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A polyherbal formulation containing *Justicia pectoralis* Jacq., *Achyrocline satureioides* (Lam.) DC., and *Eclipta prostrata* (L.) L. helped reducing symptom intensity during and after Covid-19 infection: A retrospective, uncontrolled observational study

Fabio Carmona [a,*], Lucas Chaves [a], Fabiana Cardoso Tardelli do Nascimento [b], Débora Simone Sales [b], Bianca Waléria Bertoni [b,c], Gustavo Henrique Teixeira Pinto [c], Pedro de Pádua Amatto G. [c], Ana Maria Soares Pereira [b,c]

[a] *Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil*
[b] *Nature's Pharmacy, Spiritist House Land of Ismael, Jardinópolis, SP, Brazil*
[c] *University of Ribeirão Preto, Ribeirão Preto, SP, Brazil*

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ABSTRACT

Objectives: To retrospectively describe a local experience with a polyherbal formulation on reducing the symptoms of Covid-19 and the need for hospitalization. The formulation contained three species traditionally used for respiratory symptoms: *Justicia pectoralis* Jacq. (Acanthaceae, "chambá" or "tilo"), *Achyrocline satureioides* (Lam.) DC. (Asteraceae, "macela"), and *Eclipta prostrata* (L.) L. (Asteraceae, "erva-botão" or "bhringraj", synonym *Eclipta alba* L.).
Design: Observational, retrospective study.
Methods: More than 2000 adults with suspected Covid-19 received a polyherbal formulation with *J. pectoralis*, *A. satureioides*, and *E. prostrata*. About 20% of them were contacted by phone in an observational, retrospective study to assess the effects on symptom relief and hospitalizations. Two hundred patients were included. The identities and concentrations of the main compounds in the three species were determined using high-performance liquid chromatography (HPLC).
Results: Participants reported substantial improvement in the following symptoms: weakness, pain, cough, headache, dysgeusia, anosmia, fever, dyspnea, odynophagia, diarrhea, chest pain, and conjunctivitis. The need for hospitalization was not significantly lower than the one found in the Brazilian population with Covid-19. The main compounds in the species were: quercetin, luteolin, and 3-O-methylquercetin in *A. satureioides*; umbelliferone and coumarin in *J. pectoralis*; and demethylwedelolactone and wedelolactone in *E. prostrata*.
Conclusions: A polyherbal formulation containing *J. pectoralis, A. satureioides*, and *E. prostrata* helped relieving symptoms of Covid-19 but did not reduce the need for hospitalization.

1. Introduction

The World Health Organization (WHO) declared the new coronavirus (SARS-Cov-2) disease (Covid-19) pandemics in March 2020. Since then, the scientific community has been looking exhaustively for treatments without success [1]. In Brazil, as of December 2021, 22.2 million cases and more than 600 thousand deaths were recorded (2.8% lethality) [2]. At that time, 2020 and 2021, Brazil was having numerous cases of Covid-19 and one of the highest lethality rates worldwide. São Paulo was the state with the highest mortality in the country. Although many antiviral drugs were available in Brazil, such as oseltamivir and ribavirin, their routine use was not recommended by the Brazilian Ministry of Health because they lacked scientific proof of efficacy.

Mass vaccination worldwide is gradually decreasing the number of severe cases and deaths. However, mild to moderate cases of Covid-19 are still numerous, and some patients experience symptoms lasting for

* Correspondence to: Department of Pediatrics, Ribeirão Preto Medical School, University of São Paulo, Avenida dos Bandeirantes, 3900, Monte Alegre, Ribeirão Preto 14049–900, SP, Brazil.
E-mail address: carmona@fmrp.usp.br (F. Carmona).

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more than 12 weeks, a condition coined as post-Covid-19 syndrome, or simply long-covid. The most frequent symptoms are fatigue and breathlessness [3]. Nevertheless, there is a concern that current vaccines may not protect against new variants of the virus, such as the new omicron variant [4] and others that are likely to emerge in the future. Therefore, effective treatments for Covid-19 are still needed.

Polyherbal formulations have been successfully used in cardiovascular diseases, as immunomodulators, in metabolic syndrome, and in type-2 diabetes mellitus [5–10]. Nowadays, the advent of mass spectrometry-based analytical technologies coupled with multivariate statistical methods and pharmacokinetic studies of multicomponent medicines show that synergistic actions may be responsible for the therapeutic efficacy of a large number of polyherbal products [11,12].

More recently, single and polyherbal formulations have been used to improve symptoms of Covid-19. In 2020, it was reported that patients treated with a combination of antiviral drugs and polyherbal formulations such as Huoxiang Zhengqi dropping pills and Lianhua Qingwen granules experienced a significant improvement in their overall health and clinical symptoms of COVID-19 [13]. Subsequently, a study conducted on asymptomatic patients with COVID-19 who ingested a medication called Kabasura Kudineer, consisting of 15 medicinal plants consumed in the form of tea, showed a reduction in SARS-CoV-2 viral load, suggesting that the medication could reduce the potential transmission of the virus from patients with COVID-19 [14]. Another Ayurvedic formulation called BV-4051, containing *Withania somnifera*, *Boswellia serrata*, *Zingiber officinale*, and *Curcuma longa*, was administered, in the form of tablets, to patients with moderate symptoms of COVID-19, resulting in a reduction in the duration of the disease, severity of symptoms, as well as a decrease in interleukin-6 [15]. The plant species individually investigated for the treatment of Covid-19 include *Althaea officinalis, Commiphora molmol, Glycyrrhiza glabra, Hedera helix, Sambucus nigra, Allium sativum, Andrographis paniculata, Echinacea angustifolia, Echinacea purpurea, Eucalyptus globulus, Justicia pectoralis, Magnolia officinalis, Mikania glomerata, Pelargonium sidoides, Pimpinella anisum, Salix* spp*, and Zingiber officinale* [16].

