's instructions. The diagnosis was made according to the manufacturer's instructions. The specificity of this test was 100, and its sensitivity was equal to the minimum number of detectable copies (limit of detection) of 200 copies per milliliter.

Table 1 — Biochemical analysis of herbal medicine

Indicators	POLIUM analysis	POLIUM analysis
Dry solid residue	0.05±0.01 mg/mL	0.05±0.01 mg/mL
Total Carbohydrates	10.60±1.23 µg/mL	10.60±1.23 µg/mL
Total protein	Zero	Zero
Total Phenol	30.85±1.62 µg gallic acid/mL	30.85± 1.62 µg gallic acid/mL
Antioxidant capacity (DPPH assay)	44.33±4.04% *	44.33±4.04% *

*One mL product with one mL of 0.1 mM 2, 2-diphenyl-1 picrylhydrazyl (DPPH)

Statistical analysis

The research results were statistically analyzed using SPSS/ version 21. Frequency and mean (standard deviation) indicators were applied to describe quantitative and qualitative variables. The comparison between study groups was made using Chi-square, Fisher's exact test, One-way Anova and Kruskal-Wallis. The Cox regression model was used to evaluate the hazard of getting the infection with a 95% confidence interval adjusted and non-adjusted for confounders based on clinical judgment. $p<0.05$ was considered a significant level and all analyses were performed based on ITT.

Results

In this study, 1021 individuals were assessed for eligibility, 270 were excluded for not willingness to participate in the study, pregnancy, breastfeeding, definitive coronavirus infection (n=13), age less than 7 years and renal or liver failure. Finally, 300 high-risk households were randomly allocated into three POLIUM (n=268), HYSSOP (n=231), or placebo groups (n=252) (100 households per each group). During the study, 42 people in the POLIUM group, 44 in the HYSSOP group and 38 in the placebo group discontinued intervention. All participants were included in the final analysis (Fig. 1).

The mean age (Standard Deviation) of POLIUM, HYSSOP and Placebo groups were 36.4 (17.4), 36.2 (19.6) and 37.2 (18.1), respectively (p=0.829). The ratio of male to female was about half in all three groups (p=0.423). Other socio-individual characteristics have been shown in Table 2. There were no statistically significant differences between study groups in terms of baseline characteristics (age, sex, marital status, occupation, housing status, education, number of households, smoking, smoker roommate, type of supplement received, taking other medications, having any disease, weight, and body mass index (p>0.05) other than family income (p=0.039) and supplement use (p=0.015).

Investigating the medical history among participants revealed that the highest rates of underlying diseases were related to hypertension, diabetes, heart disease and thyroid disorders, respectively. There was no statistically significant