Among these, *Justicia pectoralis* Jacq. (Acanthaceae, “chambá” or “tilo”) is widely used in Brazil and Central and South America for respiratory symptoms [17]. Coumarins present in this plant confer anti-inflammatory, bronchodilator, and expectorant activities. Flavonoids also contribute to the anti-inflammatory effect. A syrup containing *J. pectoralis* effectively alleviated cough, runny nose, and nasal congestion in children with upper viral respiratory infections [18].

Achyrocline satureioides (Lam.) DC. (Asteraceae, “macela”) is an annual herb common in the South of Brazil, and its inflorescences have been used as a remedy in folk medicine to treat flu, colds, and other respiratory problems [19]. This plant produces the flavonoids quercetin, luteolin, and 3-O-methylquercetin, all with antiviral, anti-inflammatory, and immunomodulating effects [20–26].

Eclipta prostrata (L.) L. (Asteraceae, “erva-botão” or “bhringraj”, synonym *Eclipta alba* L.) is another plant with the potential to treat Covid-19. It is historically used in Asia as an anti-inflammatory, hepatoprotective, bronchodilator, and expectorant [27]. *E. prostrata* exhibited important bronchodilator and anti-inflammatory effects in a murine model of allergic asthma [28]. A single clinical trial was published with this plant, in which *E. prostrata* decreased blood pressure and lipids and increased urine output and urinary sodium excretion [29].

In this retrospective study, we sought to describe the self-reported effects of a polyherbal formulation containing *J. pectoralis*, *A. satureioides*, and *E. prostrata* on relieving the symptoms of patients with Covid-19, reducing the need for hospitalization and, consequently, deaths.

2. Patients and methods

2.1. Study design and ethical approval

This is an observational, retrospective, telephone survey study, approved by the local institutional review board. The study was conducted under Brazilian regulations on studies with humans (CAAE: 50964721.0.0000.5498). The investigation was authorized by the Brazilian System for the Management of Genetic Heritage and Associated Traditional Knowledge (*Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado*, SisGen) (protocol number A4E4E79). We followed The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

2.2. Description and preparation of the polyherbal formulation

The plants (*J. pectoralis*, *A. satureioides*, and *E. prostrata*) were cultivated and harvested at *Farmácia da Natureza, Casa Espírita Terra de Ismael* (Nature’s Pharmacy, Spiritist House Land of Ismael), Jardinópolis, SP, Brazil (21°04′13.8″ S, 47°44′13.0″ W). A specimen of *Eclipta prostrata* was identified by Dr. Aristônio Magalhães Teles (Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Goiânia, GO, Brazil), *Achyrocline satureioides* by Dr. Ines Cordeiro (Instituto de Botânica, São Paulo, SP Brazil), and *Justicia pectoralis* by Dr. José Elvino do Nascimento Júnior (Departamento de Ciências Naturais, Universidade Federal de São João del Rei, São João del Rei, MG, Brazil). Vouchers specimens were deposited in the Herbarium of Medicinal Plants at UNAERP with numbers HPMU 848, HPMU 1533, and HPMU 3230, respectively.

The aerial parts were harvested at 9 am, washed, and dried with paper sheets. The plants were dried at 45 °C for 48 h in a circulating-air oven, and then ground to 40-mesh particle size (except *A. satureioides*). The powdered plant material was portioned in plastic bags (*J. pectoralis*, for infusion), or capsules containing 230 mg each (*E. prostrata*). For the syrup, briefly, a *J. pectoralis* tincture was made with the powdered plant material (10% w/v in ethanol 70% v/v in water). A tincture of *A. satureioides* was made with the dried inflorescences (10% w/v in ethanol 70% v/v in water). Both tinctures were added to a simple syrup (10% v/v each) [30,31]. We assessed quality control by analyzing standard compounds using high-performance liquid chromatography (HPLC).

All participants received one package containing: (a) *J. pectoralis* plus *A. satureioides* syrup (200 mL, if not diabetic), or *J. pectoralis* powdered plant material for infusion (15 g, if diabetic); plus (b) *E. prostrata*: powdered plant material in capsules (30 capsules with 300 mg each). The package also contained detailed instructions, as follows:

- *J. pectoralis* and *A. satureioides* syrup: take 10 mL (adults and adolescents >12 years) or 5 mL (children 5–12 years old) twice a day for ten days.
- *J. pectoralis* infusion: Mix 1.5 g of powder with 1 liter of hot water, wait for 5 min, then strain and store it in the refrigerator. Take 150 mL (adults and adolescents >12 years) or 3 mL/kg (children 5–12 years old, maximum 150 mL) six times a day for ten days.
- *E. prostrata* capsules: take one capsule three times a day for ten days.

All products were submitted to quality control according to the Brazilian Pharmacopeia standards and specific regulations to Living Pharmacies [32].