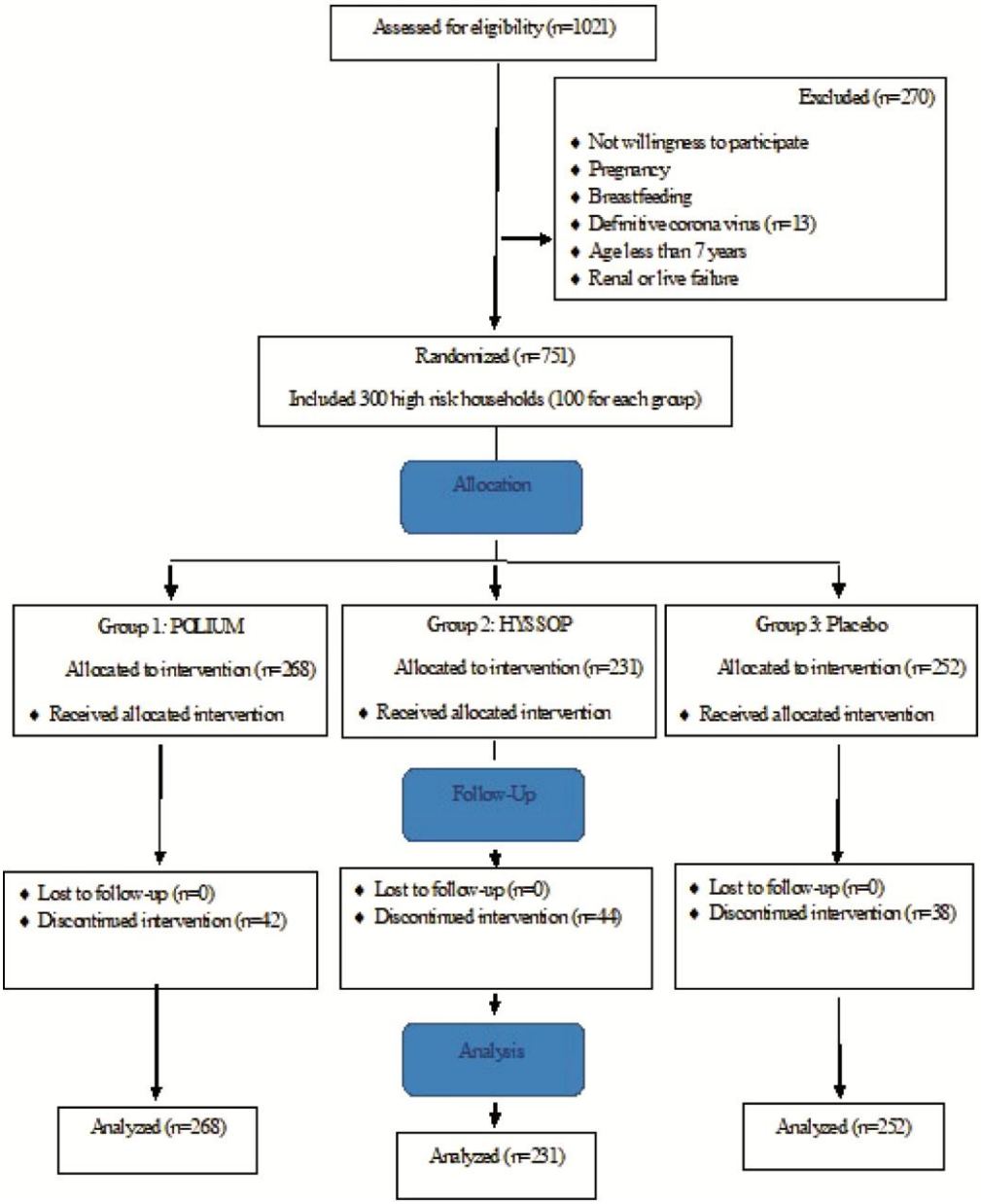

Fig. 1 — Summary of study flow diagram

difference between the study groups in terms of underlying conditions, including heart disease, diabetes, hypertension, respiratory diseases, thyroid disorders, and immune system disorders (history of taking prolonged corticosteroids or chemotherapy) ($p>0.05$) (Table 3).

Nearly 100% of people in all study groups observed the health tips related to COVID-19 according to the self-declaration.

In this study, 19 participants got infected by COVID-19 during the intervention; fifteen of them belonged to the placebo and four to the POLIUM groups. No case of the disease was observed in the HYSSOP group. There was a significant difference between the three groups in terms of getting COVID-19 ($p<0.001$). Comparing the groups with Fisher's exact test indicated that there was a significant difference between HYSSOP and placebo groups ($p<0.001$) and also between POLIUM and placebo groups ($p=0.009$). The most common associated symptoms of COVID-19 were related to fatigue and myalgia (53.3%) in the placebo group (Table 4).