2.3. Participants

In 2020 and 2021, this polyherbal formulation was freely distributed to whoever sought help at our institution, as part of our efforts to mitigate the burden of Covid-19 in our region. Potential participants

were identified in the list of patients that had received the formulation (over 2000 patients). All patients 18 years old or older that received the formulation, as recorded in our database, were eligible. A random sample (~20%) of the eligible patients was selected and contacted by phone call. They were included upon verbal consent. Participants were excluded if they refused to answer the questions after giving consent or upon later consent withdrawal.

The random sample of 20% of the eligible participants was generated using StataSE 14.0 (StataCorp LLC, College Station, Texas, USA) (command *sample*). Contact data of these patients were entered into a REDCap (*Research Electronic Data Capture*) database [33]. Two researchers (LC and PPAG) tried to contact each patient, read the consent form, and asked for consent. If consent was given, the researchers asked the questions and entered the answers directly into REDCap.

The questionnaire contained questions on demographics, the intensity of symptoms during the suspected or confirmed Covid-19 infection, the relative change in symptom intensity, and perceived side effects. The primary outcome was the perceived change on the most frequent Covid-19 symptoms. The baseline intensity of these symptoms was assessed with a numerical scale (0, absent; 1, mild; 2, moderate; 3, intense; 4, very intense), while the relative change after using the formulation was assessed with another numerical scale (−2, significant worsening; −1, slight worsening; 0, no change; +1, slight improvement; +2, significant improvement). The secondary outcome was a composite of the need for hospitalization or death.

2.4. High-performance liquid chromatography (HPLC)

2.4.1. Achyrocline satureioides

An aliquot (20 μL) of syrup was analyzed by HPLC, at room temperature (22 ± 1 °C), using a Luna C18(2) column (250 ×4.6 mm; 5 μm; Phenomex, Torrance, CA, USA) connected to a Shimadzu (Kyoto, Japan) LC-10ADvp model pump and an SPD-M10Avp PDA detector. The mobile phase consisted of HPLC grade 0.1% formic acid in water (solvent A) and methanol (solvent B) (solvent A and B; J.T. Baker), supplied at a flow rate of 0.8 mL/min. The mobile phase gradient varied as follows: initial value of 40% B (0–5 min), 40–70 - % B (5–30 min), 70–100% B (30–35 min), 100–40% B (35–40 min), and 40% B (40–42 min). Elutions of quercetin, luteolin, and 3-O-methylquercetin in the column effluent were monitored at 370 nm, 351 nm, and 357 nm, respectively. The acquired data were processed using the Shimadzu LabSolutions Multi LC-PDA software. HPLC calibration curves (Supplementary figure 1, Supplementary figure 2, and Supplementary figure 3) plotted with data from triplicate analysis of the reference standards quercetin (200–1.25 μg/mL), luteolin (300–2.50 μg/mL), and 3-O-methylquercetin (300–1.17 μg/mL) were used to determine the amount of the bioactive components in the samples. The limits of quantification and detection were, respectively, 1.04 and 0.34 μg/mL for quercetin, 1.08 and 0.35 μg/mL for luteolin, and 0.68 and 0.23 μg/mL for 3-O-methylquercetin. Standards quercetin (CAS 117–39–5, ≥98% -HPLC), luteolin (CAS 191–70–3, ≥98% -HPLC), and 3-O-methyquercetin (CAS 1486–70–0, ≥97% -HPLC) were purchased from Sigma-Aldrich Chemical Co (St. Luis, MO, USA).

2.4.2. Justicia pectoralis

An aliquot (20 μL) of syrup was analyzed by HPLC, at room temperature (22 ± 1 °C), using a Luna C18(2) column (250 ×4.6 mm; 5 μm; Phenomex, Torrance, CA, USA) connected to a Shimadzu (Kyoto, Japan) LC-10ADvp model pump and an SPD-M10Avp PDA detector. The mobile phase consisted of HPLC grade 0.1% acetic acid in water (solvent A), and methanol (solvent B) (solvent A and B; J.T. Baker) supplied at a flow rate of 1.0 mL/min. The mobile phase gradient varied as follows: initial value of 30% B (0–10 min), 30–40% B (10–20 min), 40% B (20–30 min), 40–70% B (30–35 min), 70–100% B (35–37 min), 100% B (37–40 min), and 100–30% B (40–45 min). Elutions of coumarin and umbelliferone in the column effluent were monitored at 280 nm and 310 nm, respectively. The acquired data were processed using the Shimadzu LabSolutions Multi LC-PDA software. HPLC calibration curves (Supplementary figure 4 and Supplementary figure 5) plotted with data from triplicate analysis of the reference standards coumarin (300–2.50 μg/mL), and umbelliferone (200–1.25 μg/mL) were used to determine the amount of the bioactive components in the samples. The limits of quantification and detection were, respectively, 2.02 and 0.66 μg/mL for coumarin, and 7.50 and 2.50 μg/mL for umbelliferone. Standards coumarin (CAS 117–39–5, ≥98% -HPLC) and umbelliferone (CAS 93–35–6, ≥98% -HPLC) were purchased from Sigma-Aldrich Chemical Co (St. Luis, MO, USA).