Table 2 — Baseline characteristics of individuals among study groups

Variable	POLIUM	HYSSOP	Placebo	p-value
Age (year) *n (%)*				
7-12	26 (9.9%)	31 (13.5%)	20 (8.2%)	
13-19	29 (11%)	25 (10.9%)	28 (11.4%)	
20-40	102 (38.8%)	86 (37.6%)	98 (40%)	0.386
40-65	94 (35.7%)	67 (29.3%)	83 (33.9%)	
66-90	12 (4.6%)	20 (8.7%)	16 (6.5%)	
Mean±SD	36.4 (17.4)	36.2 (19.6)	37.2 (18.1)	0.829
Gender *n (%)* Male	140 (52.4%)	112 (48.5%)	137 (54.4%)	0.423
Female	127 (47.6%)	119 (51.5%)	115 (45.6%)	
Marital status n (%)				
Single, Widow, Divorced	100 (37.6%)	105 (45.7%)	99 (39.3%)	0.164
Married	166 (62.4%)	125 (54.3%)	153 (60.7%)	
Occupation*n (%)*				
Housewife	79 (30.3%)	84 (36.4%)	75 (30.2%)	
Employed	93 (35.6%)	64 (27.7%)	76 (30.6%)	
Unemployed	20 (7.7%)	14 (6.1%)	32 (12.9%)	0.060
Retired	9 (3.4%)	16 (6.9%)	15 (6.0%)	
Student	60 (23.0%)	53 (22.9%)	50 (20.2%)	
Family income *n (%)*				
Adequate	72 (27.6%)	56 (24.8%)	62 (24.8%)	
Relatively adequate	156 (59.8%)	131 (58.0%)	130 (52.0%)	0.039
Inadequate	33 (12.6%)	39 (17.3%)	58 (23.2%)	
Housing status *n (%)*				
Private	217 (81.3%)	192 (83.5%)	211 (84.1%)	0.673
Rental or relatives house	50 (18.7%)	38 (16.5%)	40 (15.9%)	
Education *n (%)*				
Illiterate	26 (9.8%)	21 (9.3%)	27 (10.8%)	
Primary	59 (22.3%)	47 (20.7%)	49 (19.5%)	
Secondary	40 (15.1%)	45 (19.8%)	36 (14.3%)	0.803
High school	23 (8.7%)	16 (7.0%)	15 (6.0%)	
Diploma	66 (24.9%)	52 (22.9%)	70 (27.9%)	
Academic	51 (19.2%)	46 (20.3%)	54 (21.5%)	
Household numberMean±SD	4.0 (2.8)	3.6 (1.0)	3.7 (2.3)	0.161
Smoking *n (%)*				
Yes	48 (18.0%)	26 (11.3%)	45 (17.9%)	0.074
Smoker roommate *n (%)* Yes	63 (23.6%)	57 (24.7%)	54 (21.4%)	0.687
Supplement use‖ *n (%)*				
Yes	54 (20.2%)	30 (13.0%)	58 (23.0%)	0.015
Type of supplement*n (%)*				
Vit D	27 (87.1%)	24 (88.9%)	37 (87.1%)	
Calcium	1 (3.2%)	1 (3.7%)	0 (0%)	
Zinc	0 (0%)	1 (3.7%)	2 (4.8%)	0.577
Vit C	3 (9.7%)	0 (0%)	2 (4.8%)	
Vit B	0 (0%)	1 (3.7%)	1 (2.4%)	
Having any disease*n (%)*Yes	22 (8.2%)	16 (6.9%)	23 (9.1%)	0.675
Taking other medications *n (%)*Yes	15 (5.6%)	10 (4.3%)	15 (6.0%)	0.708
Weight (kg) Mean±SD	70.5 (19.8)	67.3 (19.7)	70.8 (16.70	0.076
BMI (kg/m^2) Mean±SD	32.3 (92.9)	39.4 (140.8)	26.4 (10.05)	0.333

BMI: Body Mass Index

*Chi-square †One-way Anova ‡Fisher's exact test §Linear by linear

‖Supplements containing vitamin D and calcium

Table 3 — Distribution of underlying diseases among study groups

Variable	POLIUM *n (%)*	HYSSOP *n (%)*	Placebo *n (%)*	*p-value
Heart disease	5 (1.9%)	4 (1.7%)	5 (2.0%)	0.979*
Hypertension	13 (4.9%)	13 (5.6%)	9 (3.6%)	0.554*
Mellitus Diabetes	11 (4.1%)	5 (2.2%)	10 (4.0%)	0.430*
Respiratory disease	3 (1.1%)	1 (0.4%)	1 (0.4%)	0.630‡
Thyroid disorders	2 (0.7%)	4 (1.7%)	4 (1.6%)	0.527‡
Immune system disorders (Prednisolone, chemotherapy)	1 (0.4%)	3 (1.3%)	2 (0.8%)	0.248‡
Other disease	-	-	2 (100%)	-

*Chi-square ‡Fisher's exact test

Table 4 — Frequency of Positive PCR and associated symptoms during 20 days intervention by study groups

Variable	POLIUM *n (%)*	HYSSOP *n (%)*	Placebo *n (%)*	‡p-value
PCR	4 (1.5%)	0 (0%)	15 (6.0%)	<0.001
	4 (1.5%)	-	15 (6.0%)	p=0.009
	-	0 (0%)	15 (6.0%)	<0.001
Sore throat	1 (25.0%)	-	5 (33.3%)	-
Dry cough	2(50.0%)	-	6 (40.0%)	-
Fever	0 (0.0%)	-	4 (26.7%)	-
Chilling	0 (0.0%)	-	5 (33.3%)	-
Vomiting	1 (25.0%)	-	3 (20.0%)	-
Diarrhea	1 (25.0%)	-	3 (20.0%)	-
Dyspnea /Difficulty in breathing	0 (0.0%)	-	2 (13.4%)	-
Fatigue	2 (50.0%)	-	8 (53.3%)	-
Anorexia	0 (0.0%)	-	2 (13.3%)	-
Chest pain	0 (0.0%)	-	3 (20.0%)	-
Myalgia	2 (50.0%)	-	8 (53.3%)	-
Loss of smell (anosmia)	0 (0.0%)	-	6 (40.0%)	-
Loss of taste (ageusia)	0 (0.0%)	-	6 (40.0%)	-

Table 5 — Cox regression model for getting infection after taking medicinal herbs compared to placebo

Variable	POLIUM	HYSSOP	POLIUM	HYSSOP
	HR (95% CI), p	HR (95% CI), p	HR (95% CI), p	HR (95% CI), p
	Non-adjusted		Adjusted	
Positive PCR	0.32 (0.12 to 0.86), 0.025	0.07 (0.01 to 0.52), 0.009	0.34 (0.12 to 0.94), 0.038	0.07 (0.01 to 0.56), 0.012

HR: Hazard ratio; Confounders included age, supplement use, family income, and BMI

Cox regression model adjusted for confounders illustrated that the hazard of getting infection by COVID-19 in POLIUM and HYSSOP groups decreased by 66% (OR (95% CI): 0.34 (0.12 to 0.94); p=0.038) and 93% (OR (95% CI): 0.07 (0.01 to 0.56); p=0.012) respectively, compared to placebo (Table 5, Fig. 2).

The most reported side effects following drug consumption were related to the group receiving POLIUM, which were nausea, vomiting and stomachache (by 4.9%, 4% and 3.5%, respectively). In the HYSSOP group, the most reported side effects were diarrhea (1.6%) and vomiting (1.6%). Overall, individuals in the placebo group reported the lowest complications. More than half of the participants in all three groups were satisfied and completely satisfied with the medication, in which the highest rate (63.3%) was obtained in the placebo group. Among the participants, the highest rate of dissatisfaction with the medicine was reported by the POLIUM group (6.9%), then by the placebo group (4.4%). The lowest dissatisfaction rate was in the HYSSOP group (4%) (Table 6). The rates of complete 20-day adherence to the POLIUM, HYSSOP, and placebo groups were 64.4%, 70.1% and 68.7%, respectively.

Discussion

This double-blinded clinical trial with the strict protocol was carried out to help modern medicine control the outbreak of disease and prevent mortality. In this study, 19 patients were diagnosed with definitive COVID-19 during the interventions based on PCR. Fifteen of them belonged to the placebo and 4 to the POLIUM groups. There were significant differences between the HYSSOP and placebo as well as between the POLIUM and placebo groups. No one in the group receiving HYSSOP had definite symptoms of coronavirus disease based on PCR. Most