2.4.3. Eclipta prostrata

Aerial parts (10 g) were dried for 36 h in a circulating-air oven at 45 °C, pulverized, and passed through a 40-mesh sieve. The powdered material was steeped in ethanol:water (70:30, v/v) for seven days and subsequently filtered through a Whatman No. 41 filter paper. The filtrate was reduced to dryness on a rotary evaporator and lyophilized to yield 0.32 g of dry crude extract, resulting in a drug:extract ratio of 33:1. The extraction was performed in triplicate. A sample (1 mg) of the dried hydroethanolic extract was dissolved in 1 mL of a mixture of methanol (J.T. Baker HPLC grade) and Milli-Q Ultrapure water (Merck Millipore) (80:20; v/v), sonicated for 30 min, and filtered through a 0.45 μm Millipore filter (Merck Millipore, Darmstadt, Germany). Aliquots (20 μL) of this solution were analyzed using the HPLC with a mobile phase consisting of water (solvent A) and methanol (solvent B) supplied at a flow rate of 1 mL/min. in the form of a linear gradient from 10% to 66% B between 0 and 32 min and from 66% to 10% B between 32 and 35 min, and final isocratic elution with 10% B between 35 and 40 min. Elutions of wedelolactone and demethylwedelolactone in the column effluent were monitored at 280 nm and 310 nm, respectively. The acquired data were processed using the Shimadzu LabSolutions Multi LC-PDA software. HPLC calibration curves (Supplementary figure 6 and Supplementary figure 7) plotted with data from triplicate analysis of the reference standards wedelolactone (250–1.95 μg/mL) and demethylwedelolactone (250–1.95 μg/mL) were used to determine the amount of the bioactive components in the samples. The limits of quantification and detection were, respectively, 0.89 and 0.29 μg/mL for wedelolactone, and 4.03 and 1.33 μg/mL for demethylwedelolactone. Standards wedelolactone (CAS 91–64–4, ≥98% -HPLC) and demethylwedelolactone (CAS 6468–55–9, ≥98% -HPLC) were purchased from Sigma-Aldrich Chemical Co (St. Luis, MO, USA).

2.5. Statistical analysis

Results were summarized in means [standard deviations], medians (interquartile ranges), or counts (percentages), as appropriate. The occurrence of the outcome of hospitalization and respective 95% confidence interval (95%CI) were compared to the population parameter. The effect of the formulation on the relative change in the intensity of symptoms was assessed by testing the difference from zero (no change) with Wilcoxon signed-rank tests. We adopted a significance level of 0.05. We used StataSE 14.0 (StataCorp LLC, College Station, Texas, USA) and GraphPad Prism 6.0 (GraphPad Software, San Diego, California, USA).

3. Results

3.1. HPLC analyses

The medicines were analyzed through HPLC, and the respective chromatograms are shown in Fig. 1, Fig. 2, and Fig. 3. The chromatogram of *A. satureioides* (Fig. 1) shows that the main compounds are quercetin, luteolin, and 3-O-methylquercetin. Their concentrations in the syrup—determined through HPLC—were 21.76 ± 0.51 μg/mL, 8.43 ± 0.11 μg/mL, and 88.24 ± 8.11 μg/mL, respectively. Fig. 2 presents the