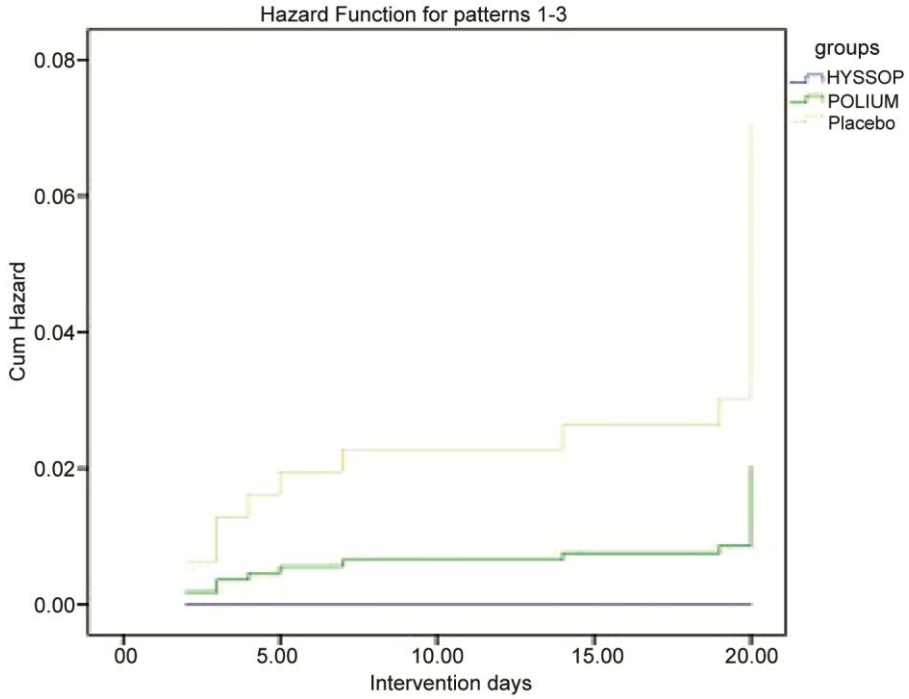

Fig. 2 — Hazard function during intervention by study groups

Table 6 — Frequency of reported side effects and satisfaction with medication among study groups

Variable	POLIUM *n (%)*	HYSSOP *n (%)*	Placebo *n (%)*
Side effects			
Nausea	11 (4.9%)	2 (1.1%)	1 (0.5%)
Vomiting	9 (4.0%)	3 (1.6%)	0 (0.0%)
Diarrhea	0 (0.0%)	3 (1.6%)	0 (0.0%)
Bloating	0 (0.0%)	2 (1.1%)	0 (0.0%)
Stomach ache	8 (3.5%)	1 (0.5%)	1 (0.5%)
Dizziness	1 (0.4%)	0 (0.0%)	0 (0.0%)
Satisfaction			
Completely satisfied	49 (18.7%)	17 (7.6%)	45 (18.1%)
Satisfied	106 (40.5%)	104 (46.2%)	112 (45.2%)
Neither dissatisfied nor satisfied	89 (34.0%)	95 (42.2%)	80 (32.3%)
Dissatisfied	18 (6.9%)	9 (4.0%)	11 (4.4%)
Completely dissatisfied	0 (0.0%)	0 (0.0%)	0 (0.0%)
p-vlaue*	0.004		

*Kruskal Wallis

COVID-19 symptoms were related to fatigue and body pain (53.3%) in the placebo group .

The pattern of infection in COVID-19 and SARS & MERS CoVs are similar, and all employ the ACE2 receptor to infect the human cells[36]. The study of SARS-CoV indicated that Traditional Chinese Medicine, in combination with western medicine, played a fundamental role in saving many lives. There is strong evidence of antiviral activities of some herbal extracts against SARS-CoV[37,38]. Also, Iranian Traditional Medicine, with or without modern medicine, has been successfully helping people to maintain health and wellbeing since the pre-modern era, which was mainly introduced by Avicenna. Furthermore, antiviral activities of some herb oil and extracts have been reported against viral infections like herpes viruses 1 & 2[39], HIV[40], influenza virusesand HBV[41] and SARS-Cov[38].

Because of the COVID-19 pandemic and high morbidity and mortality, many laboratories and clinical investigators recently focused on herbal medicine's products to use in combination with modern medicine for treating patients of COVID-19. The outcomes presented promising evidence to reduce mortalities and hospitalization and prevent further transmission of new viruses[42,43].

Hyssopus officinalis L. has been used in traditional herbal medicines due to its antiseptic, cough relieving and expectorant properties[14]. This plant has biological properties such as antimicrobial, antioxidant and antiviral properties[44]. N-hexane extract of *Hyssopus officinalis* L. indicated a strong inhibitory potency against the angiotensin-converting enzyme (ACE)[45]. SARS-CoV-2 binds to their target cells through ACE2, which is expressed by epithelial cells of the lung, kidney, intestine, and blood vessels[46].