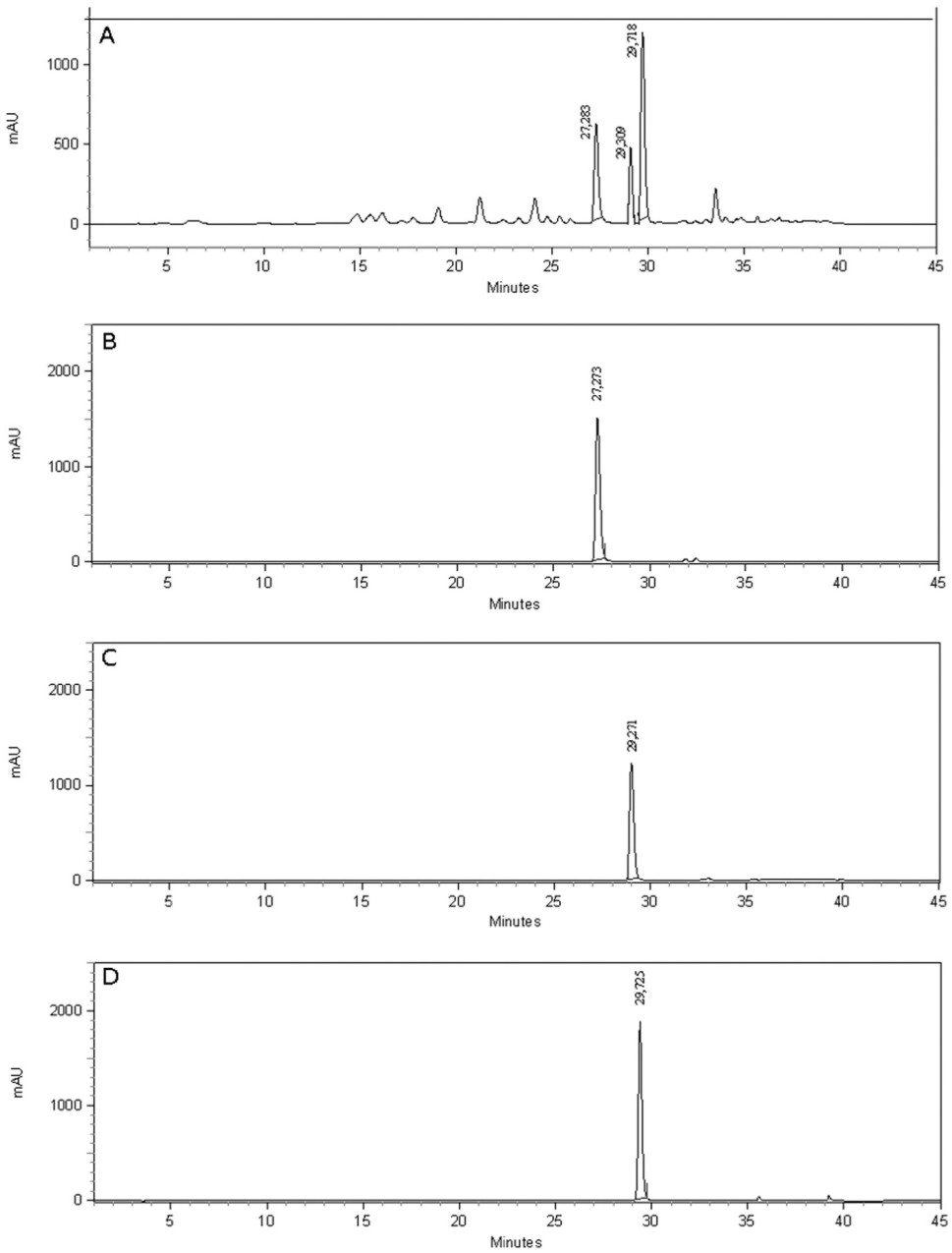

Fig. 1. HPLC-DAD chromatograms of the tincture from *Achyrocline satureioides* (A), and the standard compounds quercetin (B), luteolin (C), and 3-O-methylquercetin (D).

chromatogram of *J. pectoralis,* showing that the main compounds are umbelliferone and coumarin. Their concentrations in the syrup—determined through HPLC—were 0.99 ± 0.01 μg/mL and 70.17 ± 0.11 μg/mL, respectively. The chromatogram of *E. prostrata* (Fig. 3) shows that the main compounds are demethylwedelolactone and wedelolactone and that their concentrations in the hydroethanolic extract—determined through HPLC—were 1.11 ± 0.01 μg/mL and 2.9 ± 0.21 μg/mL, respectively.

3.2. Clinical results

A total of 445 people were contacted by telephone call, and 200 agreed with being interviewed (45%, Supplementary figure 8). Demographic characteristics and Covid-19 testing of all participants are summarized in Supplementary table 1. Briefly, they were 45 (± 16) years-old, mostly females (62.5%), from São Paulo state (62.3%), and with at least one positive Covid-19 test (74.6%). The participants presented many different symptoms, frequently of mild intensity (Fig. 4). The polyherbal formulation was correctly used by 183 participants (92%), while 14 (7%) reported not having used it, and two (1%) did not remember. Overall, there was a significant improvement (relative change from baseline) in all symptoms, except skin lesions (Fig. 5).

Regarding the need for hospitalization, one participant (0.5%) reported having been admitted in an emergency room, 14 (7.0%) in a hospital ward, and five (2.5%) in an intensive care unit (ICU). The overall need for hospitalization was 10.1% (95%CI 6.6, 15.1), which was not different from that reported in Brazil at the end of 2020 (9.7%) [34]. No deaths were reported in our study Fig. 6.

Among the side effects and adverse events reported by the participants, the main were diarrhea (n = 4), thrombosis (n = 1), colic (n = 1), and nausea (n = 1). Twelve participants (6.0%) reported having used other medications for Covid-19 concomitantly.