In traditional Iranian medicine, *Echium amoenum* Fisch & C. A. Mey has been used to treat cough,

dyspnea, sore throat, and pneumonia[15]. Numerous new therapeutic properties have been mentioned for this plant, including anti-inflammatory, antioxidant, antiviral, analgesic, treatment for common cold, stress & anxiety disorders, rheumatoid arthritis, and prevention of pneumonia. The antioxidant activity of *Echium amoenum* Fisch & C. A. Mey can be attributed to the content of polyphenols and tannins and their flavonoids[47]. The main constituents of *Glycyrrhiza glabra* L. as the third component of the HYSSOP group are triterpene saponins. Flavonoid compounds include liquiritigenin and isoliquiritigenin. *Glycyrrhiza glabra* L. is used in the treatment of sore throat, coughs, and bronchitis. It is also used in the prevention and treatment of gastric and duodenal ulcers and indigestion. As an anti-inflammatory agent, it is applied in the treatment of allergic reactions, rheumatism, and osteoarthritis[16].

Teucrium polium L. is an essential component of first combined medicine. The flowering branches of this plant have a mild tonic, stomach tonic, anti-fever and antiseptic effect, and its active ingredients are useful in the healing of digestive systems and liver disorders. It's antioxidant, antiviral, antibacterial, anti-fungal, anti-inflammatory, anti-seizure, anti-analgesic, antipyretic, wound healing and anti-cancer effects have been proven[18-23]. The medicinal impacts of the plant have been attributed to monoterpenes consisting of a-Pinene, b-Pinene, Limonene, cis-Verbenol, Carvone, Bornyl acetate, etc. which are the main constituents of plant essential oils[17].

Numerous studies have shown antimicrobial, anti-malarial, antioxidant, anti-inflammatory, anti-cancer, neuroprotective, anti-hepatotoxic, anti-diabetic and anti-allergic properties of *Cichorium intybus* L., the second important component of *Teucrium polium* L.[25-30]. *Cuscuta epithymum* Murr is traditionally used as a laxative herb. It is used in treating mental illnesses including psychosis, depression and obsessions, diseases of the urinary and gastrointestinal tract, nervous system, etc. *C. epithymum* Murr extract has shown antimicrobial, immune stimulant, liver protection, antioxidant, anticonvulsant and anti-urease effects[24].

Based on the above literature, the ingredients of these compounds individually have their own effect and when combined could improve patients' condition as a result of anti-fever, antiviral & bacterial, anti-inflammatory and antioxidant properties and improve lung performance.

The researchers did not find a similar clinical study regarding the herbs' effect on the prevention of coronavirus for comparison and discussion. There are clinical studies mainly on the prevention or treatment of SARS, MERS and influenza. Therefore, this study is a unique clinical study that has been performed on the prevention of coronavirus in a large sample size in which the effect of medicinal plants on the prevention of coronavirus has been proven.

In this study, although the antioxidant capacity and total Phenol of combined herbs were determined, the exact mechanism of plant composition on controlling the coronavirus has not been investigated. Therefore, conducting cellular and molecular studies is suggested to find the main mechanism of the plant composition. Moreover, one determined dose of the combined herbal medicine was used, which was licensed by the Vice-Chancellor for Food and Drugs. Another limitation was not assaying RT-PCR for all participants following intervention and it was carried out only for those who became symptomatic during the intervention. Besides, the reported adverse events were not exactly attributable to the drug's reaction or manifestation of COVID-19.

Conclusion

It is concluded that the administration of both POLIUM and HYSSOP herbal medicines, especially HYSSOP, in combination with the other supportive care, could protect people from getting COVID-19. However, further evaluations are required to confirm the positive effects of herbal medicines in different settings and conditions.

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Conflict of Interest

Authors declare there is no conflict of interest.

Authors' Contributions

(1) The conception and design of the study: SMBF, AFKh, KK, JAH, ABM, PS; acquisition of data: AFKh, JAH, AD,HH,BN, HP; analysis and

interpretation of data AFKh, KK, SMBF MZ, EV,YR

(2) drafting the article or revising it critically for important intellectual content: AFKh, JAH, ABM, PS, AZ, ES, NGA, HSP, SS, and (3) final approval of the version to be submitted: AFKh, JAH, KK, SMBF MZ, ES, NGA, HSP. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

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