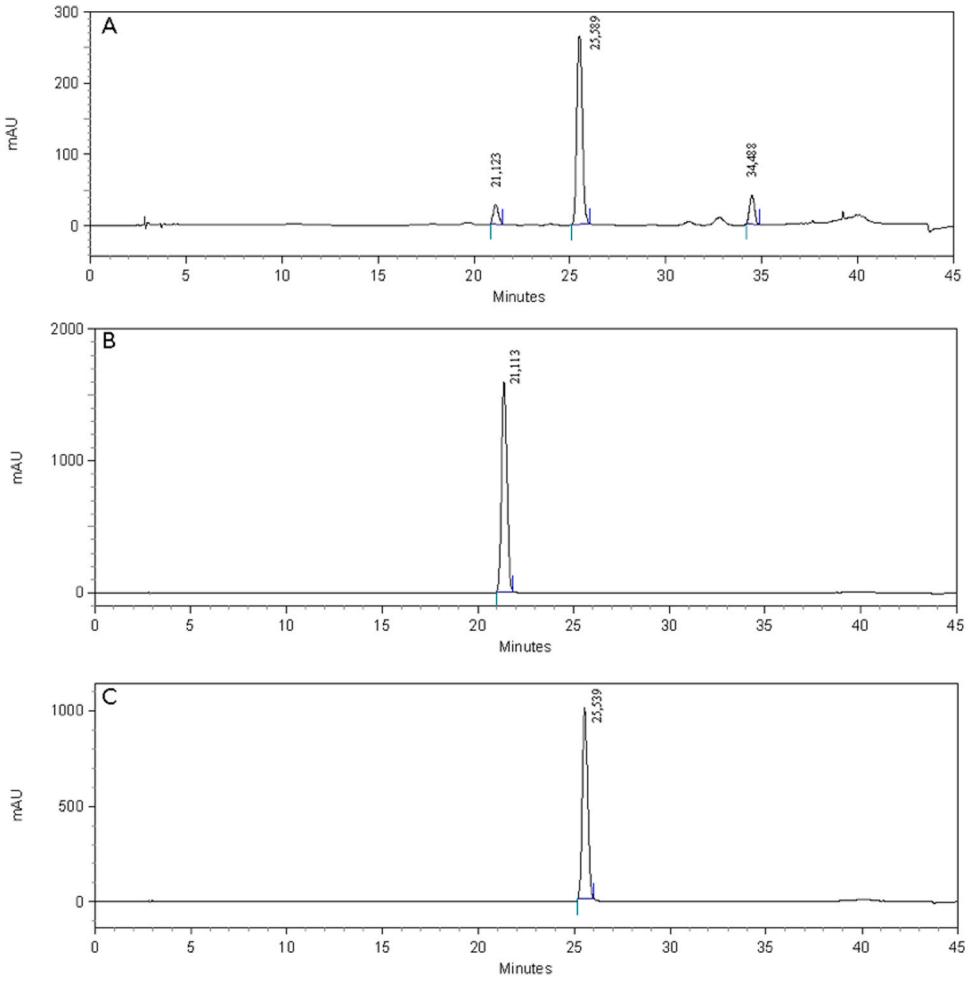

Fig. 2. HPLC-DAD chromatograms of the tincture from *Justicia pectoralis* (A), and the standard compounds umbelliferone (B) and coumarin (C).

4. Discussion

In this study, we found that a polyherbal formulation containing *J. pectoralis*, *A. satureioides,* and *E. prostrata* possibly helped alleviating symptoms of Covid-19 but did not decrease the need for hospitalization. The treatment caused only mild and transient side effects.

Many research groups from different countries have been pursuing to prove the benefits of herbal medicines in the treatment of Covid-19. These studies have focused on symptom control and prevention of hospitalization in mild to moderate cases. However, most evidence is preclinical, so translating these studies to clinical practice is difficult. Silveira et al., in their comprehensive review, recommend that *Althaea officinalis*, *Commiphora molmol*, *Glycyrrhiza glabra*, *Hedera helix*, and *Sambucus nigra* should be used as adjuvant treatments for early/mild cases of Covid-19 [16]. Other 12 species were considered promising.

Despite lots of preclinical findings of medicinal plants acting against SARS-Cov-2, only a few clinical trials on herbal medicines for Covid-19 have been published to date. In Iran, we found three studies. In the first one, a single-blind study, 100 adult patients with suspected Covid-19 infection were randomly allocated to receive a combination of *Zingiber officinale* (Tablet Vomigone II tds, Dineh Iran Pharmaceutical Company, Iran, 500 mg powdered plant material) and *Echinacea* (Tablet Rucoldup I tds, Ghaem Darou Pharmaceutical Company, species, plant part, and pharmaceutical form not described) for seven days in addition to the standard treatment, or standard treatment alone, which was hydroxychloroquine. The intervention group experienced significant improvements in symptoms such as coughing, dyspnea, and muscle pain over the control group. However, like in our study, there was no effect on the hospitalization rate [35]. In the second Iranian study, an open-label, randomized, controlled, multicenter trial enrolled 358 hospitalized adult patients with Covid-19. They were allocated to receive standard care or herbal remedies (a polyherbal decoction and two herbal capsules) plus standard care for seven days. The intervention group had a significantly shorter duration of hospital dyspnea, accelerated clinical improvement, and decreased symptoms such as dry cough, dyspnea, muscle pain, headache, fatigue, anorexia, chills, runny nose, sputum cough, and vertigo [10]. In the third Iranian study, Zufa syrup, a polyherbal formulation, was investigated in the treatment of suspected patients with mild to moderate symptoms of Covid-19. That was a triple-blind randomized controlled trial that randomized 116 adult patients to receive either Zufa or placebo syrup for ten days. However, there were no significant differences in symptom relief between the two groups [36].

In another study, an open-label, randomized, controlled clinical trial conducted in Saudi Arabia, 173 adult patients with suspected Covid-19 were randomly allocated to receive 500 mg *Nigella sativa* oil (Cuminmar, Marnys®, Cartagena, Spain) twice a day for ten days. In the intervention group, the proportion of patients who recovered (symptom-free for three days) within 14 days was significantly higher, whereas the time to recovery was significantly shorter than in the control group [37].

We chose *J. pectoralis*, *A. satureioides*, and *E. prostrata* for our polyherbal formulation because they have important anti-inflammatory effects and they are traditionally used in respiratory diseases and symptoms, as our clinical experience has confirmed. *J. pectoralis* is rich in coumarin and umbeliferone, which have important anti-inflammatory properties [38,39]. The effect of *J. pectoralis* on an

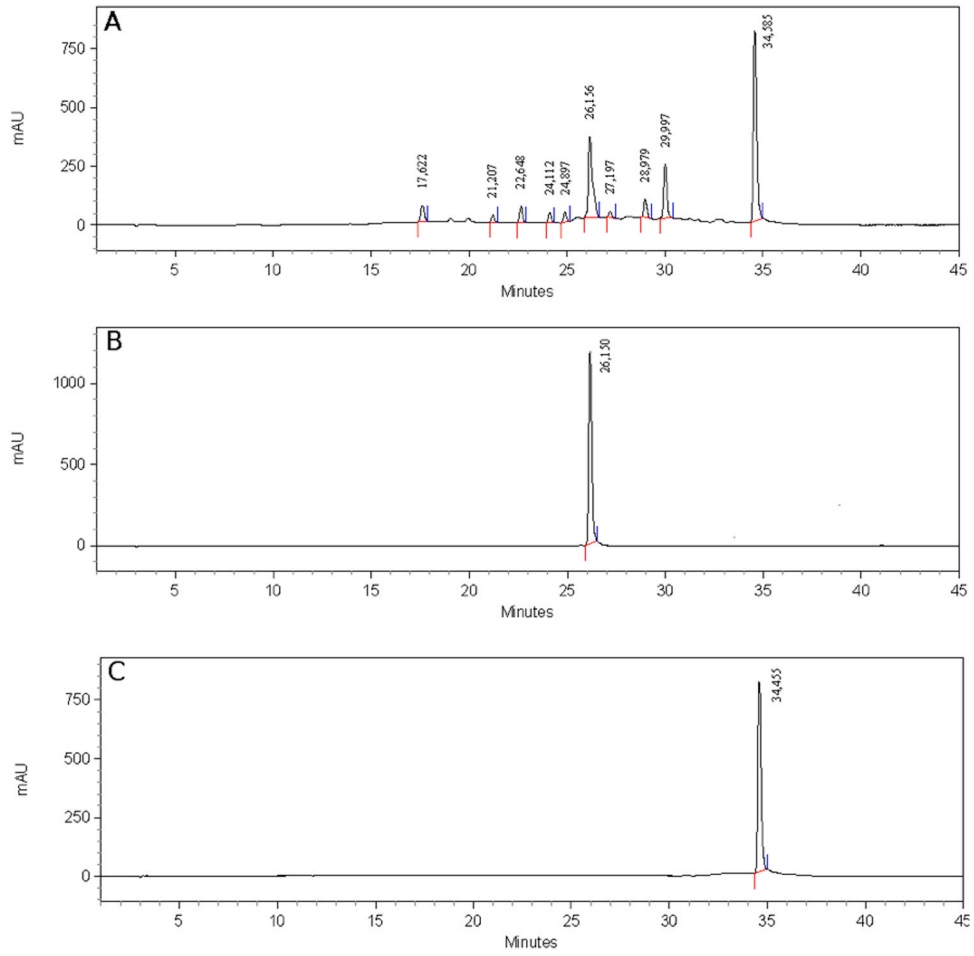

Fig. 3. HPLC-DAD chromatograms of the tincture from *Eclipta prostrata* (A), and the standard compounds demethylwedelolactone (B) and wedelolactone (C).

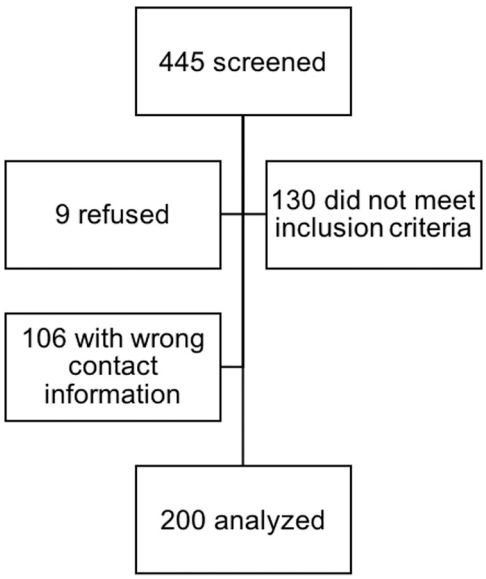

Fig. 4. Self-reported intensity of Covid-19 symptoms before using the formulation, ordered by decreasing intensity (n = 200).

animal model of asthma was already reported [40]. Indeed, a *J. pectoralis* syrup ameliorated cough and nasal congestion and running in children with upper viral respiratory infections [18]. *A. satureioides* is rich in flavonoids with many biological effects, including antiallergic, anti-inflammatory, immunomodulatory, and sedative [20,30,31,41–44]. Although there are no clinical trials with this species, it is traditionally used for respiratory ailments in Brazil [19]. *E. prostrata* is extensively used to treat respiratory diseases such as asthma, bronchitis, and cough [27]. Its beneficial effects on animal models of asthma were reported [28]. The major chemical constituents in *E. prostrata* are phytosterols, flavonoids, triterpenoids, saponins, and alkaloids, whereas the coumestans wedelolactone and demethylwedelolactone are considered the main bioactive compounds for the anti-inflammatory effect [27,28].

Our results must be interpreted with caution, given the following limitations: (a) the retrospective design, subject to selection bias (people who preferred herbal medicines and those experiencing benefit could be more likely to participate), and recall bias (since many cases occurred a year ago); (b) the lack of a control group, which we considered unethical at the time we started distributing the medicines; (c) adherence to treatment was not systematically assessed; (d) we studied a mix of suspected and confirmed cases of Covid-19; and (e) we did not record how many days passed after symptom onset and before the participants started using the formulation and, therefore, cannot rule out that the symptom relief was just the disease's natural history. We are aware of these limitations, and they would be best addressed in a randomized clinical trial. Nevertheless, given the potential benefit, low cost, and excellent safety profile of these two plants, we believe that these herbal medicines should be considered in patients with symptoms of Covid-19.

In conclusion, a polyherbal formulation containing *J. pectoralis, A. satureioides*, and *E. prostrata* helped relieving symptoms of Covid-19 without, however, decreasing the need for hospitalization. Complementary and alternative treatments based on the traditional use of species with bronchodilator, antiasthmatic, and immunomodulatory

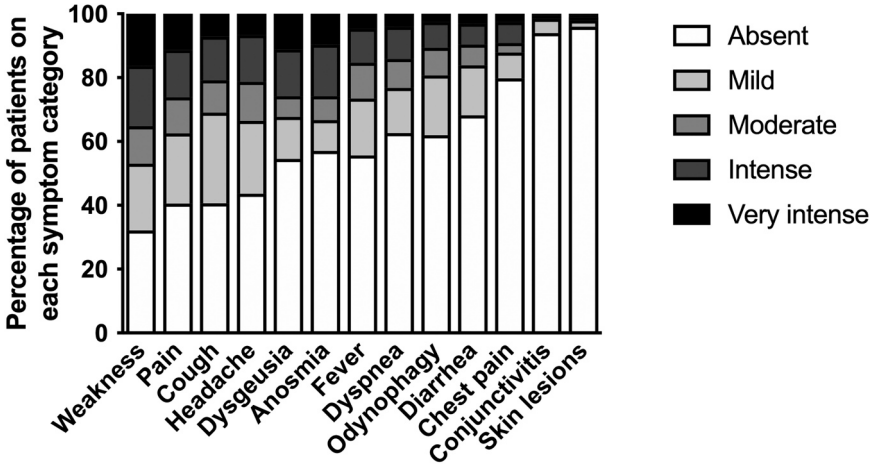

Fig. 5. Self-reported relative change in symptom intensity after using the formulation (n = 200).

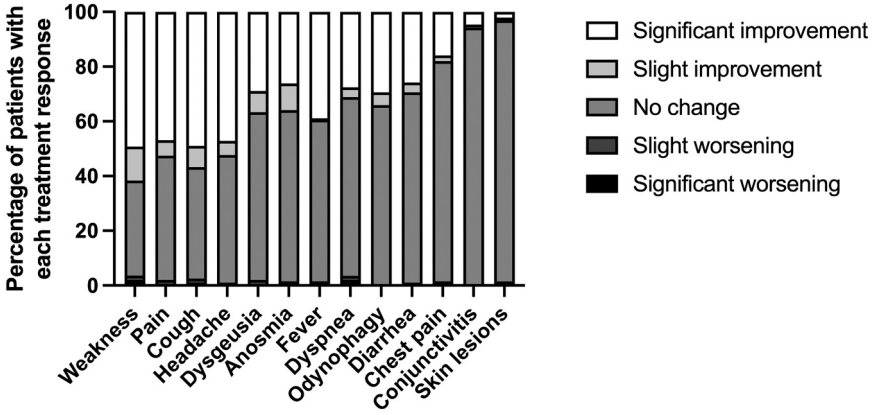

Fig. 6. Legend: All changes were significantly different from no change (Wilcoxon signed rank test, all $p < 0.001$), except for skin lesions.

effects can relieve symptoms of Covid-19, showing that polyherbal formulations are promising strategies to minimize the global burden of Covid-19.

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Ethical statement

We, authors of the manuscript entitled "**A polyherbal formulation containing *Justicia pectoralis* Jacq., *Achyrocline satureioides* (Lam.) DC., and *Eclipta prostrata* (L.) L. help reducing symptom intensity during and after Covid-19 infection**" declare that the study was conducted according to the ethical principles and the Brazilian regulations for clinical research.

CRediT authorship contribution statement

Fabio Carmona: Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Lucas Chaves:** Validation, Investigation, Writing – review & editing. **Fabiana Cardoso Tardelli do Nascimento:** Investigation, Writing – review & editing. **Débora Simone Sales:** Investigation, Writing – review & editing. **Bianca Waléria Bertoni:** Validation, Investigation, Resources, Writing – review & editing, Visualization. **Gustavo Henrique Teixeira Pinto:** Validation, Investigation, Resources, Writing – review & editing, Visualization. **Pedro de Pádua Amatto Goulart:** Validation, Investigation, Writing – review & editing. **Ana Maria Soares Pereira:** Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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What is already known about the topic?

- The new coronavirus (SARS-Cov-2) disease (Covid-19) became a pandemic in 2020. In Brazil, at the time of this study, there was still no effective treatment for it.
- Despite mass vaccination, cases were still numerous and there was a concern that vaccines would not protect against new variants of the virus.
- Several plant species have been investigated for the treatment of Covid-19, with no definite results to that date.

What this paper adds?

- A polyherbal formulation containing *Justicia pectoralis*, *Achyrocline satureioides*, and *Eclipta prostrata* was freely distributed to 2,000 + adults with suspected Covid-19, and 200 (~10%) were interviewed by phone call.
- This polyherbal formulation helped relieving symptoms of Covid-19 but did not reduce the need for hospitalization.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aimed.2023.07.004.

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